Clinical and Genetic Analysis of Patients with Cystinuria in the United Kingdom

Hannah L. Rhodes, Laura Yarram-Smith,† Sarah J. Rice,‡ Ayla Tabaksert,‡ Noel Edwards,§ Alice Hartley,‖ Mark N. Woodward,* Sarah L. Smithson,¶ Charles Tomson,**†† Gavin I. Welsh,* Margaret Williams,† David T. Thwaites,‡ John A. Sayer,§†† and Richard J.M. Coward**

Abstract

Background and objectives Cystinuria is a rare inherited renal stone disease. Mutations in the amino acid exchanger System $b^{0,+}$, the two subunits of which are encoded by *SLC3A1* and *SLC7A9*, predominantly underlie this disease. The work analyzed the epidemiology of cystinuria and the influence of mutations in these two genes on disease severity in a United Kingdom cohort.

Design, setting, participants, & measurements Prevalent patients were studied from 2012 to 2014 in the northeast and southwest of the United Kingdom. Clinical phenotypes were defined, and genetic analysis of *SLC3A1* and *SLC7A9* combining Sanger sequencing and multiplex ligation probe–dependent amplification was performed.

Results In total, 76 patients (42 men and 34 women) were studied. All subjects had proven cystine stones. Median age of presentation (first stone episode) was 24 years old, but 21% of patients presented after 40 years old. Patients had varied clinical courses, with 37% of patients having ≥10 stone episodes; 70% had evidence of CKD, and 9% had reached ESRD as a result of cystinuria and its complications. Patients with cystinuria received a variety of different therapies, with no obvious treatment consensus. Notably, 20% of patients had staghorn calculi, with associated impaired renal function in 80% of these patients. Genetic analysis revealed that biallelic mutations were present in either *SLC3A1* (n=27) or *SLC7A9* (n=20); 22 patients had only one mutated allele detected (*SLC3A1* in five patients and *SLC7A9* in 17 patients). In total, 37 different mutant variant alleles were identified, including 12 novel mutations; 22% of mutations were caused by large gene rearrangements. No genotype-phenotype association was detected in this cohort.

Conclusions Patients with cystinuria in the United Kingdom often present atypically with staghorn calculi at ≥40 years old and commonly develop significant renal impairment. There is no association of clinical course with genotype. Treatments directed toward reducing stone burden need to be rationalized and developed to optimize patient care.

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*Academic and Children's Renal Unit, School of Clinical Sciences, University of Bristol, Bristol, United Kingdom; †Bristol Genetics Laboratory—Pathology Sciences and **Richard Bright Renal Unit, Southmead Hospital, Bristol, United Kingdom; §Institute of Genetic Medicine, International Centre for Life and ‡Institute for Cell and Molecular Biosciences, Faculty of Medical Sciences, Newcastle University, Newcastle upon Tyne, United Kingdom; ‖Department of Urology, National Health Service Foundation Trust, Newcastle upon Tyne, United Kingdom; ¶Department of Clinical Genetics, University Hospitals, Bristol, United Kingdom; and ††Department of Renal Medicine, Freeman Hospital, Newcastle upon Tyne, United Kingdom

Correspondence: Dr. John A. Sayer, Institute of Genetic Medicine, International Centre for Life, Newcastle University, Central Parkway, Newcastle upon Tyne NE1 3BZ, UK, or Prof. Richard J.M. Coward, Academic and Children's Renal Unit, School of Clinical Sciences, Dorothy Hodgkin Building, University of Bristol, Bristol BS1 3NY, UK. Email: John.Sayer@ncl.ac.uk or Richard.Coward@bristol.ac.uk

Introduction

Cystinuria (OMIM 220100) is an inherited disorder characterized by the urinary loss of cystine, lysine, ornithine, and arginine. This loss leads to the formation of cystine stones in the urinary tract. Cystine is normally reabsorbed from the primary filtrate through a transporter, which consists of the two protein subunits rBAT and $b^{0,+}$AT (1). rBAT is encoded by *SLC3A1* (2), which is located at chromosome 2 (OMIM 104614), whereas $b^{0,+}$AT is encoded by *SLC7A9* (3) at chromosome 19 (OMIM 604144). Mutations in *SLC3A1*, *SLC7A9*, or both can lead to cystinuria.

The clinical presentation of cystinuria is usually before the age of 30 years. Clinical management combines lifestyle advice and medical therapy with surgical interventions, when required, to remove problematic stones from the urinary tract. The mainstays of current treatment are increased fluid intake, alkalinization of the urine, and if these measures fail, use of cystine-binding drugs, including penicillamine and tiopronin (alias Thiola), which form soluble heterodimers with cystine (4).

Patients with mutations in *SLC3A1* are known as type A, and those with *SLC7A9* are known as type B. Genotype types AA and BB denote two mutated alleles in *SLC3A1* or *SLC7A9*, respectively, whereas A and B denote the identification of only one mutated allele (5). In a third rare group, type AB, individuals have one mutation in *SLC3A1* and one mutation in *SLC7A9* (6). Occasionally, there are more than two mutated alleles present, such as AAB and BBA (5).

Cystinuria caused by mutations in *SLC3A1* is usually an autosomal recessive condition. Previous work suggests that heterozygotes for mutations in *SLC3A1* have normal urinary cystine and dibasic amino acid levels (6), except that some (but not all) heterozygotes with the duplication of exons 5–9 may have elevated levels of urinary cystine (5) and can develop recurrent cystine

stones (7). The inheritance of *SLC7A9* mutations is usually autosomal dominant with variable penetrance, with 86% of *SLC7A9* heterozygotes having abnormal urinary dibasic amino acid levels (6), and a variable proportion of these develops cystine stones.

Since linkage analysis identified cystinuria involvement of *SLC3A1* in 1994 (2,8) and *SLC7A9* in 1999 (9), 152 mutations have been reported in *SLC3A1*, and 104 mutations have been reported in *SLC7A9* (10). Cohorts of patients from Europe, Asia, and North America have been genotyped (11–21) along with a recently published group of United Kingdom patients (22).

To build up a large cohort of patients with cystinuria, a United Kingdom National Registry of Rare Kidney Diseases (RaDaR) has been established (23). A national genetic testing service has also been established, and two cohorts of patients from different geographical regions of England underwent detailed genotyping and clinical data collection. We describe the genetic mutations identified and clinical course/phenotype of these patients with a view to personalizing cystinuria management.

Materials and Methods

Patients

Patients recruited to this study all had a clinical diagnosis of cystinuria on the basis of confirmed cystine stone(s) on chemical analysis. Prevalent patients were identified and recruited to the study from October of 2012 to July of 2014 from the southwest and northeast of England (shown in Supplemental Figure 1). We believe that the majority of patients with cystinuria within these regions was identified. No patients with cystinuria declined to be included in the study.

Detailed clinical data were collected retrospectively to inform the genotype/phenotype analysis. The study was approved by the National Research Ethics Service (NRES) Committee South Central (12/SC/0456) and the NRES Committee North East (11/NE/0259), and informed consent was obtained from all participants or their parents/guardians where applicable.

Clinicians completed data collection for each patient with cystinuria under their care, including demographics (age, sex, and ethnicity), age at diagnosis, age at first stone event, number of stone events (subdivided into stones passed spontaneously, lithotripsy sessions, and invasive stone removal procedures, including open, percutaneous, and endourologic procedures), medical treatments (current and previous), and details of relevant blood and urine biochemistry, including eGFR measurements using an abbreviated Modification of Diet in Renal Disease equation (http://egfrcalc.renal.org/). The number of stone events per year was estimated by scoring all spontaneous stones passed with all stone-removing procedures performed since the age of first stone event.

For calculation of prevalence, the number of known patients with cystinuria was used together with estimates of the total population for the geographical regions of study taken from the 2011 United Kingdom National Census.

Genetic Analyses

Patients were genotyped using Sanger sequencing (ABI3730) of all coding exons and flanking intronic regions, including splice sites and branch points of *SLC3A1* and *SLC7A9*. Variant pathogenicity assignment was undertaken using Alamut (version 2.3 rev 1). In addition, all DNA samples were analyzed with multiplex ligation-dependent probe amplification (MLPA) of coding exons using an in-house high-throughput automated MLPA assay (Beckman NX/Beckman CEQ8000). Probes for *SLC3A1* and *SLC7A9* previously described by Bisceglia *et al.* (11) were used in conjunction with the Control P200 Probe Kit from MRC-Holland.

Statistical analyses of data were performed using Graphpad Prism, version 5. Where data were not normally distributed, the median and range are stated, and P values were calculated by performing Mann–Whitney U tests (two-tailed) to compare two independent groups of non-Gaussian data. For categorical data, two-tailed Fisher's exact test was performed. A P value <0.05 was considered statistically significant.

Results

Clinical Features

In total, 76 patients diagnosed with cystinuria were identified comprising 34 (45%) women and 42 (55%) men, including one pediatric patient. All patients were white British, except one patient each from Pakistan and China. Using an estimated combined population size of 7,886,100, the overall prevalence of cystinuria was estimated to be approximately one in 100,000 in this cohort. A positive family history of cystinuria was documented in 26 (34%) patients; 17 patients had only one generation affected (siblings), seven patients had two generations affected, and one patient each had cystinuria in three and four generations.

The median age at first stone event was 24 years old (range =2–62 years old). The majority of patients had their first stone event in their late teens and early 20s. However, 16 (21%) patients were over 40 years old at presentation (Figure 1A). Stone episodes per year varied widely (median frequency of 0.45 stones per year, range of 0.06–78.2), with no significant difference in stone events per year between sexes (P=0.73).

Fifty-three patients (70%) had evidence of CKD (eGFR$<$90 ml/min per 1.73 m^2), including seven patients (9%) with an eGFR$<$30 ml/min per 1.73 m^2 (Table 1). Three of these patients have undergone renal transplantation for ESRD that was attributed to a combination of multiple bilateral cystine stone episodes (including staghorn stone formation), open surgical procedures, and kidney infections.

All 76 patients had been advised to increase daily intake of fluids, and 13 (17%) patients followed diets low in animal protein. Additional alkalizing and/or cystine-binding treatments were prescribed to 52 of 76 (68%) patients (Table 2). Of 76 patients, 26 (34%) patients received monotherapy, and 24 (32%) patients were given a combination of alkalinization and cystine-binding therapies. Of 26 patients currently or historically prescribed penicillamine, 14 (54%) patients had experienced side effects (Supplemental Table 1). Of 19 patients previously or presently taking tiopronin, only one patient developed a side effect. Three patients were noted to have discontinued alkalinization medication, because they found potassium citrate unpalatable. Overall, there was evidence that 54 of 76 (71%) patients received medical therapy that followed international guidelines (24,25). Despite this, 27 (50%) of 54 patients continued to form kidney stones.

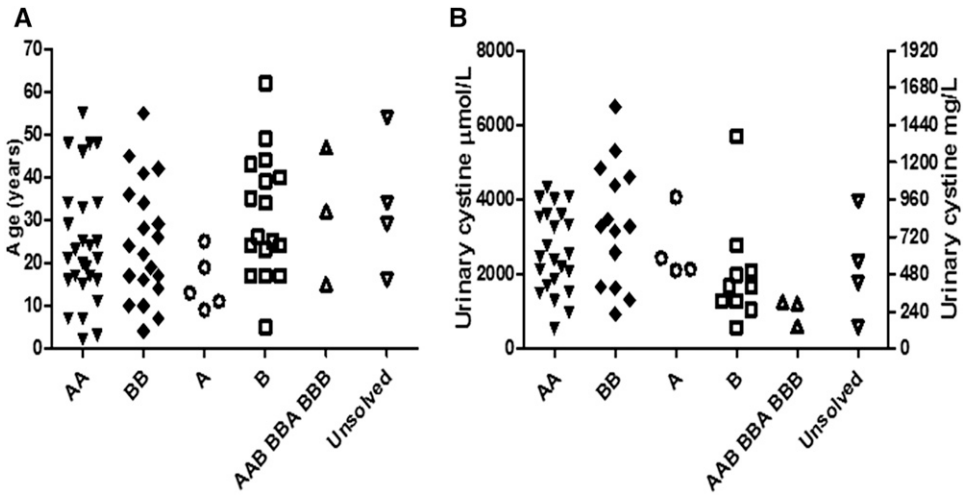

Figure 1. | **Genotype-phenotype.** A shows the age in years at first stone event for each genotyped group, and B shows 24-hour urinary cystine at first presentation, which shows the considerable overlap between the genotype groups. A, heterozygous *SLC3A1*; AA, homozygous *SLC3A1*; AAB, BBA, BBB, triallelic for *SLC7A9* mutations or *SLC7A9* and *SLC3A1* mutations; B, heterozygous *SLC7A9*; BB, homozygous *SLC7A9*; unsolved, no mutation detected in *SLC3A1* or *SLC7A9*.

Table 1. Comparison of renal function by genotype

eGFR (ml/min per 1.73 m^2)	No.	Percent	AA (B)	A	BB (A or B)	B	AB	Genotype Unknown
≥90	23	30	8		6	8		1
60–89	37	49	16	4	7	5	3	2
30–59	9	12	2		3	3		1
<30	4	5	1	1	2			
ESRD with renal transplant	3	4			2	1		

Surgical therapy included lithotripsy in 38 (50%) patients and open, endoscopic, or percutaneous stone removal in 62 (86%) patients.

Fifteen (20%) patients had documented staghorn stones (Table 3). Age at first stone, stone frequency, and sex were not associated with staghorn calculi formation, but the majority of patients had a reduction in eGFR, with 12 of 15 (80%) patients <90 ml/min per 1.73 m^2. One patient had reached ESRD and received a renal transplant.

Genetic Analyses

Genetic analysis was performed in all 76 patients (Table 4). At least two distinct genetic mutations were detected in 50 (66%) patients. Of these, three patients had evidence of triallelism. A single mutation was found in 22 (29%) patients, and in four (5%) patients, no pathogenic mutations were detected.

In total, 125 mutated alleles were identified, with 37 different distinct variants (Tables 5 and 6). Of the 125 mutated alleles, 27 (22%) were large gene rearrangements, the majority in *SLC3A1*. We identified 12 previously unreported (novel) mutations: eight in *SLC3A1* and four in *SLC7A9* (Tables 5 and 6). Only 15 (20%) patients were homozygous for a mutant allele, including seven patients with type AA, six patients with type BB, one patient with type BBA, and one patient with type BBB.

The most frequent mutant allele in *SLC3A1* was a duplication of exons 5–9 in 24% (15 of 62) of alleles followed by the missense mutation c.1400T>C, p.(Met467Thr) in 22% (14 of 62). A novel frameshift, c.2020dupT, p.(Tyr674-Leufs*20), in exon 10 was detected in 10% (6 of 62) of alleles (Table 5).

In *SLC7A9*, the most frequent allele was the frameshift c.614dupA that was observed in 24% (15 of 63). The missense mutations c.671C>T, p.(Ala224Val), c.544G>A, p.(Ala182Thr), and c.313G>A, p.(Gly105Arg) represent 16%, 13%, and 13%, respectively, of *SLC7A9* alleles. The deletion of exon 12 also accounted for 10% of *SLC7A9* alleles (Table 6).

The distributions of mutations within the exons and protein domains are represented in Figures 2 and 3. In this study, mutations in *SLC3A1* occurred throughout all exons, whereas in *SLC7A9*, mutations were limited to exons 4–6, 9, 10, 12, and 13.

One novel mutation in *SLC3A1* (c.2020dupT) was identified in four patients (two related and two unrelated patients) from the southwest of England, three of whom live in a very geographically remote location, suggestive of a founder effect for this mutation.

Genotype-Phenotype Association

We were unable to show a difference between 27 patients with type AA and 20 patients with type BB in a variety of clinical parameters, including age of first stone event, in which patients with AA presented at a median age of 21

Table 2. Medications used in the treatment of cystinuria in the English cohort

Number of Medications Used	Potassium Citrate	Sodium Bicarbonate	Captopril	Penicillamine	Tiopronin	Frequency
None	—	—	—	—	—	24
Single (*n*=26)	X	—	—	—	—	10
	—	X	—	—	—	1
	—	—	X	—	—	1
	—	—	—	X	—	9
	—	—	—	—	X	5
Dual (*n*=24)	X	X	—	—	—	1
	X	—	X	—	—	2
	X	—	—	X	—	2
	X	—	—	—	X	10
	—	X	X	—	—	2
	—	X	—	X	—	4
	—	X	—	—	X	3
Triple	X	—	X	—	X	2

Table 3. Genetic and clinical characteristics of staghorn calculi formers

ID	Type	Mutant Allele Type	DNA Variant and Predicted Protein Description	Sex	Age (yr) at First Stone	Stones per Year Since First Stone	eGFR (ml/min per 1.73 m^2)
14	AA	Com het	c.[1400T>C]; [exons 5–9 dup] p.[Met467Thr]; [exons 5–9 dup]	M	2	0.32	88.5
16	AA	Com het	c.[1400T>C]; [exons 5–9 dup] p.[Met467Thr]; [exons 5–9 dup]	W	48	0.67	>90
19	AA	Hom	c.[1400T>C]; [1400T>C] p.[Met467Thr]; [Met467Thr]	W	16	0.13	62.2
55 F4	A	Het	c.[exons 5–9dup]; [=]	W	13	0.11	65.1
30	BB	Com het	c.[368C>T] ; [671C>T] p.[Thr123Met]; [Ala224Val]	M	42	0.88	>90
32	BB	Com het	c.[414_415delGC]; [exon 12 del] p.[Pro139Leufs*69]; [exon 12 del]	M	17	2.62	80.9
37	BB	Hom	c.[614dupA]; [614dupA] p.[Asn206Glufs*3]; [Asn206Glufs*3]	W	16	0.44	49.8
38	BB	Hom	c.[614dupA]; [614dupA] p.[Asn206Glufs*3]; [Asn206Glufs*3]	M	41	0.09	18
41 F3	BB	Com het	c.[614dupA]; [exon 12 del] p.[Asn206Glufs*3]; [exon 12 del]	W	10	1.6	27
43	BB	Com het	c.[671C>T]; [997C>T] p.[Ala224Val]; [Arg333Trp]	W	4	4.47	50
46	BB	Com het	c.[1399+4_1399+7del]; [exon 12del]	M	17	2	>90
50	BBB	Com het + Hom	c.[544G>A;1060G>A]; [544G>A] p.[Ala182Thr;Ala354Thr]; [Ala182Thr]	W	47	0.73	83.4
66	B	Het	c.[671C>T]; [=] p.[Ala224Val]; [=]	M	40	0.09	39.2
69	B	Het	c.[671C>T]; [=] p.[Ala224Val]; [=]	M	5	0.06	52.6
72	B	Het	c.[exon 12 del]; [=]	M	24	?	ESRD (transplant)

ID, identification; F4, family 4; F3, family 3; Com het, compound heterozygote (two different mutations detected—one in each allele); Hom, homozygous (both alleles in the same mutation); Het, heterozygote (single mutated allele detected); M, man; W, woman.

Table 4. Frequency of genotype in cohorts

Genotype	No.	Percent of total
AA	27	36
BB	20	26
AAB	1	1
BBA	1	1
BBB	1	1
A	5	7
B	17	22
Unsolved	4	5
Total	76	

years old (range =2–55 years old) and patients with BB presented at a median of 23 years old (range =4–55 years old; P=0.96); number of stone episodes per year, in which patients with type AA had a median of 0.44 stone episodes per year (range =0.1–7.13) and patients with type BB had a median of 0.48 stone episodes per year (range =0.09–13.3; P=0.32); and renal function, with 70% of patients with type AA and 82% of patients with type BB having an eGFR<90 ml/min per 1.73 m^2.

There was overlap between all of the genotypic groups regarding age of first stone event (Figure 1A). Patients with a single mutated allele also had variable disease severity and could not be differentiated from patients with two mutated alleles. Figure 1B shows the variability both between and within the genotypic groups for the urinary cystine levels at first presentation. The level of renal impairment (Table 1) was also similar across all of the genotypes.

We found 13 subgroups who had exactly the same genotype (Supplemental Table 2): four groups for type AA genotype, three groups for type BB genotype, one group for type A genotype, and five groups for type B genotype. Each subgroup showed a wide divergence in stone frequency and age of onset of the first stone, despite having the same underlying mutations. There were pairs of siblings in groups AA, BB, and A who also all had dramatically contrasting clinical courses.

All of the patients in this study had proven cystine stones. Four type A patients (heterozygous for duplication of exons 5–9) had recurrent cystine stone events. Notably, one patient heterozygous for c.761A>C, p.(Asn254Thr) in *SLC3A1* had six cystine stone events (patient 51 in Supplemental Table 2).

Discussion

In this study, we have comprehensively studied the genetics and clinical progress of patients with cystinuria from two English regions. We have found that there is no genotype-phenotype correlation in these patients, that stones commonly present in adulthood, and that patients are on a variety of different therapies. Furthermore, our data show that, in both *SLC3A1*- and *SLC7A9*-related diseases, having a single detectable mutation is common (and sufficient for a dramatic clinical phenotype) and that a few

Table 5. Summary of *SLC3A1* alleles detected

Variant Type	*SLC3A1*	Location of Mutation	Predicted Protein Sequence	No.	
Missense	c.647C>T	Exon 3	p.Thr216Met	2	Known
Missense	c.761A>C	Exon 3	p.Asn254Thr	2	Novel
Missense	c.1093C>T	Exon 6	p.Arg365Trp	2[a]	Known
Missense	c.1354C>T	Exon 8	p.Arg452Trp	3	Known
Missense	c.1372G>A	Exon 8	p.Gly458Arg	1[b]	Novel
Missense	c.1400T>C	Exon 8	p.Met467Thr	14	Known
Missense	c.1412C>G	Exon 8	p.Thr471Arg	2	Known[c]
Missense	c.1796T>C	Exon 10	p.Phe599Ser	1	Known
Missense	c.1799G>A	Exon 10	p.Gly600Glu	1	Known
Nonsense	c.1578G>A	Exon 9	p.Trp526*	1	Novel
Nonsense	c.1975C>T	Exon 10	p.Gln659*	1	Novel
Frameshift	c.161delC	Exon 1	p.Gln55fs*51	1	Known
Frameshift	c.356dupA	Exon 1	p.Glu120Glyfs16	1	Novel
Frameshift	c.2020dupT	Exon 10	p.Tyr674Leufs*20	6	Novel
Splice site	c.1136+2T>C	Intron 6		2	Known
Splice site	c.1332+2T>A	Intron 7		1	Novel
Deletion	Del exon 2			1	Novel
Deletion	Del exon 2–3			2	Known
Deletion	Del exon 2–4			1	Known
Deletion	Del exons 5–10			1	Known
Deletion	Del exon 10			1	Known
Duplication	Dup exons 5–9			15	Known
Total				62	

Del, deletion; dup, duplication.
[a]Both alleles in a patient of Pakistani descent.
[b]Novel variants detected in a Chinese patient.
[c]Recently reported in United Kingdom patients.

Table 6. Summary of *SLC7A9* alleles detected

Variant Type	*SLC7A9*	Location of Mutation	Predicted Protein Sequence	No.	
Missense	c.313G>A	Exon 4	p.Gly105Arg	8	Known
Missense	c.368C>T	Exon 4	p.Thr123Met	1	Known
Missense	c.544G>A	Exon 5	p.Ala182Thr	8	Known
Missense	c.671C>T	Exon 6	p.Ala224Val	10	Known
Missense	c.962G>A	Exon 9	p.Cys321Tyr	1	Novel
Missense	c.997C>T	Exon 10	p.Arg333Trp	1	Known
Missense	c.1060G>A	Exon 10	p.Ala354Thr	1	Known
Missense	c.1369T>C	Exon 12	p.Tyr457His	1	Novel
Nonsense	c.1353C>A	Exon 12	p.Tyr451*	1	Novel
Frameshift	c.411_412delTG	Exon 4	p.Pro139Leufs*69	3	Novel
Frameshift	c.414_415delGC	Exon 4	p.Pro139Leufs*69	1	Known[a]
Frameshift	c.614dupA	Exon 6	p.Asn206Glufs*3	15	Known
Splice site	c.1399+4_1399+7del	Intron 12		4	Known[a]
Splice site	c.1400–2A>G	Intron 12		2	Known[a]
Deletion	Del exon 12	Exon 12		6	Known
Total				63	

Del, deletion; dup, duplication.
[a]Recently reported in United Kingdom patients.

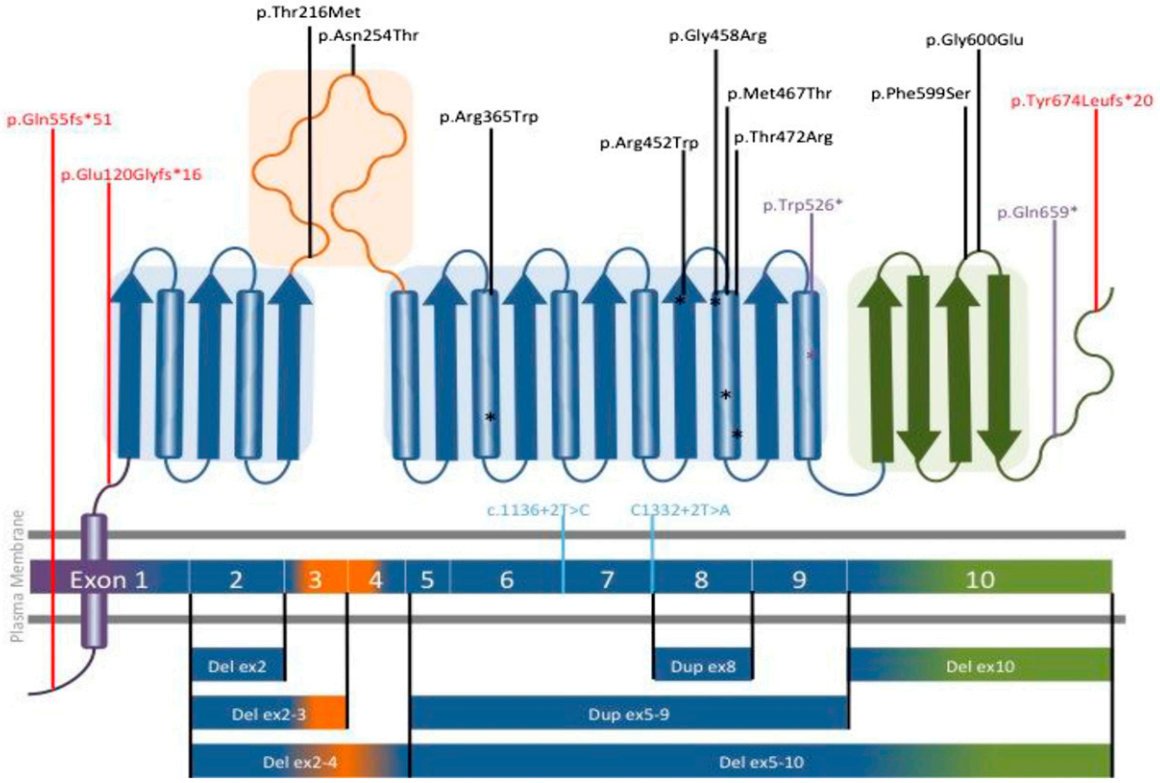

Figure 2. | Distribution of mutations detected in *SLC3A1* (rBAT) throughout the exons and protein domains. Schematic diagram of rBAT and a homology model of the extracellular domain of rBAT on the basis of the crystal structure of oligo-1,6-glucosidase from *Bacillus cereus* (Protein Data Bank ID code 1UOK). The domains of rBAT are shown in purple (TMD), blue (domain A), orange (domain B [subdomain]), and green (domain C). Mutations are labeled as follows: missense in black, nonsense in purple, frameshift in red, and splice site in pale blue. Mutations predicted to fall within α-helices are denoted by asterisks of the appropriate color. TMD, trans-membrane domain.

patients do not have an easily identifiable mutation in the known cystinuria genes.

Cystine stones can occur at any age. In previous studies, >80% of patients developed their first stones within the first two decades (12), and <1% of patients with cystinuria had their first stone when >40 years old (6). However, in this cohort, only 30 of 76 (39%) patients had their first stone by the age of 20 years old (Figure 1A); 16 of 76

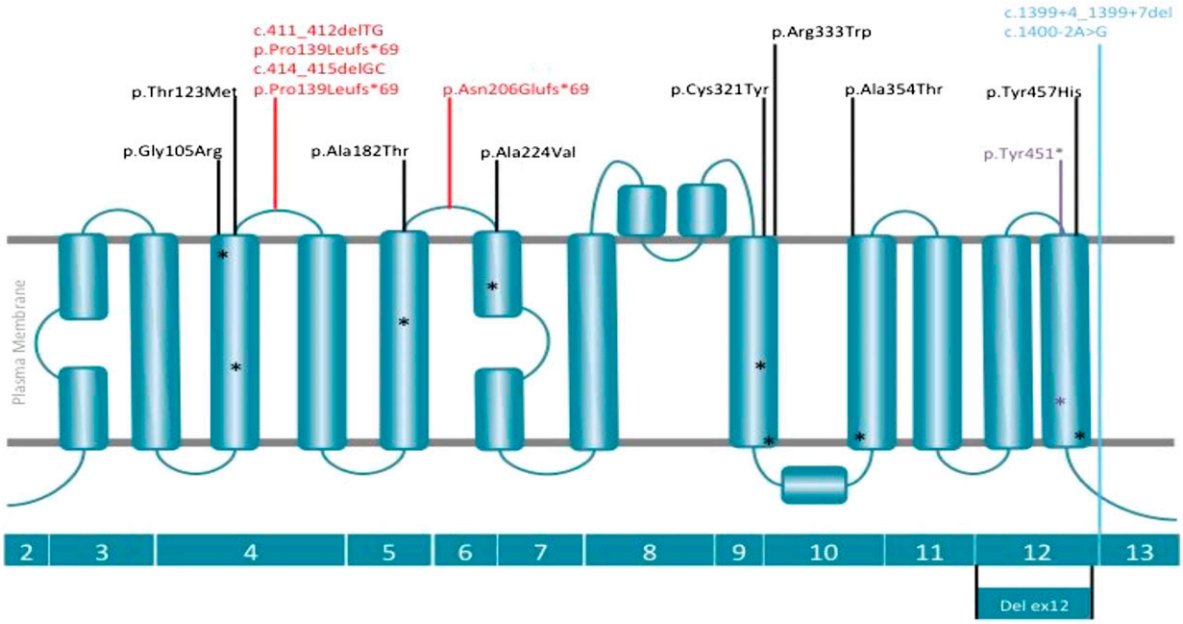

Figure 3. | Representation of the location of mutations detected in *SLC7A9* ($b^{0,+}$AT) throughout the exons and protein domains. Schematic diagram of $b^{0,+}$AT and homology model of $b^{0,+}$AT on the basis of the crystal structure of AdiC protein, an arginine:agmatine antiporter from *Escherichia coli* (Protein Data Bank ID code 3L1L). *SLC7A9* mutations are distributed in exons 4–6, 9, 10, 12, and 13. Mutations are labeled as follows: missense in black, nonsense in purple, frameshift in red, and splice site in pale blue. Mutations predicted to fall within α-helices are denoted by asterisks of the appropriate color.

(21%) patients did not pass their first stone until they were >40 years old, highlighting the importance of considering this inherited condition as an underlying cause of nephrolithiasis in older age groups. In this cohort, late presentation occurred in five patients with type AA, four patients with type BB, one patient with type BBB, five patients with type B, and one patient with an unsolved genotype.

It is also of note that 20% of patients in this study had staghorn calculi. Staghorn calculi may not always be investigated for underlying cystinuria, because it may be falsely assumed that they are infective stones, struvite in origin, and composed of magnesium ammonium phosphate. Case series reported by Soucy *et al.* (26) and Viprakasit *et al.* (27) have shown that between 21% and 44% of staghorn calculi are struvite, and for the remainder, other etiogic causes should be sought. Cystinuria is a known and important cause of staghorn calculi. Remarkably, Cupisti *et al.* (28) reported a 72-year-old patient with a cystine staghorn calculus, leading to a much delayed diagnosis of cystinuria.

Our cohort showed no associations between genotype and phenotype. There was no difference in age of onset, number of spontaneous stone emissions, or total stone events between those with type AA, BB, A, or B. These findings replicate those from other international studies (6) as well as the recent study from London (22). This clinical heterogeneity together with the fact that four patients in our study had no detectable exonic mutation in *SLC3A1* or *SLC7A9* suggest that disease-modifying single-nucleotide polymorphisms, intronic variants, or other genes may all contribute to the pathogenesis of cystinuria and should be a focus of future research.

Although there is international guidance and consensus on the optimal treatment strategy for patients with cystinuria (24,25), this was not obviously adhered to by all patients in this cohort, which was probably because of a combination of patient and clinician preferences. Furthermore, current treatments, such as penicillamine (29), often needed to be discontinued because of their serious side effect profile, which includes proteinuria and warrants close surveillance. Side effects from penicillamine in our cohort were similar to previous reports, occurring in around 50% of patients (30). We found that Tiopronin was generally much better tolerated than penicillamine, which has also been described by other groups (31,32). Even with medical therapy, continued stone formation was common. This emphasizes the importance of understanding the molecular basis of cystinuria and developing more effective and better tolerated medications. It would also be interesting to prospectively survey well defined groups of patients with cystinuria and their associated physicians in regard to the attitudes of clinicians and patients with respect to medical therapy prescribing and adherence, but this was not the focus of this study.

From a surgical perspective, we found that a high proportion of patients had received extracorporeal shock wave lithotripsy. There is conflicting evidence on the benefit of extracorporeal shock wave lithotripsy and cystine stone clearance (33,34). However, the benefit of lithotripsy in cystinuria would be better addressed in adequately powered large prospective studies and detailed international registries, such as the International Cystinuria Registry (35) (part of the Rare Kidney Stone Consortium; http://cystinuria.org/the-rare-kidney-stone-consortium-announces-the-cystinuria-registry/) and the proposed RaDaR cystinuria initiative (23).

The duplication of exons 5–9 and the missense mutation c.1400T>C, p.(Met467Thr) were the most frequent *SLC3A1*

Table 7. Summary of previous large genotype-phenotype studies of cystinuria

Author and Year	Cohort Origin	Cohort Size	Mutated Alleles Identified	Do All Subjects Have Proven Cystine Stones?	Age (yr) at First Stone/ Diagnosis (Range)	Renal Dysfunction	Is Severity of Clinical Phenotype Linked to Genotype?	No. with Staghorn Calculi	Most Common *SLC3A1* Mutation (*n*/Total)	Most Common *SLC7A9* Mutation (*n*/Total)	Large Gene Rearrangements	DNA Analysis
This study (2015)	England	76	125	Yes	Median 24 (2–62)	70% CKD, 9% with ESRD	No	15 (20%)	Dup exons 5–9 24% (15 of 62)	c.614dupA 24% (15 of 63)	22%	Sequencing, MLPA
Wong et al. 2014 (22)	England	74	143	No	Mean 23.9 (0–61)	Not studied	One or more *SLC3A1* missense mutations had lower urinary ornithine, lysine, and arginine; no other phenotype association seen	Not reported	Dup exons 5–9 27% (24 of 88)	c.614dupA 20% (11 of 55)	23% (33 of 143)	Sequencing, MLPA
Popovska-Jankovic *et al.* 2013 (18)	Southeastern Europe[a]	60	93	No	Data incomplete	Not studied	*SLC3A1* homozygous for p.Thr216Met had higher urinary dibasic amino acids[b]	Not reported	p.Thr216Met 25% (24 of 98)	p.Gly105Arg 21% (21 of 98)	Not reported	Sequencing, SSCP, RFLP
Barbosa *et al.* 2012 (37)	Portugal	12	21	No	Median 3 (0.6–14)	Not studied	No	Not reported	Dup exons 5–9 (4 of 13)	c.972G>A[c] (3 of 8)	29% (6 of 21)	Sequencing, MLPA
Bisceglia *et al.* 2010 (11)	Italy	172	308	Yes	Not studied	Not studied	Not studied	Not reported	p.Met467Thr 23% (34 of 145)	p.Gly105Arg 27% (44 of 163)	11% (35 of 308)	Sequencing, MLPA
Font-Llitjós *et al.* 2005 (5)	Italy, Spain, Israel, Belgium, Portugal, Switzerland, England, Germany	164 Probands	282	Yes	Not studied	Not studied	Not studied	Not reported	p.Met467Thr 26% (33 of 125)	p.Gly105Arg 29% (43 of 147)		SSCP, DHPLC, semiquantitative multiplex PCR, sequencing
Dello Strologo *et al.* 2002 (6)	Italy, Spain, Israel	188 had genetic analysis	302	No	Median 14	17% (30 of 176) mild renal insufficiency,[d] 1 of 176 ESRD	No	Not reported	Not reported	Not reported	Not studied	SSCP, sequencing
Botzenhart *et al.* 2002 (36)	Germany, Turkey	31	28	No	Median 7 (0.75–24)[e]	Not studied	No	Not reported	p.Met467Thr	p.Gly105Arg	Not studied	SSCP
Font *et al.* 2001 (38)	Italy, Spain, North America, Libya (Jewish)	175		No	Not studied	Not studied	Not studied	Not reported	Not reported	p.Gly105Arg 25% (29 of 114)	Not reported	Sequencing, RNA SSCP, SSCP

Table 7. (Continued)

Author and Year	Cohort Origin	Cohort Size	Mutated Alleles Identified	Do All Subjects Have Proven Cystine Stones?	Age (yr) at First Stone/ Diagnosis (Range)	Renal Dysfunction	Is Severity of Clinical Phenotype Linked to Genotype?	No. with Staghorn Calculi	Most Common *SLC3A1* Mutation (*n*/Total)	Most Common *SLC7A9* Mutation (*n*/Total)	Large Gene Rearrangements	DNA Analysis
Gitomer *et al.* 1998 (15)	United States (Texas)	33	34	No	3–75	Not studied	Not studied	Not reported	p.Met467Thr 18% (12 of 66)	Not studied	Not reported	RNA SSCP, RNA MisMatch

dup, duplication; MLPA, multiplex ligation–dependent probe amplification; SSCP, single–strand conformation polymorphism analysis; RFLP, restriction fragment length polymorphism; DHPLC, denaturing high performance liquid chromatography.
[a]Macedonia, Serbia, Turkey, Kosovo, Montenegro, Croatia, Bulgaria, and Slovenia.
[b]Mutation specific to Gypsy families (Popovska-Jankovic *et al.* [18] concluded that environmental factors and compliance may influence disease severity).
[c]Mutation that might contribute to expression of disease.
[d]Renal insufficiency defined as plasma creatinine >120 μmol/L.
[e]Majority of subjects were children.

mutations in our cohort, and they were also observed in another United Kingdom cohort (22). The missense mutation c.1400T>C, p.(Met467Thr) is the most common mutation in studies of Italian (11), German (12), Swedish (13), Spanish (14), North American (Texas) (15), and Czech and Slovak (16) cohorts. However, the duplication of exons 5–9 in *SLC3A1* is reported much less frequently around the world, except in Germany, where it accounts for 18.8% of *SLC3A1* mutations (12). This may be because of some studies failing to incorporate MLPA in their genomic analysis of cystinuria genes. The novel frameshift c.2020dupT, p.(Tyr674Leufs*20) in exon 10 of *SLC3A1* was detected in 9% (6 of 64) of alleles in our cohort.

In *SLC7A9*, the most frequent allele was the frameshift c.614dupA, p.(Asn206Glufs*3) occurring in 23% (15 of 64) of alleles. This allele was also frequently detected in another United Kingdom cohort (22) and a Spanish cohort (14). The *SLC7A9* mutation c.671C>T, p.(Ala224Val) described in a German family in 2002 (36) is rare in reported literature (12) but had a high frequency (15%) in our cohort. The deletion of exon 12 accounts for 11% of *SLC7A9* alleles in this study but was not described in the recent United Kingdom study (22). Previous cystinuria studies have focused on different clinical aspects of this disease, but many have analyzed genotype-phenotype associations (5,6,11,15,18,22,36–38). These are summarized in Table 7.

We identified five patients with type A genotype who have developed cystine stones. One patient who was heterozygous for the novel missense mutation c.761A>C in exon 3, p.(Asn254Thr) had multiple stone events since 11 years of age, and four patients with a single duplication of exons 5–9 were cysteine stone formers. This challenges the usual paradigm that those with single heterozygous *SLC3A1* mutations are unaffected carriers (except, as previously noted, some heterozygotes of duplication exons 5–9 [5,7]). This is important in assessing stone risk in children, genetic counseling, and when considering initiating preventative therapies.

The clinical data were collected retrospectively, which can limit accuracy, but our findings were comparable with those from the prospective study of patients from London (22). Moreover, a singular clinical and genetic database for United Kingdom patients with cystinuria is currently being established using the RaDaR initiative. Establishing national and international patient cohorts for the study of rare diseases, like cystinuria, is beneficial to develop collaborative research ideas, support patients and their families, and enable the effectiveness of medications to be assessed. Indeed, by genotyping these patients and correlating this information with clinical information held in the database, we hope that it may be possible to individualize therapies and establish treatment algorithms on the basis of specific mutations.

This study broadens the clinical and genetic spectrum of cystinuria, which is not a simple autosomal recessive disease. Rather, it is likely to result from a more complex genetic model involving at least two genes and additional unknown genetic modifiers together accounting for the variable severity of the phenotype.

We also conclude that focused medical and surgical management of patients with cystinuria is needed. Our aim is to encourage national and international comprehensive clinical research networks to study this condition more completely.

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J.A.S. and R.J.M.C. contributed equally to this work.

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