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MUTATION UPDATE

CTNS Mutations in Patients With Cystinosis

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Cystinosis is an autosomal recessive lysosomal storage disease caused by mutations in the gene *CTNS*. The *CTNS* gene product, cystinosin, has 367 amino acids and seven transmembrane domains and is thought to transport cystine out of lysosomes. The most common form of cystinosis, the nephropathic or infantile type, is characterized by renal failure at 10 years of age and other systemic complications. To date, 32 different *CTNS* mutations have been described in nephropathic cystinosis patients. Intermediate cystinosis, with later onset of renal disease, has been associated with three different *CTNS* mutations. Benign or nonnephropathic cystinosis, with symptoms related only to corneal crystals and photophobia, has been associated with two other *CTNS* mutations. In general, only certain splicing or missense mutations are associated with milder cystinosis phenotypes. Hum Mutat 14:454–458, 1999. Published 1999 Wiley-Liss, Inc.†

KEY WORDS: **cystinosis; lysosomal storage disease; transport; deletions; variants**

INTRODUCTION

In cystinosis, the disulfide amino acid cystine accumulates to crystal-forming levels within cellular lysosomes. [Gahl et al., 1982a,b, 1983; Jonas et al., 1982b]. There are two basic cystinosis phenotypes—nephropathic and nonnephropathic. The nephropathic form can be further subdivided based upon the age at presentation [Gahl et al., 1995].

Classical nephropathic or infantile cystinosis (MIM# 219800) presents in infancy and is the most common and severe variant of the disorder. An autosomal recessive disease, nephropathic cystinosis has an incidence of approximately 1 per 100,000 live births in North America and is the most common identifiable cause of renal Fanconi syndrome in children [Gahl, 1986; Krasnewich and Gahl, 1991]. Patients are normal at birth, but develop renal tubular Fanconi syndrome at 6–12 months of age, accompanied by failure to thrive, polyuria and polydypsia, dehydration, and hypophosphatemic rickets. Photophobia and hypothyroidism generally appear in the first decade of life. Glomerular damage results in renal failure at approximately 10 years of age [Gahl, 1986; Gahl et al., 1995]. Continued accumulation of cystine in the host tissues after renal transplantation can result in retinal blindness, diabetes mellitus, swallowing difficulties, and neurologic deterioration. The main form of treatment consists of oral therapy with the free thiol cysteamine, which lowers the cystine content of leukocytes. Cysteamine participates in a disulphide interchange reaction with the accumulated cystine in lysosomes and the products of the reaction leave the lysosome via a carrier system that is not defective in cystinosis. Cysteamine [Thoene et al., 1976] has proven efficacy in preventing renal deterioration and enhancing growth if treatment is implemented early and adequately [Gahl et al., 1987; Markello et al., 1993]. Cysteamine eye drops can dissolve corneal cystine crystals [Kaiser-Kupfer et al., 1987, 1990].

In the intermediate or late-onset form of cystinosis (MIM# 219900), the age of presentation is usually 12–15 years, but ranges from 2–26 years. Crystalline cystine deposits are found in the cornea, conjunctiva, and bone marrow. The complete renal tubular Fanconi syndrome often does not develop, but progression to end-stage renal failure generally occurs by the third decade of life [Goldman et al., 1971; Gahl et al., 1995].

Patients with the benign or adult, nonnephro-

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pathic type of cystinosis (MIM# 219750) never suffer renal disease and do not show a retinal pigment abnormality, but do have crystals in their cornea and bone marrow [Lietman et al., 1966; Gahl et al., 1995].

Each type of cystinosis represents a different but allelic disorder, and there likely exists a continuum of disease severity. Heterozygotes for cystinosis are clinically normal, regardless of the type.

The basic defect in cystinosis is impaired transport of cystine out of lysosomes. The gene for cystinosis was mapped to chromosome 17p13 by linkage analysis [Cystinosis Collaborative Research Group, 1995], followed by isolation of the cystinosis gene, *CTNS* [Town et al., 1998]. This gene has 12 exons spanning 23 kb of genomic DNA. The *CTNS* gene product, cystinosin, has 367 amino acids, seven predicted transmembrane domains, a GY dipeptide for lysosomal targeting near the C-terminus, and eight potential glycosylation sites. Cystinosin has homology to a 55.5 kD *C. elegans* protein and to a yeast protein, ERS1 [Town et al., 1998].

NEPHROPATHIC CYSTINOSIS

Large Deletions

Although 32 different *CTNS* mutations have been identified, the most common is a 57-kb deletion whose 3′ border cuts exon 10. Homozygotes for this deletion are detectable by the absence of the polymorphic marker *D17S829*. The frequency of homozygotes for this deletion was 33% in a European study and 44% in American-based patients. Overall, 56% of cystinosis alleles examined contained the deletion. Three families had smaller deletions that included *D17S829* but were shorter than 57 kb [Town et al., 1998; Shotelersuk et al., 1998; Forestier et al., 1999]. Of 96 alleles in 48 homozygous deletion patients, 38 (46%) derived from Germany and the rest were from Ireland, England, Iceland, Italy, and Spain [Shotelersuk et al., 1998]. The 57-kb deletion has not been detected outside of patients with European ancestry. This implicates a Germanic founder, with migration circa 700 AD. The deletion size and breakpoints appear identical in all deletion patients, supporting the concept of a founder effect. A multiplex PCR system using primer pairs flanking the breakpoints and other primer pairs inside the deletion has proven useful for diagnosis of homozygosity and heterozygosity in patients of European origin [Anikster et al., 1999a; Forestier et al., 1999]. Northern blots from seven homozygous deletion patients showed no *CTNS* expression in fibroblasts.

Other Mutations

Thirty-one other mutations, present in the homozygous or compound heterozygous states, are spread throughout the coding area of the gene, with no mutation hot spot (Table 1, Fig. 1). Several different kinds of mutations have been reported, including insertions, small deletions, nonsense, splicing, and missense mutations. Each of the seven reported missense mutations gives rise to an amino acid substitution within a transmembrane region or one amino acid before a transmembrane region (Fig. 2). Mutations have been named according to the recommendations for a nomenclature system for human gene mutations [Antonarakis et al., 1998].

INTERMEDIATE CYSTINOSIS

CTNS mutations have been identified in six cases of intermediate cystinosis from four different families. Three of the four sibships showed the combination of a severe, nephropathic type of mutation (i.e., W138X, 57 kb deletion) and a milder missense (K280R) or splicing mutation. The fourth family showed homozygosity for the N323K missense mutation. Neither the K280R nor the N323K mutation involves a transmembrane domain. Presumably, alleles carrying the milder mutations provide some residual cystinosin and attenuate the disease [Thoene et al., 1999].

BENIGN CYSTINOSIS

The molecular basis for benign cystinosis has been determined in four individuals. Each had one severe nephropathic type of mutation (i.e., 545delTCCTT or the 57 kb deletion) and one mild mutation. The latter consisted of either a missense (G197R) mutation far from any transmembrane domain, or a splicing mutation resulting in the insertion of 182 bp of IVS10 into the *CTNS* mRNA. The mild mutations appear to allow for residual normal *CTNS* mRNA production, providing sufficient cystinosin to prevent renal disease altogether [Anikster et al., 1999b].

CTNS POLYMORPHISMS

Five possible polymorphisms have been reported, including two silent base changes (843A→G and 1299C→T), one (1214A→G) in which the amino acid charge was conserved (K292R), and two in introns (669-5T→C and 1020+9A→G) [Shotelersuk et al., 1998].

BIOLOGICAL RELEVANCE

Cystinosis is the first reported disease due to a lysosomal membrane transporter defect and, as

TABLE 1. Cystinosis Mutations

No.	Exon/intron	Base change	New system[a]	AA change	Stop codon	Reference
Infantile nephropathic						
1	Ex 1-10	57kb del	—	—	—	Town et al., 1998
2	Ex 3	357delGACT	18	T7F	13	Town et al., 1998
3	Ex 3	371delT	32	L14X	14	Town et al., 1998
4	Ex 3	397delTG	58	C20X	20	Town et al., 1998
5	In 4	479+1G→T	140+1	splice	—	Town et al., 1998
6	—	skip exon 4 and 5[b]	—	splice	—	Lucero et al., submitted
7	Ex 5	537del21bp	198	del 67-73[c]	—	Shotelersuk et al., 1998
8	Ex 5	545delTCCTT	206	I69R	73	Shotelersuk et al., 1998
9	In 5	564+5+6GT→CC	225+5+6	splice	54	Lucero et al., submitted
10	Ex 6	622G→T	283	G95X	95	Town et al., 1998
11	Ex 6	651delTCAC	312	H105P	116	Shotelersuk et al., 1998
12	Ex 7	721C→T	382	Q128X	128	Town et al., 1998
13	Ex 7	753G→A	414	W138X	138	Town et al., 1998
14	Ex 8	845G→A	506	G169D	—	Shotelersuk et al., 1998
15	Ex 8	857delAC	518	Y173X	173	Town et al., 1998
16	Ex 8	883T→C	544	W182R	—	Shotelersuk et al., 1998
17	Ex 8	900delG	561	splice	—	Town et al., 1998
18	In 8	901-1G→C	562-1	splice	—	Town et al., 1998
19	Ex 9	908delTTCTCCTCA	569	F190X	190	Town et al., 1998
20	Ex 9	950delACG	611	D205del	—	Shotelersuk et al., 1998
21	Ex 9	952G→A	613	D205N	—	Shotelersuk et al., 1998
22	Ex 9	985insA	646	T216N	227	Shotelersuk et al., 1998
23	Ex 10	1033insCG	694	V233A	253	Shotelersuk et al., 1998
24	Ex 10	1035insC	696	V233R	296	Shotelersuk et al., 1998
25	Ex10	1080delC	741	F247L	252	Shotelersuk et al., 1998
26	Ex 11	1209C→G	870	Y290X	290	Shotelersuk et al., 1998
27	Ex 11	1232G→A	893	S298N	—	Shotelersuk et al., 1998
28	Ex 11	1253A→G	914	D305G	—	Shotelersuk et al., 1998
29	Ex 11	1261insG	922	S310Q	364	Shotelersuk et al., 1998
30	Ex 11	1261G→A	922	G308R	—	Shotelersuk et al., 1998
31	Ex 12	1354G→A	1015	G339R	—	Shotelersuk et al., 1998
32	Ex 12	1367TCGTCTTC→A	1028	I343K	364	Shotelersuk et al., 1998
Intermediate						
33	Ex 10	1178A→G	839	K280R	—	Thoene et al., 1999
34	Ex 11	1308C→G	969	N323K	—	Thoene et al., 1999
35	In 11	1309+2T→C	970+2	splice	289	Thoene et al., 1999
Benign						
36	Ex 9	928G→A	589	G197R	—	Anikster et al., 1999b
37	In 10	1192-3C→G	853-3	splice	313	Anikster et al., 1999b
Polymorphisms						
38	In 6	669-5T→C	329-5	—	—	Shotelersuk et al., 1998
39	Ex 8	843A→G	504	T168T	—	Shotelersuk et al., 1998
40	In 9	1020+9A→G	681+9	—	—	Shotelersuk et al., 1998
41	Ex 11	1214A→G	875	K292R	—	Shotelersuk et al., 1998
42	Ex 11	1299C→T	960	S320S	—	Shotelersuk et al., 1998

[a]The A of the ATG of the initiator Met codon is denoted nucelotide +1 [Antonarakis et al., 1998].
[b]Mutation at the genomic DNA level was not determined.
[c]Amino acids ITILELP deleted.

such, it serves as the prototypic disorder in which the synthesis, targeting, and processing of functional integral lysosomal membrane proteins can be examined. In this regard, it is useful to note that the *CTNS* gene product, cystinosin, differs considerably from the cystine binding protein used in the assay of intracellular cystine levels in leukocytes [Oshima et al., 1974]. The *E. coli* cystine-binding protein, called *FLIY,* is a heatshock protein encoded by the *fliY* gene, which has no homology to *CTNS* [Butler et al., 1993; Mytelka and Chamberlin, 1996]. The binding site of cystinosin appears to be highly specific for cystine [Gahl et al., 1983].

The peculiar phenomenon of kidney sparing in benign cystinosis can be explained in at least two ways. First, sufficient cystinosin may be produced by the mutant allele to spare the kidney but not other tissues. Alternatively, expression of splicing factors may be different in the kidney compared with other tissues, so that more properly spliced, functional cystinosin is provided in renal tissue. The molecular

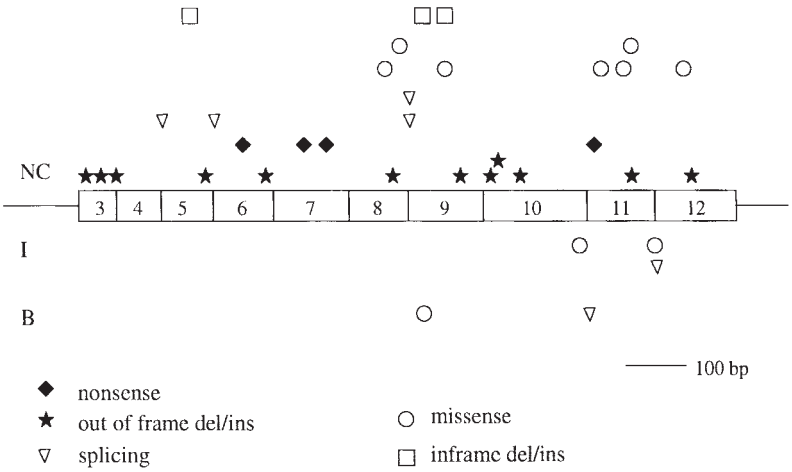

FIGURE 1. ***CTNS* mutations in cystinosis variants. Different symbols designate different types of mutations. Numbers indicate exons. Above the gene are mutations causing nephropathic cystinosis (NC); below are those resulting in intermediate (I) or benign (B) cystinosis.**

resolution of this issue will reveal the reason for the distinct, tissue-specific involvement characteristic of the different types of cystinosis.

CLINICAL AND DIAGNOSTIC RELEVANCE

Some genotype–phenotype correlation exists both within and between subtypes. Presumably, the greater the expression of cystinosin, the milder the disease. The specific combination of a severe nephropathic cystinosis allele and a milder allele leads to a milder phenotype, i.e., intermediate or benign cystinosis. Milder mutations produce enough functional protein to provide some residual transporting capacity.

The molecular basis of the 57 kb deletion makes it easy to diagnose a high percentage of patients, especially those of European origin. Molecular diagnosis may also be useful for siblings or fetuses at

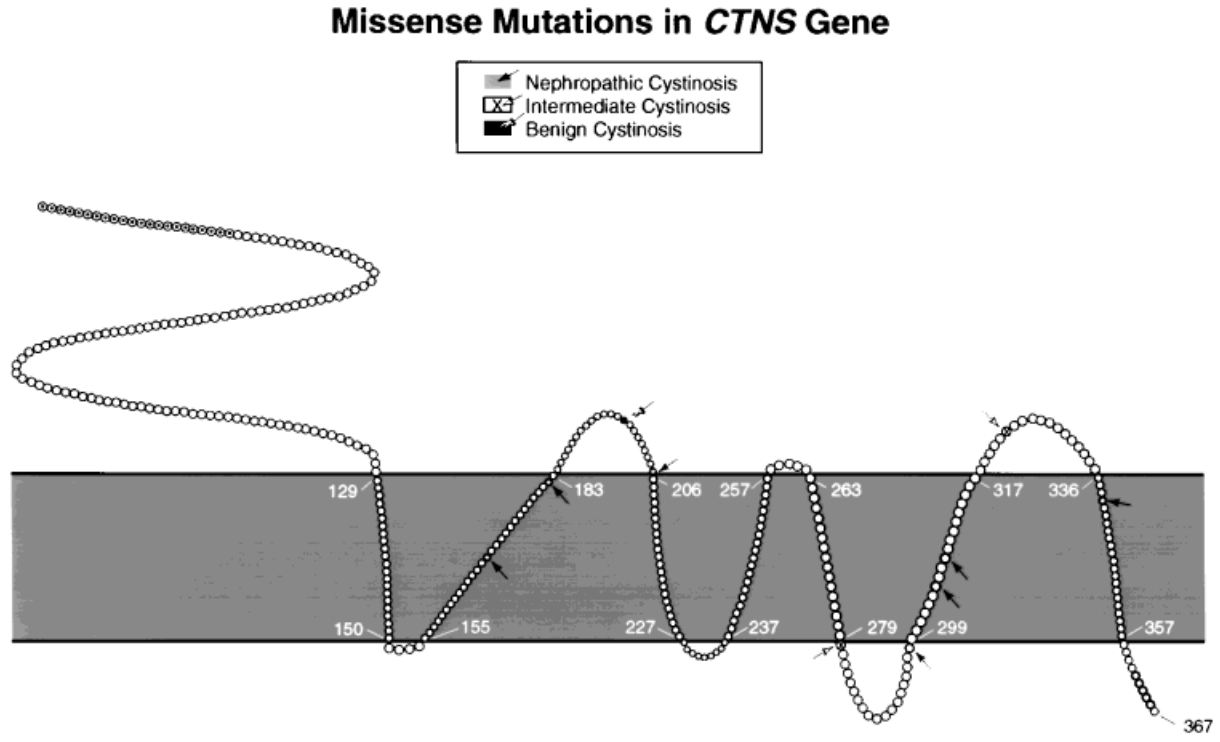

FIGURE 2. **The predicted structure of cystinosin, showing the location of the 10 different amino acids altered by individual *CTNS* missense mutations. The dotted circles in the N-terminal portion represent the predicted signal peptide and the bold circles in the C-terminal portion represent the predicted lysosomal targeting sequence. Numbers indicate the amino acid residue, counting from the initial methionine at position 1.**

risk when the family mutations are known. However, the majority of diagnoses will continue to rely upon leukocyte or fibroblast cystine measurements, which are elevated in all patients with *CTNS* mutations involving both alleles.

FUTURE PROSPECTS

Knowledge of the molecular basis of cystinosis opens new horizons for research. For example, normal and mutant *CTNS* genes can be fused with detection markers and transfected into fibroblasts containing homozygously deleted *CTNS*. This will allow determination of the movement of cystinosin from synthesis to incorporation into the lysosomal membrane. Site-directed mutagenesis will help to determine which targeting signals in the cystinosin protein are essential for it to reach its destination. A mouse model for cystinosis will allow the comprehensive examination of which tissues are involved in this disease, and to test different treatments such as gene therapy and alternative cystine-depleting agents. In addition, a mouse model will reveal the extent of CNS involvement in cystinosis and whether cysteamine can deplete the brain of cystine.

The genomic structure of *CTNS* will provide data covering its promoter region, which may lead to discovery of binding sites for possible transcription factors. The entire sequencing of the 57 kb deletion may reveal another gene involved in the phenotype of nearly half the reported cystinosis patients.

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