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Identification and Detection of the Common 65-kb Deletion Breakpoint in the Nephropathic Cystinosis Gene (*CTNS*)

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The most common mutation in the cystinosis gene, *CTNS*, is a 65-kb deletion thought to have originated in Germany. Although homozygotes for this deletion are detectable by the absence of the *D17S829* polymorphic marker, no method exists to identify heterozygotes. We identified the 65-kb deletion breakpoints and used flanking PCR primers to amplify a 423-bp fragment present only in the deletion alleles. Using this method, we determined that 121 of 216 (56%) cystinosis alleles examined bore the 65-kb deletion. We found no non-Europeans with the deletion, and the deletion size and breakpoints appeared identical in all patients studied, supporting the concept of a founder effect. The addition of *D17S829* primers (266 bp apart) to the PCR created a multiplex PCR system useful for diagnosing cystinosis patients homozygous and heterozygous for the 65-kb deletion.

Key Words: **cystinosis; deletion; multiplex PCR; sample sequencing; founder effect.**

Nephropathic cystinosis (MIM 219800) is a storage disorder due to the defective transport of the disulfide amino acid, cystine, out of lysosomes (1–3). Its incidence has been estimated to be 1 in 100,000–200,000 live births. Affected patients are normal at birth, but typically exhibit failure to thrive, acidosis, dehydration, and hypophosphatemic rickets at 6 to 12 months of age (4, 5). These symptoms reflect renal tubular Fanconi syndrome, but renal glomerular damage soon supervenes, leading to kidney failure at approximately 10 years of age unless long-term cystine-depleting therapy is instituted. Hypothyroidism and photophobia due to corneal crystals often manifest late in childhood. After renal transplantation, a variety of nonrenal complications, including a distal vacuolar myopathy (6), impaired swallowing (7), retinal blindness (8), pancreatic insufficiency (9, 10), neurological deterioration (11, 12), and gonadal insufficiency in males (13), occur with differing frequencies.

The treatment of cystinosis includes replacement of renal losses due to the Fanconi syndrome and supplementation with L-thyroxine for those patients with hypothyroidism (4, 5). However, the mainstay of therapy involves early initiation of treatment with cysteamine (14), a free thiol that reacts with intralysosomal cystine to form cysteine and the mixed disulfide cysteine–cysteamine (15). These compounds exit the cystinotic lysosome via a process not requiring the defective cystine carrier (16). The consequent cystine depletion prevents renal deterioration and growth retardation (17, 18), depletes muscle of cystine (19), and obviates the need for thyroid hormone replacement (20). Cysteamine eyedrops effectively dissolve existing corneal crystals (21, 22).

In 1995, the cystinosis gene, presumed to encode the defective cystine carrier, was mapped by linkage analysis to chromosome 17p13 (23). After progressive narrowing of the critical region (24–26), a polymorphic marker in the area, *D17S829,* was found to be absent in several cystinosis patients (27). This apparent deletion facilitated the isolation of the cystinosis gene, called *CTNS*. The mRNA of *CTNS* is

1096-7192/99

~2.6 kb in size. *CTNS* contains 12 exons that are distributed across ~23 kb of genomic DNA and is predicted to encode a 367 amino acid protein called cystinosin. Cystinosin has seven predicted transmembrane domains and eight potential glycosylation sites (27).

Although 31 different mutations in *CTNS* have already been identified, between 33 and 44% (27, 28) of patients are reported to be homozygous for a 65-kb deletion that encompasses the *D17S829* marker located within intron 3. The homozygous deletion state is associated with the failure to amplify a product with PCR primers specific for *D17S829,* but a method for establishing the heterozygous state for this deletion has yet to be reported. Therefore, we determined the breakpoints of the common 65-kb deletion and used primers immediately distal to these breakpoints for designing a PCR assay that detects heterozygotes for the deletion. Our method has allowed the frequency of this common deletion among cystinosis alleles to be established. It also provides a diagnostic test for the most common *CTNS* mutation in individuals of Northern European descent.

MATERIALS AND METHODS

All patients were enrolled in an Institutional Review Board-approved protocol, and they or their parents gave informed consent. DNA extraction and agarose gel electrophoresis were performed as described (28, 29). BAC RG147P12 (Research Genetics, Huntsville, AL) was sequenced using the "shotgun" method (30). Direct, automated DNA sequencing was performed using the ABI Prism dRhodamine terminator cycle sequencing kit (Applied Biosystems, Foster City, CA) according to the manufacturer's recommendations. The conditions for multiplex PCR amplification are provided in the Results. The PCR primers used in this study are listed in Table 1. Sequence comparisons were performed using PowerBLAST (31).

RESULTS

Determination of the deletion breakpoints. The 3′ breakpoint of the common 65-kb deletion was reported by Town *et al.* (27) to reside within intron 10 of *CTNS*. We chose two reverse sequences in IVS 10 and IVS 11 that flanked exon 11 (see Table 1). These sequences served as the 3′ primers for PCR amplification across the deletion. Since these primers were 174 bp apart, the resulting PCR products generated using these primers along with suitable 5′ primers should differ in size by ~170 bp.

TABLE 1
Primers for Multiplex PCR Amplification

Name	Sequence
Flanking exon 11	
Reverse-1 (IVS10)	5′-AGACAGACGGACAGACAGAC-3′
Reverse-2 (IVS11)[a]	5′-GCCCGATGCCCCAGC-3′
LDM_1[b]	
Forward	5′-CTAACAGTATCACCGGAGTC-3′
Reverse	5′-GGCCATGTAGCTCTCACCTC-3′
LDM_2	
Forward	5′-ACCTCTCTGATGTGTCCAAG-3′
Reverse	5′-AGCCAAAGGCATCAGGAAAG-3′
D17S829	
Forward	5′-CTAGGGGAGCGTGTTAGCAT-3′
Reverse	5′-TGTAAGACTGAGGCTGGAGC-3′
Exon 4	
Forward	5′-GTCATTGATTTGGGTCC-3′
Reverse	5′-TAGGGCTTGTCTTACAGGTA-3′

[a] Reverse 2 (IVS11) is identical to the reverse primer for exon 11 of *CTNS,* previously described (27).

[b] LDM, large deletion marker. The reverse primer of LDM_1 is identical to the reverse primer for exon 10 of *CTNS* (27).

Potential PCR primers for the 5′ end of the breakpoint were selected based upon data acquired in the search for the cystinosis gene. Sequence data for BAC RG147P12, which contains the 5′ portion of the deleted sequence, was available (manuscript in preparation). In addition, the 5′ deletion breakpoint had been placed within 4 kb of EST T85505 (NID g713857) by Town *et al.* (27). Hence, candidate 5′ primer sequences, chosen at 1-kb increments on either side of T85505, were screened using the two 3′ primers flanking exon 11; DNA from a homozygous deletion patient served as template.

One 5′ primer, LDM_1 Forward (Table 1), yielded PCR products that were 2.22 and 2.05 kb in size (data not shown), indicating amplification across the deletion. The 2.22-kb product was sequenced from both ends. Its 5′ portion matched the sequence of BAC RG147P12 for 360 bases (or 361 bases, depending upon whether the C nucleotide at the junction was from the 5′ or the 3′ border of the deletion) (Fig. 1). The remaining sequence contained the last 5 (or 4) bases of exon 10 and the beginning of IVS 10. (Note that the entire 1683-bp sequence of IVS 10 was previously determined by sequencing the PCR product formed using primers from exon 10 and exon 11.) The 3′ portion of the 2.22-kb fragment was