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CTNS Mutations in an American-Based Population of Cystinosis Patients

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Summary

Nephropathic cystinosis is an autosomal recessive lysosomal storage disease characterized by renal failure at 10 years of age and other systemic complications. The gene for cystinosis, *CTNS*, has 12 exons. Its 2.6-kb mRNA codes for a 367–amino-acid putative cystine transporter with seven transmembrane domains. Previously reported mutations include a 65-kb "European" deletion involving marker *D17S829* and 11 small mutations. Mutation analysis of 108 American-based nephropathic cystinosis patients revealed that 48 patients (44%) were homozygous for the 65-kb deletion, 2 had a smaller major deletion, 11 were homozygous and 3 were heterozygous for 753G→A (W138X), and 24 had 21 other mutations. In 20 patients (19%), no mutations were found. Of 82 alleles bearing the 65-kb deletion, 38 derived from Germany, 28 from the British Isles, and 4 from Iceland. Eighteen new mutations were identified, including the first reported missense mutations, two in-frame deletions, and mutations in patients of African American, Mexican, and Indian ancestry. *CTNS* mutations are spread throughout the leader sequence, transmembrane, and nontransmembrane regions. According to a cystinosis clinical severity score, homozygotes for the 65-kb deletion and for W138X have average disease, whereas mutations involving the first amino acids prior to transmembrane domains are associated with mild disease. By northern blot analysis, *CTNS* was not expressed in patients homozygous for the 65-kb deletion but was expressed in all 15 other patients tested. These data demonstrate the origins of *CTNS* mutations in America and provide a basis for possible molecular diagnosis in this population.

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Introduction

Classical nephropathic or infantile cystinosis (MIM 219800) is a rare autosomal recessive disorder characterized by lysosomal storage of free, nonprotein cystine (Gahl 1986; Gahl et al. 1995). Cystinosis patients are normal at birth but develop renal tubular Fanconi syndrome at 6–12 mo of age, accompanied by failure to thrive, polyuria and polydipsia, dehydration, and hypophosphatemic rickets. Photophobia and hypothyroidism generally appear in the 1st decade of life. Glomerular damage results in renal failure at ~10 years of age (Gahl et al. 1995). After renal transplantation, progressive cystine storage can cause a distal vacuolar myopathy (Charnas et al. 1994), swallowing difficulty (Sonies et al. 1990), retinal blindness (Kaiser-Kupfer et al. 1986), diabetes mellitus (Fivush et al. 1987), and neurological deterioration (Ehrich et al. 1979; Fink et al. 1989). Milder variants of the classical disease include an intermediate form with late-onset renal disease and a benign form with corneal involvement but no renal impairment (Gahl et al. 1995). Oral therapy with the free thiol cysteamine lowers the cystine content of leukocytes (Thoene et al. 1976) and a variety of cell types, including muscle (Gahl et al. 1992). Cysteamine has proven efficacy in preventing renal deterioration, enhancing growth, and obviating the need for thyroid-hormone replacement (Gahl et al. 1987; Markello et al. 1993; Kimonis et al. 1995).

The basic defect in cystinosis is deficiency of a cystine carrier in the lysosomal membrane (Gahl et al. 1982*a*, 1982*b*; Jonas et al. 1982). The cystine-transport system is saturable and bidirectional (Gahl et al. 1983), and it displays a gene-dosage effect in obligate heterozygotes for cystinosis (Gahl et al. 1984). The therapeutic action of cysteamine involves its reaction with cystine to produce the free thiol amino acid cysteine and the mixed disulfide cysteine-cysteamine, both of which exit the cystinotic lysosome by a process not requiring the defective carrier system (Gahl et al. 1985; Pisoni et al. 1985).

The gene for cystinosis was first mapped to a 4-cM region of chromosome 17p13, by linkage analysis (Cystinosis Collaborative Research Group 1995). The critical

Table 1

Severity Score Parameters

PARAMETER	SEVERITY SCORE 1	2	3
Age at onset (mo)	>15	9–15	<9
Leukocyte cystine (nmol half-cystine/mg protein)[a]	<5	5–15	>15
Fanconi syndrome index (μmol/kg/d)[b]	<500	500–2,000	>2,000
Age at renal failure (years)	>10	8–10	<8
Age at nonrenal complication (years)	None	⩾20	<20

[a] Not receiving cysteamine therapy. Normal value is <0.2 nmol half-cystine/mg protein.

[b] Normal value is 95 ± 45 μmol/kg/d (Charnas et al. 1991).

region was progressively narrowed (Jean et al. 1996; McDowell et al. 1996; Peters et al. 1997), and several cystinosis patients were found to have deletions in a specific microsatellite marker, *D17S829*, in the region. Physical mapping near this deletion led to isolation of the cystinosis gene, *CTNS* (Town et al. 1998; Genome Database, accession number 700761). The gene has 12 exons spanning 23 kb of genomic DNA. Its 2.6-kb mRNA codes for a 367–amino acid protein, cystinosin, with homology to a 55.5-kD *Caenorhabditis elegans* protein and to a yeast transmembrane protein, ERS1 (Town et al. 1998). Cystinosin has seven transmembrane domains, a GY dipeptide near the C terminus, and eight potential glycosylation sites. Eleven different mutations in exons 3, 4, and 6–9 have been described, with no correlation with clinical phenotype (Town et al. 1998). The most common mutation, involving *D17S829*, consists of a deletion extending from intron 10 upstream ⩾65 kb.

We report the *CTNS* mutations identified in an American-based population of 108 nephropathic cystinosis patients, with concomitant phenotype correlations employing a clinical severity score. We trace the possible origin of the 65-kb deletion, describe 18 previously unreported mutations, and delineate the mutations present in cystinosis fibroblasts available from the Human Genetic Mutant Cell Repository (HGMCR). Finally, we identify the extent of *CTNS* gene expression in a sample of cystinosis fibroblasts with various mutations.

Patients, Materials, and Methods

Patients

All patients or their parents gave informed consent to participate in Institutional Review Board–approved protocols for the study of cystinosis at the National Institutes of Health (NIH) Clinical Center or the University of Michigan Medical Center. Of 108 patient DNA samples, 102 were obtained from individuals who could potentially provide clinical information; 5 samples were from skin-biopsy fibroblasts stored at the NIH, and one patient was unreachable. The subjects of this investigation had classical nephropathic cystinosis, with onset of their disease in early childhood, and came from throughout the United States, Mexico, and Iceland.

Severity was determined in accordance with five parameters (table 1), each assigned a score of 1 (mild), 2 (average), or 3 (severe). The final severity score was the mean of all applicable parameter scores, which were obtained by history and chart review. Age at onset was the age at the first clear appearance of symptoms. The leukocyte cystine value refers to a level obtained prior to initiation of cysteamine therapy. For a sample of 66 patients, the mean leukocyte cystine value, in nanomoles half-cystine/milligram protein, was similar in three different age groups: 10.1 for 33 patients <5 years of age; 8.9 for 16 patients 5–15 years of age; and 12.2 for 17 patients >15 years of age. The normal value is <0.2 nmol half-cystine/mg protein. The Fanconi syndrome index offers an objective measure of the severity of Fanconi syndrome, which is not influenced by cysteamine therapy (Thoene et al. 1976: Gahl et al. 1987), by quantitating the daily urinary excretion of 21 amino acids (Charnas et al. 1991). Age at renal failure (i.e., dialysis or transplantation) and nonrenal complications (i.e., myopathy, swallowing difficulty, retinal blindness, CNS deterioration, and diabetes mellitus requiring insulin therapy) applied only to patients who had not received previous cysteamine therapy. For 102 individuals for whom a clinical history was available, a total of 277 parameters were applicable, for a mean of 2.7 parameters/patient. At least two parameters were required in order to assign a se-

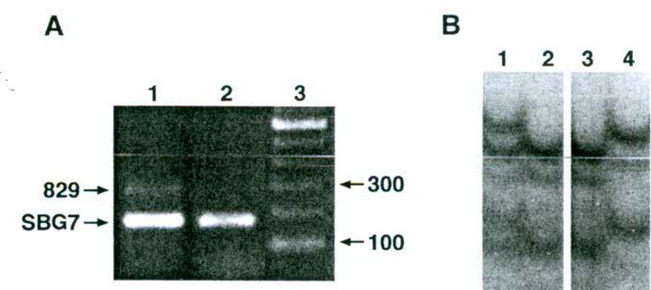

Figure 1 *A*, Gel-electrophoresis pattern for markers *D17S829* (266 bp; denoted by "829"), within the common 65-kb deletion region, and SBG7 (168 bp), outside the deletion region. Samples were from a nondeletion patient (lane 1) and a deletion patient (lane 2). Lane 3 shows the size markers. *B*, SSCP analysis of exon 7. Lanes 2 and 3 show the normal band pattern. Lane 4 contains DNA from a patient subsequently shown by sequencing to be homozygous for 753G→A (W138X). DNA from a patient heterozygous for this mutation was run in lane 1. All the lanes are from the same gel; intervening lanes were removed.