Heterogeneous mutations in the *SLC3A1* and *SLC7A9* genes in Chinese patients with cystinuria

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Cystinuria is a recessively inherited aminoaciduria that leads to recurrent urolithiasis. It is caused by the defective transport of cystine and dibasic amino acids in the proximal renal tubules and intestinal epithelium. Two genes responsible for this, *SLC3A1* and *SLC7A9*, are known. Patients with two *SLC3A1* mutations are classified as type A cystinuria, whereas patients with two *SLC7A9* mutations are classified as type B cystinuria. Few clinical and molecular data have been reported for Asian cystinuria patients. In this study, we determined the molecular basis of cystinuria in eight unrelated Chinese subjects. Coding exons and flanking introns of the *SLC3A1* and *SLC7A9* genes were directly sequenced after amplification by polymerase chain reaction. Five different *SLC3A1* mutations were found. Two missense mutations, D210G and S547L, were novel. The other three *SLC3A1* mutations (IVS6 + 2T > C, R181Q and R365W) have been described previously. In addition, four novel *SLC7A9* mutations, C137R, c.730delG, IVS10 + 2_3delTG and IVS12 + 3insT, together with two previously reported mutations (A70V and G195R) were found. All patients except one carried compound heterozygous mutations. IVS12 + 3insT was detected in patients from two families. This is the first molecular genetic study on Chinese cystinuria patients. Three patients with type A cystinuria, two with type B cystinuria, and three carriers of type B cystinuria were identified. Our results suggest that the molecular basis of cystinuria is heterogeneous in our local population.

Kidney International (2006) **69,** 123–128. doi:10.1038/sj.ki.5000003

KEYWORDS: cystinuria; *SLC3A1*; *SLC7A9*

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Received 14 April 2005; revised 30 June 2005; accepted 15 September 2005

Cystinuria (MIM220100) is a recessively inherited aminoaciduria caused by the defective transport of cystine and dibasic amino acids (arginine, lysine, and ornithine) in the renal tubular and intestinal epithelial cells. Patients with cystinuria excrete cystine amounts that are 50–200% of the filtered load.[1] Cystine has a low solubility threshold and it precipitates easily when urine becomes more acidic and concentrated in the renal tubules. Traditionally, cystinuria was classified into three subtypes based on the amino-acid excretion patterns in obligate heterozygotes. Type I cystinuria is the fully recessive form and its heterozygotes (I/N) have normal urinary amino-acid excretion. Type II and type III heterozygotes (II/N, III/N), however, excrete high or moderate amounts of urinary cystine. These carriers are at risk of renal stone formation if the urinary cystine levels exceed the solubility threshold.[2]

Two genes responsible for cystinuria have been identified. *SLC3A1*, located on chromosome 2p16.3–21, was first identified to be the cause of type I cystinuria in 1994.[3] The gene responsible for type non-I cystinuria was mapped to chromosome 19q12–13.1 by linkage analysis[4,5] and later identified as *SLC7A9* by the International Cystinuria Consortium.[6] The protein products of *SLC3A1* (rBAT) and *SLC7A9* ($b^{0,+}$ AT) form the heterodimeric amino-acid transporter system $b^{0,+}$, which is responsible for the uptake of cystine and dibasic amino acids in the renal tubular and intestinal epithelial cells.[7] Over 100 *SLC3A1* mutations have been identified, and all, except one (dupE5-E9), were restricted to patients with type I cystinuria.[3,8–12] At least 66 *SLC7A9* mutations were known and these mutations were found in both type I and type non-I patients.[6,10,13–15] In one study, 14% of *SLC7A9* carriers have normal urinary amino-acid patterns.[13] Thus, a new classification system for cystinuria was proposed, based on the genetic defects: (i) type A cystinuria, caused by two mutations in *SLC3A1*; (ii) type B cystinuria, caused by two mutations in *SLC7A9*; and (iii) type AB cystinuria, caused by one mutation in each gene.[13]

Cystinuria is a global disorder and has variable prevalence in different populations.[1] This inherited disease accounts for about 1–2% of urolithiasis in adult patients and about 6–8% of urolithiasis in pediatric patients.[1] Despite its relatively high prevalence ($\sim$1:7000)[1] and easy recognition by urine amino-acid analysis, there were few clinical and genetic data reported for Asian cystinuria patients. Ito *et al.*[16] have reported the incidence of cystinuria in Japan and investigated 36 Japanese cystinuria patients for *SLC3A1* mutations.[9,16] In this study, we analyzed both the *SLC3A1* and *SLC7A9* genes in a group of Chinese patients with hyperexcretion of cystine and dibasic amino acids. The results were compared with those reported in other ethnic groups.

RESULTS

SLC3A1 gene

Five different *SLC3A1* mutations were found in patients 1–3. Two of the five mutations were novel. Patient 1 was compound heterozygous for a novel mutation c.629A>G (Figure 1a) and a previously reported splice site mutation IVS6+2T>C.[12] c.629A>G changes the highly conserved codon 210 from aspartate to glycine (D210G). In all, 50 control subjects were screened for this missense mutation by a restriction enzyme method and none of them showed a positive result (Figure 2). Patient 2 carried the second novel mutation c.1640C>T, which changes codon 547 from serine to leucine (S547L) (Figure 1b). He was also heterozygous for a mutation c.542G>A (R181Q) reported previously.[3] In patient 3, homozygosity of a known disease-causing mutation c.1093C>T (R365W) was observed (Tables 1 and 2).[17]

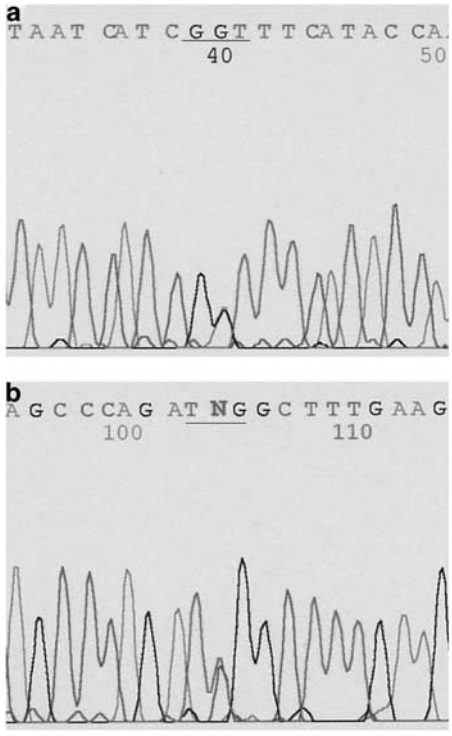

Figure 1 | Electrophoretograms of novel mutations in *SLC3A1*. (**a**) c.629A>G (D210G). (**b**) c.1640C>T (S547L).

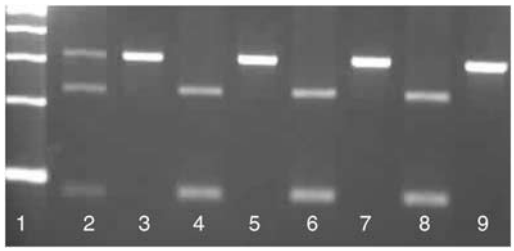

Figure 2 | Restriction enzyme analysis for c.629A>G in *SLC3A1*. Wild-type allele has one *Taq*$^{\alpha}$I restriction site. The PCR product (313 bp) will be cut into two fragments (228 and 85 bp) after *Taq*$^{\alpha}$I digestion. This *Taq*$^{\alpha}$I restriction site is abolished in the presence of c.629A>G, so the PCR product remains unchanged after *Taq*$^{\alpha}$I digestion. A 10 μl portion of PCR product was mixed with 0.2 μl *Taq*$^{\alpha}$I (20 000 U/ml; New England BioLabs, Beverly, MA), 1.5 μl 10 $\times$ NEBuffer3 (New England BioLabs) and bovine serum albumin (final concentration 100 μg/ml). The reaction mix was made up to 15 μl with water. After an overnight incubation at 65°C, the products were electrophoresed in 4% agarose gel. Lane 2: PCR product from patient 1 after *Taq*$^{\alpha}$I digestion. The presence of three bands confirmed that patient 1 was heterozygous for c.629A>G. Lane 3: PCR product from patient 1 without *Taq*$^{\alpha}$I digestion. Lanes 4, 6, and 8: PCR products from three control subjects after *Taq*$^{\alpha}$I digestion. The corresponding undigested PCR products are shown in lanes 5, 7, and 9, respectively. Lane 1: 100-bp marker.

SLC7A9 gene

Patient 4 carried two *SLC7A9* mutations, c.730delG (Figure 3a), which leads to a frameshift after codon 243, and IVS12+3insT (Figure 3b). Both mutations were novel. IVS12+3insT was also found in patient 8 and his elder sister in a heterozygous state. Two more novel *SLC7A9* mutations, c.409T>C (Figure 3c) and IVS10+2_3delTG (Figure 3d), were detected in patient 5. c.409T>C changes the amino-acid residue at codon 137 from cysteine to arginine (C137R). Primer-introduced restriction analysis (PIRA)-polymerase chain reaction (PCR) did not detect this mutation in 50 control subjects (Figure 4). Patients 6 and 7 were heterozygous for previously reported missense mutations c.768G>A (G195R) and c.394C>T (A70V), respectively (Tables 1 and 2).[6,14]

DISCUSSION

In this study, we determined the molecular basis of cystinuria in eight unrelated Chinese subjects by analyzing the *SLC3A1* and *SLC7A9* genes. For patients 1–5, who were either classified as having type I cystinuria or unclassified, *SLC3A1* was analyzed first. A negative result would be followed by analysis of *SLC7A9*. Patients 6–8 were likely to be carriers of type non-I cystinuria based on their urinary cystine levels; hence, only the *SLC7A9* gene was analyzed. Our results showed that patients 1–3 carried two *SLC3A1* mutations (type A cystinuria), patients 4 and 5 carried two *SLC7A9* mutations (type B cystinuria), whereas patients 6–8 carried a single *SLC7A9* mutation (carriers of type B cystinuria).