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Short communication

Two novel *CTNS* mutations in cystinosis patients in Thailand

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ABSTRACT

Cystinosis is an autosomal recessive disorder characterized by defective transport of cystine across the lysosomal membrane and resulting in renal, ophthalmic, and other organ abnormalities. Mutations in the *CTNS* gene cause a deficiency of the transport protein, cystinosin. We performed mutation analysis of *CTNS* in six cystinosis patients from four families in Thailand. Using PCR sequencing of the entire coding regions, we identified all eight mutant alleles, including two mutations, p.G309D and p.Q284X, that have not been previously reported. This study expands the mutational and population spectrum of nephropathic cystinosis.

1. Introduction

Cystinosis is an autosomal recessive lysosomal storage disorder caused by impaired transport of free cystine out of lysosomes; it affects many organs and tissues, but early manifestations involve the kidney (Gahl et al., 2002). The different severities of the disease include classical nephropathic cystinosis, with renal tubular Fanconi syndrome in the first year of life, glomerular failure by 10 years of age, and later involvement of other organ systems. Intermediate cystinosis is characterized by all the clinical manifestations of nephropathic cystinosis, but with later onset. Non-nephropathic, or ocular cystinosis manifests with only corneal crystals and photophobia (Gahl et al., 2001; Nesterova and Gahl, 2008).

In 1995, the cystinosis gene was mapped to chromosome 17p13 (McDowell et al., 1995), and in 1998 Town et al. found that mutations in the *CTNS* gene caused cystinosis (Town et al., 1998). *CTNS* consists of 12 exons with coding regions of 1104 bp. The *CTNS* gene product, cystinosin, contains 367 amino acids and 7 transmembrane domains and serves as an integral lysosomal membrane protein. At least 85 different mutations in *CTNS* have been identified in the Human Gene Mutation Database (http://www.hgmd.org/).

We identified six affected individuals with cystinosis from four families in Thailand, and performed mutation analysis of the *CTNS* gene. Two novel mutations were found in this, the first evaluation of the molecular biology of cystinosis in Thailand.

2. Materials and methods

2.1. Patients

Six patients from four different families were studied. Patients 1, 2 and 3 were single cases. Patients 4, 5 and 6 were siblings originally from Cambodia and had intermediate cystinosis; their clinical characteristics were previously reported (Kitnarong et al., 2005). These siblings had short stature and leg deformity. They did not have renal dysfunction or ocular symptoms. Further investigations demonstrated Fanconi syndrome and rickets. In all cases, the diagnosis was based upon finding typical corneal crystals and elevated leukocyte cystine levels. Clinical details and leukocyte cystine levels are in Table 1. Parental DNA was available only for the fourth family.

2.2. CTNS mutation analysis

Genomic DNA and total RNA were extracted from peripheral leukocytes according to standard protocols. Direct sequencing of PCR-amplified DNA representing the entire coding region of *CTNS* was performed as previously described (Shotelersuk et al., 1998). The PCR products were treated with ExoSAP-IT (USP Corporation, Cleveland, Ohio) and directly sequenced. A novel missense mutation,

Abbreviations: CTNS, cystinosin; PCR, polymerase chain reaction; RFLP, restriction fragment length polymorphism.

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Table 1
Clinical and molecular characteristics of Thai patients with cystinosis.

Patient/Ethnic group	Age of onset	Type	Inbred	WBC cystine[a]	DNA change	Amino acid change	Novel
1/Thai	9 mo	Nephropathic	No	4.5	c.926G>A/c.969C>G	p.G309D/p.N323K	Yes No
2/Thai	7 mo	Nephropathic	Yes	NA	c.850C>T/c.850C>T	p.Q284X/p.Q284X	Yes Yes
3/Thai	15 mo	Nephropathic	No	NA	c.18-21del/c.971-12G>A	T7fsX13	No No
4/Cambodian	13 y	Intermediate	Yes	0.5	c.969C>G/c.969C>G	p.N323K/p.N323K	No
5/Cambodian	10 y	Intermediate	Yes	0.5	c.969C>G/c.969C>G	p.N323K/p.N323K	No
6/Cambodian	18 mo	Intermediate	Yes	0.6	c.969C>G/c.969C>G	p.N323K/p.N323K	No

[a] Nanomoles of half-cystine/mg of WBC protein. Normal values are <0.2 and heterozygous values are less than 1.0. See text for details.

c.926G>A, was analyzed by PCR-RFLP, using *Mwo*I restriction enzyme.

3. Results

Patient 1 was compound heterozygous for c.926G>A (p.G309D) and c.969C>G (p.N323K) (Fig. 1). The former mutation has never been described, and was not detected in 50 Thai controls (100 chromosomes), using PCR-RFLP with *Mwo*I. Patient 2 had a history of parental consanguinity and was homozygous for a nonsense mutation, c.850C>T (p.Q284X) (Fig. 1), not previously described. Patient 3 was compound heterozygous for a 4 bp deletion (c.18-21del) and a G>A substitution at the −12 position of exon 11 (c.971-12G>A). Both mutations were previously reported (Town et al., 1998).

Patients 4, 5, and 6 were homozygous for a previously described missense mutation at nucleotide position c.969C>G (p.N323K) (Thoene et al., 1999). Both parents were carriers for the mutation. The affected siblings had leukocyte cystine levels approximately 5 times the normal level (Table 1), which is always <0.2 nmol half-cystine/mg protein but averages ~0.1 nmol half-cystine/mg protein.

4. Discussion

We report the *CTNS* mutations in 6 patients from 4 families in Thailand; three had classical nephropathic cystinosis and three had intermediate cystinosis. Patients 1 and 3, who were products of non-consanguineous parents, had compound heterozygous mutations, while patient 2 and the 3 siblings from family 4, from consanguineous matings, were homozygous. Five different mutations were identified, including a 4 bp deletion, 2 missense mutations, 1 nonsense and a single base pair substitution in intron 11. One missense (c.926G>A, p.G309D) and the nonsense (c.850C>T, p.Q284X) mutations were not previously reported.

The newly identified missense mutation in patient 1 (c.926G>A; p.G309D) was predicted by PolyPhen (http://coot.embl.de/PolyPhen/) to be probably damaging with a score of 0.999. ClustalX showed the nucleotide was conserved from *Homo sapiens* to *Oryza sativa* (Fig. 2). Patient 2 was homozygous for a single base pair transition (c.850C>T), expected to result in changing a glutamine at amino acid position 284 into a stop codon (p.Q284X).

Several studies supported the correlation of genotype with phenotype in cystinosis. Among our patients, p.N323K was present in the homozygous state in patients 4, 5, and 6, who had intermediate cystinosis. This is consistent with a previous study reporting patients with intermediate cystinosis, who were also homozygous for p.N323K (Thoene et al., 1999). Both patients had fibroblast cystine values of 1.1 and 1.2 nmol half-cystine/mg protein; our patients' values, 0.5 and 0.6 nmol half-cystine/mg protein, are within the range for individuals who are heterozygous for severe *CTNS* mutations (i.e., <1.0 nmol half-cystine/mg protein). This discrepancy might be related to the difference in cell types assayed, but in any event, it suggests that p.N323K allows for considerable residual cysteine-transporting

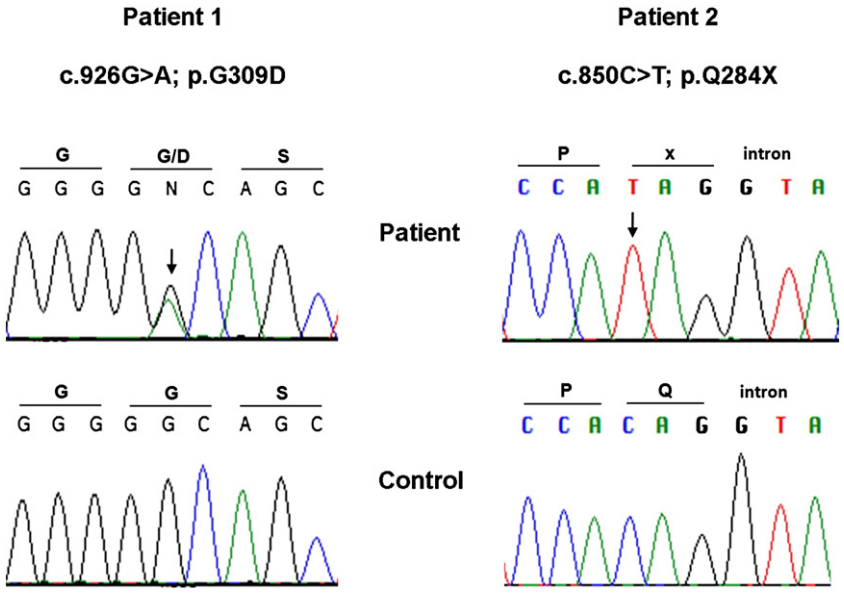

Fig. 1. *CTNS* mutation analysis for patients (upper panels) compared with controls (lower panels). Left panel: Electropherogram of patient 1 using complementary DNA as template shows a heterozygous novel missense mutation c.926G>A (p.G309D) (arrow). Right panel: Electropherogram of patient 2 using genomic DNA as template reveals a homozygous novel nonsense mutation c.850C>T (p.Q284X) (arrow).

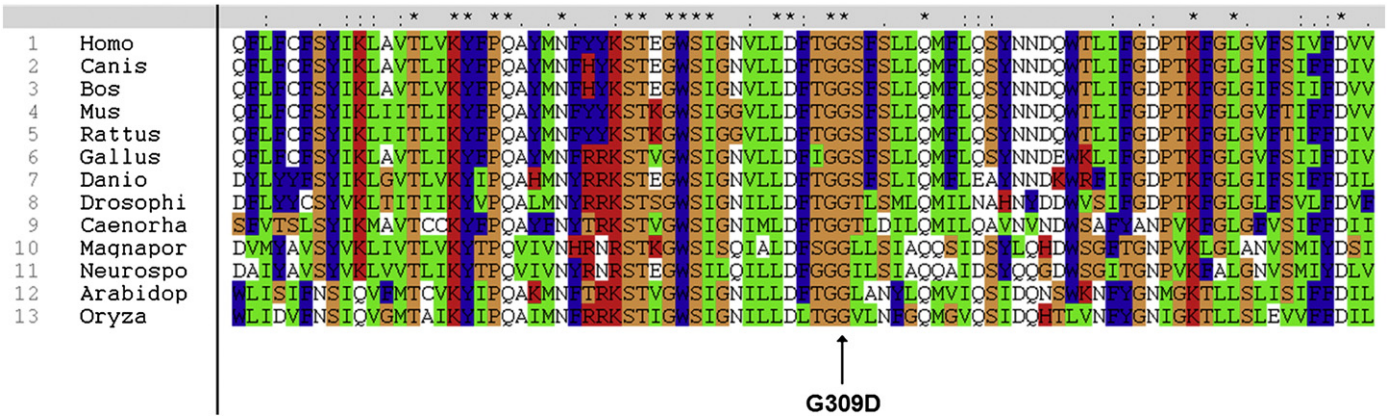

Fig. 2. ClustalX. Multiple sequence alignment of CTNS in different species. The position of amino acid (G309D) is 100% conserved across all sequences (arrow).

activity, perhaps as much as 50%. The p.N323K was also found in the heterozygous state in our patient 1 with nephropathic cystinosis. The other two previously reported mutations in our cases, c.18-21del and c.971-12G>A, have been reported in patients with classical nephropathic cystinosis (Attard et al., 1999; Town et al., 1998). Our findings support a correlation between *CTNS* mutations and clinical severity in cystinosis, and identify *CTNS* mutations that are, to date, novel to Thai families.

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