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ARTICLES

Ocular Nonnephropathic Cystinosis: Clinical, Biochemical, and Molecular Correlations

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ABSTRACT

Ocular nonnephropathic cystinosis, a variant of the classic nephropathic type of cystinosis, is an autosomal recessive lysosomal storage disorder characterized by photophobia due to corneal cystine crystals but absence of renal disease. We determined the molecular basis for ocular cystinosis in four individuals. All had mutations in the cystinosis gene *CTNS*, indicating that ocular cystinosis is allelic with classic nephropathic cystinosis. The ocular cystinosis patients each had one severe mutation and one mild mutation, the latter consisting of either a 928 G→A (G197R) mutation or an IVS10–3 C→G splicing mutation resulting in the insertion of 182 bp of IVS10 into the *CTNS* mRNA. The mild mutations appear to allow for residual *CTNS* mRNA production, significant amounts of lysosomal cystine transport, and lower levels of cellular cystine compared with those in nephropathic cystinosis. The lack of kidney involvement in ocular cystinosis may be explained by two different mechanisms. On the one hand (*e.g.* the G197R mutation), significant residual cystinosin activity may be present in every tissue. On the other hand (*e.g.* the IVS10–3 C→G mutation), substantial cystinosin activity may exist in the kidney because of that tissue's specific expression of factors that promote splicing of a normal *CTNS* transcript. Each of these mechanisms could result in minimally reduced lysosomal cystine transport in the kidneys. (***Pediatr Res* 47: 17–23, 2000**)

Abbreviations

SSCP, single-stranded conformational polymorphism
RT-PCR, reverse-transcription polymerase chain reaction

Cystinosis is an autosomal recessive lysosomal storage disorder in which the disulfide amino acid cystine accumulates to crystal-forming levels within cellular lysosomes (1). Patients with the classic infantile nephropathic form of this disease are normal at birth but typically present with renal tubular Fanconi syndrome in infancy, characterized by failure to thrive, dehydration, polyuria and polydipsia, acidosis, hypophosphatemic rickets, and hypokalemia (1, 2). Hypothyroidism and photophobia due to corneal cystine crystals develop at variable times, but renal glomeruli lose function progressively, resulting in kidney failure at approximately 10 y of age. After a renal allograft procedure, cystine accumulation continues to destroy the nonrenal organs of cystinosis patients (3), frequently causing a distal vacuolar myopathy (4, 5), swallowing difficulty (6), pancreatic endocrine (7) and exocrine (8) insufficiency, CNS deterioration (9, 10), testicular dysfunction (11), and ophthalmic complications that include severe retinal dysfunction (12). The treatment of cystinosis consists of replacement of renal losses and cystine depletion by using cysteamine orally (13–17) or topically (18, 19).

Variants of classic nephropathic cystinosis contribute to a broad spectrum of disease severity. Individuals with intermediate, or juvenile, cystinosis have the same signs and symptoms of classic cystinosis only at a later age (1). Patients with ocular nonnephropathic cystinosis, first reported in 1957 (20) and termed "benign" or "adult" cystinosis, never suffer renal disease and do not exhibit a retinal pigment abnormality but do have crystals in their cornea and bone marrow (1). Of the

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approximately 15 ocular cystinosis patients reported, most presented in childhood with mild photophobia or with corneal crystals on routine ophthalmologic examination. No ocular cystinosis patient has experienced any consequence of the disorder besides photophobia. Ocular cystinosis patients generally have leukocyte cystine levels of 1–3 nmol half-cystine per milligram of protein, compared with 3–23 for classical patients (normal, ≤0.2). Based upon fibroblast complementation studies, ocular cystinosis has been considered allelic with the classic nephropathic disease (21).

The basic defect in cystinosis involves deficiency of a cystine transporter in the lysosomal membrane (22–24). The normal cystine carrier displays characteristics of both egress (22, 23) and countertransport (25), a phenomenon in which a small radiolabeled ligand appears to traverse a membrane against its concentration gradient. The lysosomal cystine carrier is presumably encoded by the cystinosis gene *CTNS*, mapped to chromosome 17p in 1995 (26) and isolated in 1998 (27). *CTNS* is known to contain 12 exons distributed across ~23 kb of genomic DNA; the *CTNS* gene product cystinosin has 367 amino acids with seven predicted transmembrane domains and eight potential glycosylation sites (27). To date, 31 distinct mutations in *CTNS* have been reported (27, 28), but the most common is a 65-kb deletion found frequently in patients of northern European descent. This deletion, now known to be 57 kb (J. Touchman and E. Green, personal communication), was found in 56% of the alleles of 108 cystinosis patients seen in the United States (29).

We performed mutation analysis of the *CTNS* gene on four ocular cystinosis patients. The results indicate that the nonnephropathic variant is, indeed, allelic with the classic disorder. Moreover, ocular cystinosis patients exhibit a unique array of novel mutations combined with known classic mutations.

METHODS

Patients. All patients and their families were enrolled in a protocol approved by the Institutional Review Board at the National Institutes of Health Clinical Center or the University of California, San Diego Center for the Health Sciences. The patients were examined and treated for various durations at these institutions.

Clinical biochemical studies. Leukocyte cystine values were determined using the cystine-binding protein method (30). The severity of the renal tubular reabsorption defect was gauged by the Fanconi syndrome index; a measure of the daily urinary excretion of 21 amino acids per kilogram body weight (31) was used (28). Patients were also evaluated using a recently devised clinical severity scale involving five parameters, *i.e.* age at presentation, leukocyte cystine value, Fanconi syndrome index, age at renal failure, and age of nonrenal complications. According to this scale, a value of 1 is extremely mild and a value of 3 is very severe. Typical nephropathic cystinosis patients have a value of 2.0.

Molecular studies. Genomic DNA of each patient was initially screened for mutations by performing SSCP analysis on exons 3–12 of *CTNS* as previously described (28). Any exons showing abnormal bands were subjected to direct DNA sequencing using the ABI Prism dRhodamine Terminator Cycle Sequencing Kit (Applied Biosystems, Foster City, CA) according to the manufacturer's recommendations.

Detection of the 57-kb common deletion in the heterozygous state was accomplished using primers flanking the deletion. This gave a 423-bp product if the deletion was present, and no product if the deletion was absent (29). In this multiplex PCR reaction, primers for the polymorphic marker *D17S829* were included to verify the presence of a nondeleted allele.

Analysis of the polymorphic microsatellite marker *D17S829* in cases 3 and 4 was performed as previously described (28). Northern blot analysis was performed as previously described (28) using human *CTNS* cDNA (exons 3–10) random primer labeled with $\alpha[^{32}P]$-dCTP (DuPont/NEN, Wilmington, DE) as probe.

RT-PCR was performed after total RNA extraction from cultured fibroblasts by using Trizol reagent (Life Technologies, Grand Island, NY). cDNA was synthesized by reverse transcription by using 5 μg of total RNA according to the manufacturer's protocol (GIBCO-BRL, Grand Island, NY). PCR amplification was performed using 2 μL of the first-strand cDNA, 1x PCR buffer (GIBCO-BRL), 1.5 mM $MgCl_2$, 200 μM dNTP, 0.2 μM of each primer, and 2.5 U *Taq* DNA polymerase in a final volume of 50 μL. After an initial denaturation step at 94°C for 4 min, PCR was conducted for 30 cycles, each with a denaturation step at 94°C for 30 s, annealing at 55°C for 40 s, and extension at 72°C for 1.5 min. (The extension time was 2.5 min when the entire coding cDNA was amplified.) An elongation step at 72°C for 10 min finalized the procedure. The PCR products were electrophoresed in 1.4% agarose and were stained with ethidium bromide. The primers used for amplification of the coding cDNA were 5′-CCTCTTCCAGTAACATTGAGG-3′ (in exon 2) and 5′-AGAAAAGAGATGGCGGTGTC-3′ (in the 3′ UTR), yielding a 1431-bp product. The primers used to create the probe for Northern blot analysis were 5′-TGAAGCTCGTAGAGAAATGTG-3′ and 5′-GCTTGATGTAGGAGAAGCAG-3′, yielding a 779-bp band. The primers used to amplify the paternal allele of case 1, excluding the maternal allele, were 5′-CTATCCTTGAGCTCCCCG-3′ and 5′-GGTTGGGTCTCCGAAGATC-3′, yielding a 799-bp product.

CASE HISTORIES

Case 1. This 26-y-old male was patient 2 of a previous report (32). Briefly, he presented with corneal crystals on routine ophthalmologic examination at 6 y 9 mo of age. At that time, a renal biopsy, fixed in absolute ethanol to preserve cystine crystals, showed no crystals, but a bone marrow biopsy showed typical crystals on light microscopy (Fig. 1*A*) and under birefringent light (Fig. 1*B*). Serum creatinine was 0.7 mg/dL, growth and bone ages were normal, and there was no evidence of renal tubular Fanconi syndrome. By age 14 y 6 mo, the serum creatinine was 1.0 mg/dL, height was 166 cm (50%), and weight was 73 kg (90%). Renal tubular function was normal. At this time, the leukocyte cystine level was 2.85 nmol half-cystine/mg protein, and the countertransport of cystine across the membranes of leukocyte granular fractions (*i.e.*