

An Indian Boy with Nephropathic Cystinosis: A Case Report and Molecular Analysis of CTNS Mutation

Sha Tang¹, Sumita Danda², Mehrdad Zoleikhaeian³, Mariella Simon³, Taosheng Huang^{1,3,4,5*}

¹*Division of Human Genetics/Department of Pediatrics;* ²*Clinical Genetics Unit Christian Medical College and Hospital, Vellore 632004, Tamil Nadu, India;* ³*Center for Molecular and Mitochondrial Medicine and Genetics;* ⁴*Department of Developmental and Cell Biology, University of California, Irvine, CA, USA,* ⁵*Department of Pathology, University of California, Irvine, CA, 92697*

*Address for correspondence and reprints:

Taosheng Huang, MD, Ph.D.

Division of Genetics, Department of Pediatrics

314 Robert R. Sprague Hall

University of California, Irvine, CA 92697

E-mail: huangts@uci.edu

(tel) (949) 824-9346 (fax) (949) 824-9776

Abstract Cystinosis is a rare autosomal recessive lysosomal storage disorder characterized by excessive accumulation of cystine within the lysosome. Cystinosis is caused by mutations in the lysosomal cystine transporter, cystinosin (CTNS). The *CTNS* gene consists of 12 exons and encodes for an integral lysosomal membrane protein with seven transmembrane domains. A majority of cystinotic patients are European descents and only a few cases have been reported from other ethnic groups. Here we report a case of nephropathic cystinosis in an Indian boy born to consanguineous parents. Major symptoms of the patient include gross loss of weight, vomiting, dehydration and cystine crystals in the cornea. Ichthyosis on the arms and legs is also observed. Sequencing analysis of all the *CTNS* exons revealed that the proband is homozygous for a 3-bp in-frame deletion in exon 10, resulting in the loss of a conserved Ser270 within the fifth transmembrane domain of CTNS. His parents are both heterozygous for the same mutation. This work represents the first molecular characterization of cystinotic patients from India. Interestingly, an identical S270del deletion in *CTNS* had been identified in a European patient. Therefore, it appears that this mutation arose independently in the two different continents.

Introduction

Cystinosis is a rare autosomal recessive disease caused by abnormal accumulation of cystine in the lysosome (Schneider et al. 1967a; Schneider et al. 1967b). Typically, cellular cystine levels are elevated 10 to 1,000 times and cystine crystals form in most tissues. Clinically, cystinosis is classified as nephropathic (MIM 219800) or non-nephropathic (MIM 219900). Nephropathic cystinosis is associated with multi-organ damage, particularly renal failure. Without cystine-depleting therapy, cystinotic patients develop signs of Fanconi's syndrome between 6 and 12 months of age and progress to end-stage renal failure at approximately ten years of age (Gahl 1986; Gahl et al. 2002). At the molecular level, cystinosis is caused by a defective lysosomal cystine transporter, cystinosin (CTNS). CTNS is predicted to be an integral lysosomal membrane protein with seven transmembrane domains. The *CTNS* gene, consisting of 12 exons, is located on chromosome 17p13.3 and spans 23 kb of genomic DNA. Various mutations have been identified throughout the gene and the most common one is a large 57 kb deletion, which removes the first 9 exons, most of exon 10, plus a large amount of the upstream sequence (Shotelersuk et al. 1998; Town et al. 1998; Touchman et al. 2000). The phenotypes of the *CTNS* mutations range from complete absence of the gene product, incorrect intracellular localization of CTNS to CTNS mutant proteins with residual or aberrant function (Shotelersuk et al. 1998). The genotypic variation is correlated with the age at onset and severity of the disease (Attard et al. 1999).

Cystinosis has a birth prevalence of about 1 in 100,000 to 200,000 in European and US populations (Meikle et al. 1999) and most patients are fair-skinned individuals of European descent. Incidence of cystinosis has also been reported from American patients of African

American, Mexico and Indian ancestry (Shotelersuk et al. 1998), Iranian children (Madani et al. 2001), Taiwan sisters (Thoene et al. 1999), and a Japanese infant (Ota et al. 2000). In addition, infantile nephropathic cystinosis was proposed to cause early onset of renal failure in an Indian girl from a non- consanguineous marriage (Kumar and Subramanyam 2004), but the underlying *CTNS* mutation was not characterized in this case. In this study, we report an Indian cystinotic patient and the molecular analysis of mutations in the *CTNS* gene of both the proband and his parents.

Subjects and Methods

Patient

The patient is a four year and six month old boy from a rural background of Tamil Nadu, South India. He was the second child born to consanguineous parents (uncle-niece) by vaginal delivery with birth weight of 3,100 g. Family history was significant with the first sibling dying with a diagnosis of cystinosis on the basis of clinical features, renal impairment, proximal renal tubular acidosis and metabolic acidosis. The proband presented at 6/12 age with gross loss of weight, vomiting, dehydration and found to have metabolic acidosis with compensated respiratory alkalosis. His height and weight are below the 3rd centile. The patient had been repeatedly admitted and treated for dehydration. He has pallor, light colored skin, hypopigmented hair and iris with nystagmus (Fig. 1a). The child also has ichthyotic skin on the legs and arms (Fig. 1b). He is also noted to have bowing of arms and legs and Harrison sulcus (Fig. 1a). Additional observations include distended abdomen with prominent venous markings, just-palpable spleen, and cystine crystals in the cornea. Therefore, clinical diagnosis of cystinosis was made. He was found to have hypothyroidism and was treated as such. Genomic DNA from blood samples of

the proband and his parents was obtained using the QIAamp DNA extraction kit (QIAGEN, Valencia, CA) and sent to the MITOMED in University of California, Irvine for molecular studies.

Detection of the common 57-kb CTNS deletion

The common 57-kb deletion represents 76% of cystinotic patients of European origin (Kalatzis and Antignac 2002). To detect the presence or absence of this common deletion, duplex PCR assays employing two sets of primers are used in a single PCR reaction to produce amplicons of varying sizes specific to detect the 57-kb deletion. The first primer set, D17S829F and D17S829R, flank a 250 bp fragment in intron 3. The second pair, LMD1F and LMD1R, will generate a 424 bp fragment upon the 57-kb deletion (Anikster et al. 1999) (Table 1).

Mutation screening in all the 12 CTNS exons

For alleles without the 57-kb deletion, mutation screening of all the coding exons was performed. Exons 1-12 from the proband were amplified using 13 PCR reactions (exon 12 is a large exon, which is amplified by two PCR reactions) and the products were directly sequenced in both orientations in an ABI PRISM™ sequencer (Applied Biosystems, Foster City, CA). Primers used in this procedure are listed in Table 2. PCR cycling started with 95°C for 2 min, followed by 35 cycles that included 95°C for 30 sec, 59°C for 20 sec, and 72°C for 45 sec. A final extension step was then conducted at 72°C for 5 min. After the mutation was identified in the proband, sequence of the corresponding exon was also analyzed for his parents.

Results

Absence of the common 57-kb deletion in the patient and his parents

The duplex PCR reaction to detect the presence of the 57-kb deletion only yielded a single band of 250 bp in the proband and his parents (data not shown), indicating the absence of this common deletion in this family.

A 3-bp in-frame deletion in exon 10 in the patient (homozygote) and his parents (heterozygote)

Molecular analysis revealed a homozygous 3-bp in-frame deletion in exon 10 in *CTNS* for the proband (Fig. 2a). Both of his parents were heterozygous for the same mutation (Fig. 2b).

Discussion

Here we report the first molecularly characterized nephropathic cystinosis in India. He was born to consanguineous parents. Sequencing analysis of the *CTNS* exons revealed that the proband is homozygous for a 3-bp in-frame deletion in exon 10, resulting in the loss of a conserved Ser270 within the fifth transmembrane domain of *CTNS*. Interestingly, the proband in this study presents with features of cystinosis and a certain phenotype not reported previously. The boy displays skin and hair hypopigmentation, which is consistent with the speculation that *CTNS* dysfunction may impair pigment formation in the melanosomes (Schneider and Dohil 2008). Moreover, the patient has ichthyotic skin on the legs and arms. Ichthyosis is a genetically heterogeneous disorder and has not been associated with cystinosis. One major type of non-congenital ichthyoses is X-linked ichthyosis caused by mutations or deletions in the steroid sulfatase enzyme gene on Xp22.3 (Ballabio et al. 1987). Whether the cystinotic Indian boy also has this genetic defect needs further cytogenetic characterization.

With an incidence of one in 100,000 -200,000, nephropathic cystinosis is a rare disease in the European and American population. The diagnosis of cystinosis is even more unusual among other ethnic groups, which may indicate either the disorder occurs at a lower frequency in these populations or is under-diagnosed. Molecular basis of *CTNS* mutations in these developing countries remains even less investigated. No work at the molecular level was conducted in the only case of infantile cystinosis reported in India (Kumar and Subramanyam 2004) prior to this study. Our report represents the first effort to characterize the molecular defects in Indian cystinotic patients.

The incidence and frequency of genotype of cystinosis partly depend on the dispersal of “founder” defective allele in the population. For example, the common 57-kb deletion is found in 76% of cystinotic patients of European origin and has been associated with a founder effect that probably occurred in the middle of the first millennium (Kalatzis and Antignac 2002). A significantly higher prevalence (1 in every 26,000 live births) of the disease in the province of Brittany in France can be attributed to a founder 898-900+24del27 mutation (Kalatzis et al. 2001). In addition, consanguineous marriage can significantly increase the disease frequency. For example, cystinosis incidence among Pakistanis was more than 10-fold higher compared to the white children in the West Midlands in UK although carrier frequency appeared similar in the two groups (Hutchesson et al. 1998). The real incidence of cystinosis and frequency of mutated *CTNS* alleles has not been studied in the Indian population. However, a high occurrence of cystinosis is anticipated because consanguineous marriage is a widely practiced social custom in certain regions of India. In the family in this case, the parents are uncle and niece both harboring one rare defective *CTNS* originated from the same founder mutation. Both of their

children are homozygous for the mutated gene and present with the serious nephropathic cystinosis.

The mutation identified in the proband and his parents, a 3-bp in-frame deletion in exon 10, results in the loss of Ser270 (S270del) within the fifth transmembrane domain in the predicted structure of cystinosin. Along evolution, Ser270 is well-conserved from human, mouse to fly and nematode, implying the functional importance of this amino acid residue. Although S270del did not affect the lysosomal localization of cystinosin (Kalatzis et al. 2004), the loss of the hydroxyl group on the conserved serine residue might impair the interaction between cystinosin and the membrane (Attard et al. 1999). Such disruption in the transmembrane domain can lead to loss-of-function of the protein and be manifested as the severe, infantile nephropathic phenotype as observed in the proband. Interestingly, identical amino acid change in CTNS was reported in a European cystinotic patient (Attard et al. 1999). However, they differ at the genomic level. Since the proband in this study is from a rural background in South India and is probably derived from a pure Indian gene pool, the S270del mutation most likely arose independently in the two different continents.

Acknowledgements: We thank our patient and his families for participating in our study. This project is supported in part by the Cystinosis Research Foundation to (ST)

LITERATURE CITED

- Anikster Y, Lucero C, Touchman JW, Huizing M, McDowell G, Shotelersuk V, Green ED, Gahl WA (1999) Identification and detection of the common 65-kb deletion breakpoint in the nephropathic cystinosis gene (CTNS). *Mol Genet Metab* 66:111-116
- Attard M, Jean G, Forestier L, Cherqui S, van't Hoff W, Broyer M, Antignac C, Town M (1999) Severity of phenotype in cystinosis varies with mutations in the CTNS gene: predicted effect on the model of cystinosin. *Hum Mol Genet* 8:2507-2514
- Ballabio A, Parenti G, Carrozzo R, Sebastio G, Andria G, Buckle V, Fraser N, Craig I, Rocchi M, Romeo G, et al. (1987) Isolation and characterization of a steroid sulfatase cDNA clone: genomic deletions in patients with X-chromosome-linked ichthyosis. *Proc Natl Acad Sci U S A* 84:4519-4523
- Gahl WA (1986) Cystinosis coming of age. *Adv Pediatr* 33:95-126
- Gahl WA, Thoene JG, Schneider JA (2002) Cystinosis. *New England Journal of Medicine* 347:111-121
- Hutchesson AC, Bunday S, Preece MA, Hall SK, Green A (1998) A comparison of disease and gene frequencies of inborn errors of metabolism among different ethnic groups in the West Midlands, UK. *J Med Genet* 35:366-370
- Kalatzis V, Antignac C (2002) Cystinosis: from gene to disease. *Nephrol Dial Transplant* 17:1883-1886
- Kalatzis V, Cherqui S, Jean G, Cordier B, Cochat P, Broyer M, Antignac C (2001) Characterization of a putative founder mutation that accounts for the high incidence of cystinosis in Brittany. *J Am Soc Nephrol* 12:2170-2174
- Kalatzis V, Nevo N, Cherqui S, Gasnier B, Antignac C (2004) Molecular pathogenesis of cystinosis: effect of CTNS mutations on the transport activity and subcellular localization of cystinosin. *Hum Mol Genet* 13:1361-1371
- Kumar PA, Subramanyam G (2004) Early onset of chronic renal failure in infantile nephropathic cystinosis. *Indian Pediatr* 41:1172-1173
- Madani K, Otoukesh H, Rastegar A, Van Why S (2001) Chronic renal failure in Iranian children. *Pediatr Nephrol* 16:140-144
- Meikle PJ, Hopwood JJ, Clague AE, Carey WF (1999) Prevalence of lysosomal storage disorders. *Jama* 281:249-254
- Ota H, Nomura T, Ogawa M, Toyoda H, Yamamoto H, Taki M (2000) A Japanese Infant with Nephropathic Cystinosis. *Journal of the Japan Pediatric Society* 104:78-81
- Schneider JA, Bradley K, Seegmiller JE (1967a) Increased cystine in leukocytes from individuals homozygous and heterozygous for cystinosis. *Science* 157:1321-1322
- Schneider JA, Dohil R (2008) First International Cystinosis Research Symposium. *Pediatric Nephrology* 23:1907-1907
- Schneider JA, Rosenbloom FM, Bradley KH, Seegmiller JE (1967b) Increased free-cystine content of fibroblasts cultured from patients with cystinosis. *Biochem Biophys Res Commun* 29:527-531
- Shotelersuk V, Larson D, Anikster Y, McDowell G, Lemons R, Bernardini I, Guo J, Thoene J, Gahl WA (1998) CTNS mutations in an American-based population of cystinosis patients. *Am J Hum Genet* 63:1352-1362
- Thoene J, Lemons R, Anikster Y, Mullet J, Paelicke K, Lucero C, Gahl W, Schneider J, Shu SG, Campbell HT (1999) Mutations of CTNS causing intermediate cystinosis. *Mol Genet Metab* 67:283-293
- Touchman JW, Anikster Y, Dietrich NL, Maduro VV, McDowell G, Shotelersuk V, Bouffard GG, Beckstrom-Sternberg SM, Gahl WA, Green ED (2000) The genomic region encompassing the nephropathic cystinosis gene (CTNS): complete sequencing of a 200-kb segment and discovery of a novel gene within the common cystinosis-causing deletion. *Genome Res* 10:165-173

Town M, Jean G, Cherqui S, Attard M, Forestier L, Whitmore SA, Callen DF, Gribouval O, Broyer M, Bates GP, van't Hoff W, Antignac C (1998) A novel gene encoding an integral membrane protein is mutated in nephropathic cystinosis. *Nat Genet* 18:319-324

Figure Legends:

Figure 1. Clinical symptoms of the Indian boy.

Figure 2. Sequence Analysis of Exon 10 of *CTNS* in the proband (a) and his parents (b). The proband is homozygous for a 3-bp in-frame deletion (CCT). Both parents are heterozygous for the 3-bp deletion and the trace data for the mother is shown here.