Comparison between SLC3A1 and SLC7A9 Cystinuria Patients and Carriers: A Need for a New Classification

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Abstract. Recent developments in the genetics and physiology of cystinuria do not support the traditional classification, which is based on the excretion of cystine and dibasic amino acids in obligate heterozygotes. Mutations of only two genes (SLC3A1 and SLC7A9), identified by the International Cystinuria Consortium (ICC), have been found to be responsible for all three types of the disease. The ICC set up a multinational database and collected genetic and clinical data from 224 patients affected by cystinuria, 125 with full genotype definition. Amino acid urinary excretion patterns of 189 heterozygotes with genetic definition and of 83 healthy controls were also included. All SLC3A1 carriers and 14% of SLC7A9 carriers showed a normal amino acid urinary pattern (i.e., type I phenotype). The rest of the SLC7A9 carriers showed phenotype non-I (type III, 80.5%; type II, 5.5%). This makes the traditional classification imprecise. A new classification is needed: type A, due to two mutations of SLC3A1 (rBAT) on chromosome 2 (45.2% in our database); type B, due to two mutations of SLC7A9 on chromosome 19 (53.2% in this series); and a possible third type, AB (1.6%), with one mutation on each of the above-mentioned genes. Clinical data show that cystinuria is more severe in males than in females. The two types of cystinuria (A and B) had a similar outcome in this retrospective study, but the effect of the treatment could not be analyzed. Stone events do not correlate with amino acid urinary excretion. Renal function was clearly impaired in 17% of the patients.

Cystinuria is an autosomal recessive disorder that is characterized by an impaired transport of cystine, lysine, ornithine, and arginine in the proximal renal tubule and in the epithelial cells of the gastrointestinal tract. High cystine concentration in the urinary tract most often causes the formation of recurrent renal stones that are resistant to medical treatment. Urine acidification and concentration facilitate stone formation (1).

Traditionally, cystinuria has been divided into three subtypes: types I, II, and III (2). Type I heterozygotes show a normal amino acid urinary pattern, whereas type II and III are characterized by an increase of cystine, lysine, ornithine, and arginine urinary excretion. Type II has been described as having a more severe impairment of intestinal transport than type III, as demonstrated by a lack of increase in plasma level after an oral load of cystine or lysine (2,3). Subtype non-I grouped previous subtypes II and III (4).

In 1994 a first gene, SLC3A1 in chromosome 2 (5,6) encoding rBAT, the mutations of which are responsible for type I cystinuria, was identified (7–9). Thereafter, the defect associated with the most common cystinuria-specific SLC3A1 mutation was described (10) and over 50 mutations in SLC3A1 were reported (11). A second locus gene that is responsible for type non-I cystinuria was mapped on chromosome 19 (12–15). In 1999, the gene responsible for type non-I cystinuria, SLC7A9 encoding for bo,+AT, was identified (15,16). This second gene encodes a subunit that associates with rBAT to form the active transporter (17,18). This gene appears to cause both type II and III cystinuria (13), supporting the grouping of these subtypes in type non-I (16,19). Over 30 mutations in SLC7A9 have been described so far (19).

Despite the increasing knowledge of the genetic and physi-
ologic characteristics of the carriers involved in cystinuria, clinical data in homozygotes are very scanty. Large series are not available in the literature. In this study, we present clinical data combined with mutation analysis of 224 patients and the amino acid urinary patterns of 47 and 142 SLC3A1 and SLC7A9 heterozygotes, respectively, compared with those of 83 healthy control relatives of the affected subjects in whom the mutation identified in the proband was not detected. Excretion patterns in heterozygotes with known mutations do not fully correspond to the classification used up to now. We propose a new cystinuria classification based on molecular analysis and not on urinary cystine and dibasic amino acid excretion patterns.

Material and Methods

The Institutional Review Board at each of the participating centers approved the study and participants gave informed consent.

All subjects meeting the following criteria were reported to the database:

- Cystine urinary excretion higher than 1040 μmol/g creatinine in a single urine sample or in a 24-h collection and at least one identified cystine stone (emitted or surgically removed).
- Siblings of cystinuric patients defined as above in whom the same genetic mutations were identified or with an amino acid urinary pattern compatible with the diagnosis of cystinuria, even in the absence of stone identification.

Two classifications were attempted:

1. On the basis of parents’ amino acid urinary excretion, as previously published (19). Patients were classified as type I when both parents showed a normal urine amino acid pattern, type non-I when urinary excretion of both parents was above normal, and mixed type when urinary excretion was above normal in one of the parents and normal in the other. If urinary excretion in one or both parents was not available, they were defined as unclassified.

Type non-I patients were classified as type II or III according to Kelly (20). Patients were therefore classified as type II when obligate heterozygotes in their families had urine excretion of cystine over 750 μmol/g of creatinine, lysine over 4000 μmol/g of creatinine, and the sum of cystine and the three dibasic amino acids over 4500 μmol/g of creatinine. Patients were classified as type III when obligate heterozygotes in their families had urine excretion of amino acids above normal but below the above-mentioned limits.

2. On the basis of genetic findings. Patients with two mutations in SLC3A1 (one on each allele) were defined as type A, whereas patients with two mutations in SLC7A9 were defined as type B. Patients with one mutation on SLC3A1 and one on SLC7A9 were defined as type AB. The remaining patients with only one or zero mutations detected were not considered for this classification.

The database includes the following entries: personal data (gender, age, and presence of siblings), anamnestic data (age at first stone emission, number of emitted stones, type and number of surgical interventions [extracorporeal shock wave lithotripsy being excluded due to its low efficacy in treating the stones]), laboratory data (including creatinine plasma levels and amino acid urinary excretion), and genetic data (screening for mutations in SLC3A1 and SLC7A9).

To compare patients of different ages, number of emitted stones and surgical interventions were considered as events per year of life.

Heterozygotes

The urinary levels of cystine and dibasic amino acids of the parents of the patients (obligated heterozygotes) and of siblings whose carrier status was genetically proven were also introduced when available in the database.

Controls

Amino acid urinary excretions were measured in 83 healthy subjects, relatives of the affected patients, who agreed to undergo genetic analysis and who were found not to carry the mutations of the probands and/or the affected chromosome.

Genetic Analysis

All patients included in the database agreed to undergo genetic analysis. So far, 210 patients have been analyzed by means of single strand conformation polymorphism (SSCP) analysis and/or direct sequencing, as described previously (16,19).

Statistical Analyses

Comparisons between proportion of males and females have been performed through the binomial test. Comparison between groups was performed by means of Mann-Whitney or with t test as appropriate. ANOVA for multiple comparisons (Bonferroni test) was used when needed. Correlation between amino acid urinary excretion and stone events was evaluated with Spearman rank correlation coefficient. Each test was considered significant if \( P < 0.05 \).

Results

Database

Data on 224 patients from 150 families were collected in the database; 125 were male patients, and 99 were female patients. Most were from Italy (69.6%); 16.5% were from Spain, and 13.9% were from Israel.

According to the parents’ urinary amino acids, classification was as follows: 31% of the patients where type I, 30% where type non-I, 10% were mixed type, and the remaining 29% were unclassified. In 125 patients, a complete genetic classification was possible: 45.2% were type A, 53.2% were type B, and only 1.6% were type AB (i.e., two patients from one family).

Clinical symptoms were almost identically represented in the groups when either the clinical or genetic classification was considered; to provide data from a larger series, the figures concerning clinical aspects of the disease refer to the urinary amino acid–based classification. Clinical manifestations of cystinuria were early events in all patients. Average age at detection of first renal stone was 13.1 yr (median, 15; SD, ± 9.3) for type I and 11.7 yr (median, 14; SD, ± 8) for type non-I (\( P = NS \)). Male and female patients had a similar average age at onset (13.6 yr for type I in male patients [median, 15] and 12.4 yr for type I in female patients [median, 12]; 12.1 yr for type non-I in male patients [median, 14] and 11.08 yr for type non-I in female patients [median, 10.5]), but if we look at the gender distribution in the first 3 yr of life, 35 male patients and only 14 female patients reported having had disease-related symptoms (\( P = 0.003 \)). All patients except one had their first renal stone detected before the age of 40 (Figure 1). Twelve siblings of affected patients with increased urinary excretion of cystine and dibasic amino acids who did not develop renal
stones were also included in the database. Two of them are over 40 yr of age (one is 43 and the other is 65). In 10 of the 12 patients, full genetic confirmation of the disease was obtained (i.e., two mutations were identified in SLC3A1 or SLC7A9). In two patients, a single mutation was identified in SLC3A1 in one and in SLC7A9 in the other.

On average, renal stone emissions occurred one every 4 yr, with no difference between type I and non-I. Male patients had on average one emitted stone every 3 yr, and female patients had one every 5 yr (P = 0.02; Figure 2). Total stone events (spontaneously emitted stones plus those surgically removed) resulted as follows: male patients had on average 0.42 episodes per year (type I, 0.47/yr; type non-I 0.44/yr; P = NS), and female patients averaged 0.21 episodes per year (type I, 0.17/yr; type non-I, 0.27/yr; P = NS). Disease figures are similar if we only consider fully genotyped patients with a full report of data (Table 1).

Urinary amino acid excretion in the patients was very similar in the two types of cystinuria. Excretion patterns in patients with genetic confirmation of the disease are reported in Table 2; no significant difference was evident between type A and B patients in the urinary excretion of cystine and dibasic amino acids. Type A male and female patients had similar amino acid urinary excretion, whereas type B female patients in this set of patients excreted a higher amount of cystine than male patients. The sum of cystine and dibasic amino acids was also significantly higher. No significant correlation was found between the level of amino acid urinary excretion and the incidence of stone events.

Seventeen percent of the patients had mild renal insufficiency, but only one of them reached end-stage renal failure: 146 patients (out of 176 with reported data) had a plasma creatinine below 120 μmol/L and in 6 it was higher than 200 μmol/L. In Fig 3 plasma creatinine for all patients older than 15 is reported.

Genetic Analysis

Genetic analysis has so far been completed in 188 out of 224 patients: in 125 patients (67%) two mutations have been identified, in 52 one mutation has been detected and in 11, from 6 families, no mutations have yet been detected. This means that 84.5%, 83.7%, and 74.0% of type I, type non-I, and unclassified cystinuria independent alleles have been explained. Stratifying the genotyped patients according to amino acid urinary excretion reveals that 34.6% were type I, 33.5% were type non-I, 12.2% were mixed type (I/non-I), and 19.7% remained unclassified. The mixed-type group was composed of 23 patients from 14 families. In two of these families, we detected mutations in both SLC3A1 and SLC7A9: one mutation in each gene in one family and one mutation in SLC3A1 and two mutations in SLC7A9 in each allele in the other family. Two and one SLC7A9 mutations were identified in four and six families, respectively. In the remaining two families one SLC3A1 mutation was identified.

Unexplained alleles in the 188 studied patients could be due to mutations outside the coding region or mutations that escape SSCP analysis. Linkage studies of the families with an incomplete genotype do not exclude the SLC3A1 and SLC7A9 loci (data not shown). Therefore, additional cystinuria loci for these families is unlikely. A manuscript with a complete report of the newly identified mutations is in preparation.

Controls

Amino acid urinary excretion of the controls is reported in Table 3.

Heterozygotes with Genetic Classification

Table 3 provides a report of the cystine and dibasic amino acid urine levels of 47 type A and 142 type B carriers. The attribution of these subjects to each group was genetically confirmed. The difference in urinary excretion between the two sets is obviously very significant, but it is worth noting that, although the sums of cystine and dibasic amino acids in type A
heterozygotes were within the range of the controls, these values were within the 95th percentile of the controls in 14% of those of type B (Figure 4). If the classification were based solely on urinary excretion patterns, these findings would lead to an erroneous classification of the offspring. The overlapping was also confirmed when we log plotted cystine and lysine according to Rosenberg (21) (Figure 5). It is important to note that 5.5% (i.e., eight individuals) and 80.5% of type B heterozygotes had amino acid urine levels within the range of type II and type III heterozygotes, respectively, in accordance with the classification of Kelly (20). Therefore, according to the old classification system, mutations in \textit{SLC7A9} were associated with the three phenotypic types of cystinuria.

### Discussion

Cystinuria prevalence has been estimated to vary from 1:2500 in a Libyan-Jewish population to 1:100,000 in Sweden (1). Screening tests for cystinuria in Japan identified six patients out of 110,000 students. None of these showed stone formation in a 7-yr follow-up (22). In Quebec, 17 patients were diagnosed through a neonatal screening program (23). These patients were followed, and four of eight patients with type I cystinuria developed cystine stone in their first decade (24). The relative rarity of this condition accounts for the absence of large series reports in the literature. Most of the available clinical data concerning cystinuria deal with specific problems: surgical techniques, the effect of medical or surgical treatment on the occurrence of stone relapses, and the effect of diet (25–30). Our multinational database presently collects data on 224 patients, most of whom have been screened for genetic mutations, allowing a complete genetic definition of the two alleles in 60%. This represents the largest series reported so far.

### Table 1. Disease figure for types A and B

<table>
<thead>
<tr>
<th>Type</th>
<th>n</th>
<th>Age at First Stone Average (± SD)</th>
<th>Spontaneous Emissions Average (± SD)</th>
<th>Total Stone Events Average (± SD)</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>48</td>
<td>12.2 (± 8.9)</td>
<td>0.31 (± 0.72)</td>
<td>0.35 (± 0.73)</td>
</tr>
<tr>
<td>B</td>
<td>59</td>
<td>12.8 (± 10.8)</td>
<td>0.34 (± 0.6)</td>
<td>0.39 (± 0.63)</td>
</tr>
</tbody>
</table>

### Table 2. Excretion patterns

<table>
<thead>
<tr>
<th>Type A µmol/g Creatinine</th>
<th>Type B µmol/g Creatinine</th>
</tr>
</thead>
<tbody>
<tr>
<td>M (± SD)</td>
<td>M (± SD)</td>
</tr>
<tr>
<td>(n = 33)</td>
<td>(n = 43)</td>
</tr>
<tr>
<td>F (± SD)</td>
<td>F (± SD)</td>
</tr>
<tr>
<td>(n = 22)</td>
<td>(n = 29)</td>
</tr>
<tr>
<td>P</td>
<td>P</td>
</tr>
<tr>
<td>Overall (± SD)</td>
<td>Overall (± SD)</td>
</tr>
<tr>
<td>(n = 55)</td>
<td>(n = 72)</td>
</tr>
</tbody>
</table>

**Figure 3.** Plasma creatinine in patients over 15 yr of age. One patient, in renal replacement therapy, is not reported in the figure.
patients were indeed detected when the siblings of symptomatic patients were being studied.

Our data show that renal stones occur early in life when cystinuria is symptomatic; the great majority of the patients had their first stone identified before the age of 30 yr (82.9% in the first two decades). Only one patient was diagnosed after the age of 40; however, two other patients included in the database with a negative history for renal stones are older than that. The proportion of the two subtypes of cystinuria (I and non-I) is almost identically represented in the database. The age at onset, the clinical outcome and the severity of the disease do not differ in the two subtypes. Indeed, no difference in stone emissions or number of interventions per year is evident between the two subtypes. We may not, however, exclude that the treatment received by the patients might have influenced the outcome, blunting differences between patients. Severe cases may indeed have received a stronger treatment.

Severe cases may indeed have received a stronger treatment. Male patients are more severely affected than female patients. In fact, male patients produce significantly more renal stones. The differences in severity between the genders, in addition to marked intrafamilial differences between siblings sharing the same mutations (31) suggest that other lithogenic factors, both genetic and environmental, play a role in determining the final phenotype.

Amino acid urine excretion was also similar for patients with mutations in \( \text{SLC3A1} \) and those with mutations in \( \text{SLC7A9} \). Stone event frequency did not correlate with urinary excretion of any of the considered amino acids or with the sum of urinary dibasic amino acid and cystinuria. As urinary excretion of cystine is always well above the solubility threshold, mild differences in urinary excretion of cystine and/or dibasic amino acid might not induce significant differences in stone formation and, again, other factors and/or treatment, may play a determinant role.

Type B female patients (i.e., bearing two mutated \( \text{SLC7A9} \) alleles) excreted a higher amount of cystine than did the male patients. We have no explanation for this finding.

Table 3. Amino acid urinary excretion

<table>
<thead>
<tr>
<th></th>
<th>Controls ( n = 83 )</th>
<th>Type A ( n = 47 )</th>
<th>Type B ( n = 142 )</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>( \mu \text{mol/g creatinine} )</td>
<td>( \mu \text{mol/g creatinine} )</td>
<td>( \mu \text{mol/g creatinine} )</td>
</tr>
<tr>
<td></td>
<td>Mean</td>
<td>5\textsuperscript{th} to 9\textsuperscript{5}</td>
<td>SD</td>
</tr>
<tr>
<td>Cystine</td>
<td>52</td>
<td>23 to 109</td>
<td>26</td>
</tr>
<tr>
<td>Lysine</td>
<td>181</td>
<td>35 to 499</td>
<td>151</td>
</tr>
<tr>
<td>Ornithine</td>
<td>28</td>
<td>7 to 70</td>
<td>23</td>
</tr>
<tr>
<td>Arginine</td>
<td>17</td>
<td>0 to 48</td>
<td>24</td>
</tr>
<tr>
<td>Total</td>
<td>277</td>
<td>93 to 620</td>
<td>172</td>
</tr>
</tbody>
</table>

Figure 4. Sum of cystine, lysine, ornithine, arginine in parents of probands (obligated heterozygotes), with a precise genetic characterization and in controls (relatives of the affected, without the mutation of the probands).

Figure 5. Distribution of genetically confirmed heterozygotes according to log plotting of cystinuria and lysinuria.
Renal insufficiency, defined as plasma creatinine higher than 120 μmol/L, was identified in 17% of our patients. It is important to note that renal function was only studied by measuring plasma creatinine, which is a rather imprecise method, but it is reliable enough to obtain a picture of the occurrence of chronic renal failure in this disease. Renal function was previously studied in 40 patients (19 female and 21 male) (32) by means of a gamma camera renography (indicators were 51Cr-EDTA in 29 cases, 99mTc-DTPA in 5, and iohek in 1). The conclusion of the authors was that renal function was impaired in 70% of the patients after a mean follow-up of 25.9 yr (range, 1 to 52 yr).

In previous studies, mutations in SLC3A1 have been associated with type I disease, and type II and III disease (type no-I) have been attributed to mutations in SLC7A9. However, in this study an analysis of the urinary amino acid excretion patterns in heterozygotes demonstrated that a SLC7A9 carrier status was compatible with a normal amino acid urinary pattern in 14% of the cases. This makes the classification of cystinuria used so far imprecise. Genetic classification would, therefore, be a more appropriate way to classify patients with cystinuria. The classification we propose is therefore as follows:

**TYPE A:** Cystinuria caused by mutations in both alleles of SLC3A1 (chromosome 2). In this type, heterozygotes show a normal amino acid urinary pattern.

**TYPE B:** Cystinuria caused by mutations in both alleles of SLC7A9 (chromosome 19). In this type heterozygotes usually (but not always) show an increase of cystine and dibasic amino acid urinary excretion but may also have a normal pattern as demonstrated in 14% of our cases.

**TYPE AB:** Cystinuria caused by one mutation in SLC3A1 and one mutation in SLC7A9. This type would involve the offspring of one parent carrier of a mutation on chromosome 2 and of another parent with a mutation on chromosome 19. Interestingly, the observed prevalence of AB patients was much lower than expected. Considering a similar frequency of mutations in SLC7A9 and SLC3A1, we would expect one third of the patients to suffer from type A disease, one third from B disease, and one third from AB disease. Indeed, the prevalence of type A disease is similar to that of type B disease; however, type AB is extremely rare (only two patients from one family in our database). Two explanations could account for this low prevalence. The first, type AB patients may suffer from a mild phenotype and therefore, in most cases, escape detection. Alternatively, these patients may actually represent type B disease (two mutations in SLC7A9, one of which was detected, the other yet to be defined) and a coincidental carrier state for an SLC3A1 mutation. Indeed, one patient from our database has three mutations, M467T in SLC3A1 and two missense mutations in SLC7A9, one in each allele.

In conclusion, no clinical differences were evident in this retrospective study between cystinuria type A (due to SLC3A1 mutations) and B (due to SLC7A9 mutations). Male patients are more severely affected than female patients. Renal function is clearly impaired in 17% of patients. Renal stone formation cannot be directly correlated with amino acid urinary excretion, suggesting the importance of cofactors of lithogenesis. A type B carrier status was compatible with a normal amino acid urinary pattern in 14% of cases. A reliable classification of cystinuria requires the identification of the mutations in both alleles. The existence of type AB cystinuria is possible but needs to be confirmed.

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