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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.†

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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