Clinical Manifestations in Israeli Cystinuria Patients and Molecular Assessment of Carrier Rates in Libyan Jewish Controls

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Abstract

Background: Cystinuria is an autosomal recessive disease that is manifested by the development of kidney stones. Mutations in SLC3A1 cause type I disease, while mutations in SLC7A9 are associated with non-type I disease. In Israel, cystinuria is especially common among Libyan Jews who suffer from non-type I disease.

Objectives: To compare clinical manifestations of patients with mutations in SLC3A1 to those with mutations in SLC7A9, and to assess the carrier rate among unaffected Libyan Jewish controls.

Methods: Clinical manifestations were evaluated in patients with mutations in SLC3A1 and in patients with mutations in SLC7A9. Carrier rates for two SLC7A9 mutations were assessed in 287 unaffected Libyan Jewish controls.

Results: Twelve patients with mutations in SLC3A1 were compared to 15 patients with mutations in SLC7A9. No differences were detected between the patients with mutations in SLC3A1 and those with mutations in SLC7A9 in relation to the age of disease onset, the estimated number of stones, the number of invasive procedures, the number of patients receiving drug therapy, or the patients' urinary pH. Eleven of the unaffected Libyan Jewish controls were found to be heterozygotes for the V170M mutation, establishing a carrier rate of 1:25. The 1584+3 del AAGT mutation was not found in any of the Libyan Jewish controls.

Conclusion: Mutations in SLC3A1 and SLC7A9 cystinuria patients result in indistinguishable disease manifestations. The high carrier rate among Libyan Jews is a result of a single missense mutation, V170M.

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Cystinuria is an autosomal recessive disease characterized by the formation of kidney stones [1]. The disease is caused by a defect in a renal tubular amino acid transporter resulting in impaired reabsorption of cystine and the dibasic amino acids, lysine arginine and ornithine [2]. Cystine, which has a low solubility, can precipitate and form kidney stones, causing pain, obstruction, infection, and sometime renal failure [3]. Based on urine cystine excretion in heterozygotes, previous studies have defined three subtypes of cystinuria [4,5]. Type I heterozygotes excrete normal amounts of cystine in the urine, while types II and III excrete high and moderate cystine levels, respectively. Mutations in SLC3A1 located on the short arm of chromosome 2 cause type I disease [6–8], while mutations in SLC7A9 are associated mainly with what was formally defined as type II and type III disease (non-type I) [9,10]. Since cystine tends to precipitate at low pH, treatment includes alkalinization of the urine [11], and compounds such as D-penecillamine [12] and mercaptoproprionylglycine [13] that bind to cystine and increase its solubility. Cystinuria accounts for 1–3% of all urolithiasis among adults [14] and for about 6–8% of urolithiasis in children [15]. Urolithiasis in cystinuria tends to follow a more severe course compared to other forms of renal stones. Martin et al. [16] reported that only 44% of their patients remained stone-free 3 months after removal of a cystine stone by surgery or lithotripsy.

In Israel, cystinuria is especially common among Libyan Jews, with an estimated prevalence of 1:2,500 [17]. A founder mutation in SLC7A9, V170M, was found in most of the Libyan Jewish patients who suffer from the disease [9]. Libyan Jewish heterozygotes show increased, but widely variable urine cystine levels, from a small increase above the normal range to very high levels overlapping those of homozygotes, findings compatible with non-type I disease [18]. In Israel, cystinuria is also found in other ethnic groups though at a lower frequency. The disease has been described in Ashkenazi Jews (East European origin), in the Druze, and in Muslim Arabs – all of whom suffer from type I disease. We have previously shown that in Ashkenazi Jews the disease is caused by a founder mutation in SLC3A1 [7]. A premature stop codon, R270X, was found in all the carrier chromosomes of Ashkenazi cystinuria patients. This mutation was also found in Druze patients. The marked phenotypic differences between heterozygotes of the two genes allow a clear distinction between them, but whether a clinical distinction could be made between homozygotes (or compound hetetozygotes) for the two genes is less clear.

In this report we present the clinical characteristics of the disease in a group of patients fully analyzed for mutations and urine amino acid profile. We also present the screening results of normal Libyan Jewish controls for the prevalence rate of two mutations, V170M and 1584+3 del AAGT.

Patients and Methods

The Helsinki Committee at the Sheba Medical Center approved the study and participants gave informed consent. Patients included in this study were previously analyzed for mutations and urine amino acid profiles. Home visits were made and the patients completed a

detailed questionnaire that included the age at onset, an estimation of the number of stones excreted in the urine, the number of invasive procedures (shockwave lithotripsy, percutaneous nephrolithotomy or open surgery), and medical treatment at the time of the study. A fresh urine sample was assessed for pH. Results were analyzed using Student's *t*-test.

For determining the frequency of the two Libyan Jewish mutations, DNA samples were obtained from 287 normal Libyan Jewish controls. The frequency of the V170M mutation was determined using a restriction assay. In the carrier chromosomes this mutation creates a *Rsa*I restriction site. A segment containing the mutation was amplified using the primers 5'-CGG GGA GGC AAA GAT TGC TGA GAT CGT-3'and 5'-CCA GCT GGC CGC GGT GAA GAT GTT CTG GAC GTA GCT TCC CAG CCG TA-3'. Amplification was carried out in a 25.7 µl reaction volume containing 50 ng of DNA, 10 pmol of each primer, 1.5 mM dNTPs, 1.5 mM of polymerase chain reaction buffer with 1 U of *Taq* DNA polymerase. After initial denaturation of 5 minutes at 95°C, 30 cycles were performed (95°C for 45 seconds, 56°C for 45 sec, and 72°C for 1 min), followed by a final extension time of 5 min at 72°C. Ten microliters of the PCR reaction were incubated at 37°C for a total of 15 hours with the *Rsa*1 restriction enzyme. Finally, the digested DNA was run on a 3% agarose gel. In the mutated allele the 258 bp PCR product was digested to yield a 211 bp and a 47 bp fragment. The 1584+3 del AAGT mutation was detected using a radioactive assay. A DNA segment containing this mutation was amplified with the primers 5'-AGC AAG CCC ACC TGG GAG TAC-3' and 5'-TGG AGT CAG GAC AGG TGA GG-3'. The amplification was carried out in a 10.76 µl reaction volume containing 50 ng of DNA, 4 pmol of each unlabeled primer, 1.5 mM dNTPs, 0.08 µg ^{32}P-labeled primer in 1.5 mM $MgCl_2$ PCR buffer, with 1 U of *Taq* polymerase (Bio-Line, London). After an initial denaturation of 5 min at 95°C, 31 cycles were performed (94°C for 2 min, 52°C for 3 min, and 72°C for 1 min), followed by a final extension of 7 min at 72°C. Samples were mixed with 9 µl of loading buffer, denatured at 95°C for 5 min and then electrophoresed on a 6% denaturing polyacrylamide gel. A 4 bp deletion could be detected in carrier alleles.

Results

Twelve of the 27 patients who took part in this study had homozygous mutations in SLC3A1, while 15 were homozygotes or compound heterozygotes for mutations in SLC7A9 [Figure 1]. Eighteen of the patients were males and 9 were females. Clinical features are presented in Table 1. No differences were noted between patients with mutations in SLC3A1 and those with mutations in SLC7A9. When looking at both groups combined, 13 patients began suffering from symptoms in the first decade, 8 in the second and 4 in the third. Two patients, one aged 15 with an SLC3A1 mutation and the other aged 30 with an SLC7A9 mutation, were asymptomatic. Only 12 of the 27 patients were receiving medical treatment at the time the study was conducted. This treatment included urinary alkalinizing agents, D-penicillamine, and α-mercaptopropionylglycine. These drugs were taken as single therapy modalities or in combination. Patients who were receiving medical treatment seem to suffer from a more severe disease, manifested by a higher rate of stone emission and invasive procedures [Table 2a]. Even though eight of them received urinary alkalinizing agents, their average urinary pH was lower than that of the patients who were not on medical treatment. Male patients had an older age at disease onset, but a higher rate of stone emission,

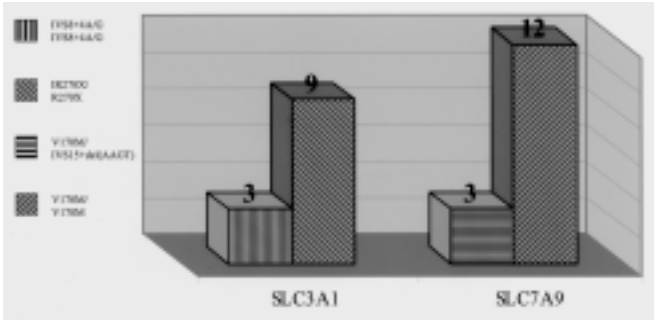

Figure 1. Distribution of SLC3A1 and SLC7A9 mutations among 27 cystinuria patients

Table 1. Comparison of clinical features between patients with mutations in SLC3A1 and patients with mutations in SLC7A9

	All patients (n=27)	SLC3A1 mutations (n=12)	SLC7A9 mutations (n=15)
Age at onset (yrs)	10.8 ± 8	8 ± 7	11 ± 8.5
Estimated number of stones per patient	27 ± 35	25 ± 37	28 ± 33
No. of procedures per patient	5.5 ± 5.6	5.4 ± 5	5.6 ± 6
No. of patients receiving drug treatment	12	4	8
Urinary pH	7.45	7.45	7.44

Table 2. Stratification of patients according to treatment and gender.

a: Treated vs. untreated patients

	Treated (n=12)	Untreated (n=15)
Age at onset (yrs)	10.5	11.8
No. of stones (per patient)	41*	16
No. of procedures (per patient)	7.6	3.9
Urinary pH	7.22	7.62

*$P < 0.02$

b: Stratification according to gender

	Males (n=18)	Females (n=9)
Age at onset (yrs)	12 ± 8	7 ± 5
No. of stones (per patient)	31 ± 36	19 ± 30
No. of procedures (per patient)	6.9 ± 6.2	2.7 ± .7
Drug treatment	10	2
Urinary pH	7.50	7.33

PCR = polymerase chain reaction

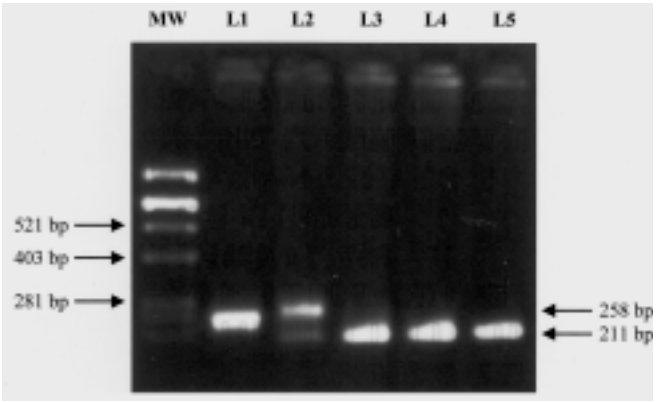

Figure 2. Restriction assay for the detection of V170M carriers. In the carrier allele the 258 bp PCR product was digested to yield a 211 bp and a 47 bp fragments. MW = molecular weight ladder, L1 = an unaffected individual, L2 = a carrier, L3 to L5 = three cystinuria homozygotes.

more invasive procedures compared to females, and were more likely to be on medical treatment [Table 2b].

To assess the carrier rate for the two mutations among Libyan Jews we screened 287 unaffected individuals from this community. Using the *Rsa*I restriction assay [Figure 2], the V170M mutation was found in 11 individuals, thus determining a carrier rate of 1:25. The 1584+3 del AAGT was not found in any of the control population.

Discussion

This study represents the first attempt to detect clinical differences between homozygote cystinuria patients with mutations in SLC3A1 and those with mutations in SLC7A9. Although caused by different genes, the clinical manifestations are indistinguishable.

Heterozygotes for mutations in these two genes can be easily distinguished based on urinary amino acid excretion patterns. However, in homozygotes or compound heterozygotes urinary dibasic amino acids and cystine levels are universally high and therefore could not be used for this purpose.

In reviewing 25 years of experience at St. Bartholomew's hospital in London, Stephens [19] estimated that 25–30% of symptomatic patients report their first kidney stone in the first decade and another 30–35% first experience nephrolithiasis in their teens. In the present report almost 50% reported nephrolithiasis in their first decade and 22% in the second. The early age of onset in Israeli cystinuria patients is consistent with a relatively severe phenotype previously described with the two predominant mutations in these patients – R270X in SLC3A1 [20] and V170M in SLC7A9 [9]. The warmer and drier weather conditions in Israel compared to the UK may also account for this difference.

Stratification of patients into a treated and untreated group revealed that treated patients suffer from a more severe disease compared to untreated patients. This may reflect a natural tendency of the more severe patients to seek medical treatment and adhere to the drug regimens prescribed. However, the low urinary pH of the treated patients, most of whom receive urinary alkalinizing agents, raises concern as to the efficacy of drug treatment in these patients. Most drug protocols for cystinuria patients are based on relatively small retrospective studies. Large well-designed, double-blind studies are needed to assess the optimally effective drug combination for these patients.

Stratification of symptoms according to gender revealed that males suffer from a more severe disease compared to females. Although results in this study did not reach statistical significance, a recent study on more than 200 patients confirmed these results [Dello Strologo et al., submitted]. The reason for these differences is not clear, but may suggest that female hormones have a protective effect in cystinuria.

Screening normal controls of Libyan Jewish ancestry revealed a carrier rate of 1:25 for the V170M mutation. This rate is extremely close to the rate described by Weinberger et al. [17], who found 11 carriers among 285 random schoolchildren of Libyan Jewish origin. These researchers' findings, which were based on urinary cystine profiles assessed by thin layer chromatography, led them to the assumption that the high carrier rate in Libyan Jews is caused by both type II and type III carriers. Today we know that a single mutation is responsible for the vast majority of carriers, and that this mutation can present with a wide range of urinary cystine levels in the heterozygotes. In contrast, the 1584+3 del AAGT was not found in any of the 287 control subjects that we screened. The 1584+3 del AAGT mutation probably represents a rare event, and the more common V170M mutation acts as a detector for this mutation.

A carrier rate of 1:25 results in a disease incidence of 1:2,500, which is one of the highest described for cystinuria. Screening programs performed on newborns demonstrated a high incidence of the disease in Britain (1:2,000) [21] and in Australia (1:4,000) [22]. However, these studies most probably overestimated the real incidence of the disease. Non-type I newborn heterozygotes excrete large amounts of urinary cystine due to renal tubular immaturity and thus cannot be differentiated from homozygotes in the first few months of life [23].

In the next few decades we would expect the prevalence of non-type I cystinuria in Israel to decrease. Intra-ethnic marriages were very popular in the 1950s and 1960s, in the first years after immigration to Israel, but today about 70% of all marriages in Israel are inter-ethnic. Since non-type disease in Israel has been described only among Libyan Jews, the prevalence of non-type I disease will steadily decrease in the next few generations.

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