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Molecular Characterization of *CTNS* Deletions in Nephropathic Cystinosis: Development of a PCR-Based Detection Assay

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Summary

Nephropathic cystinosis is an autosomal recessive disorder that is characterized by accumulation of intralysosomal cystine and is caused by a defect in the transport of cystine across the lysosomal membrane. Using a positional cloning strategy, we recently cloned the causative gene, *CTNS,* and identified pathogenic mutations, including deletions, that span the cystinosis locus. Two types of deletions were detected—one of 9.5–16 kb, which was seen in a single family, and one of ~65 kb, which is the most frequent mutation found in the homozygous state in nearly one-third of cystinotic individuals. We present here characterization of the deletion breakpoints and demonstrate that, although both deletions occur in regions of repetitive sequences, they are the result of nonhomologous recombination. This type of mechanism suggests that the ~65-kb deletion is not a recurrent mutation, and our results confirm that it is identical in all patients. Haplotype analysis shows that this large deletion is due to a founder effect that occurred in a white individual and that probably arose in the middle of the first millenium. We also describe a rapid PCR-based assay that will accurately detect both homozygous and heterozygous deletions, and we use it to show that the ~65-kb deletion is present in either the homozygous or the heterozygous state in 76% of cystinotic patients of European origin.

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Introduction

Nephropathic cystinosis (MIM 219800), an autosomal recessive disorder, is the most common inherited cause of proximal renal tubular dysfunction (the renal Fanconi syndrome) and is due to defective lysosomal transport of cystine (Gahl et al. 1982). Affected individuals typically present with symptoms of severe fluid and electrolyte disturbance (polyuria and polydypsia, vomiting, poor growth, and rickets) at age 6–12 mo. Without specific treatment, increasing growth retardation occurs, with end-stage renal failure by age ~10 years (Gahl et al. 1995). After renal transplantation, cystine continues to accumulate in other organs, leading to multisystem disease. The cystinosis locus was mapped to the short arm of chromosome 17 (The Cystinosis Collaborative Research Group 1995), and, using a positional cloning strategy, we recently cloned the causative gene, *CTNS* (Town et al. 1998). The *CTNS* gene encodes a protein of 367 amino acids, cystinosin, which is predicted to be an integral lysosomal membrane protein. The identification of *CTNS* was greatly facilitated by the detection of deletions spanning the cystinosis locus in affected individuals. Two types of deletions were detected—one of ~65 kb, which is found in the homozygous state in nearly one-third of cystinotic individuals; and a smaller one of 9.5–16 kb, carried by a single family. Both the larger deletion and the smaller one span the 5′ end of *CTNS,* covering exons 1–10 and 1–3, respectively, and STS analysis indicated that the larger deletion was the same size in all patients. In this study, we characterize the deletion breakpoints and describe a rapid, PCR-based assay to detect both homozygous and heterozygous deletions. Our results confirm that the ~65-kb deletion is identical in all patients and suggest an ancient, common ancestry for this mutation.

Subjects and Methods

Patients

We studied 167 individuals, from 147 families, with nephropathic cystinosis. The families were divided into

two cohorts. The first group of 67 families, with 84 patients, comprised families for which the STS content of the *CTNS* region had been studied, and haplotype analysis was done by means of polymorphic markers flanking the *CTNS* locus. Thirty-nine of these unrelated individuals were homozygous or heterozygous for the large deletion and one (family P11) was apparently homozygous for the smaller deletion (Town et al. 1998). The second cohort of 80 families, consisting of 83 patients, comprised unstudied cases of nephropathic cystinosis. Informed consent was obtained from all individuals or their parents. The patients were from a variety of ethnic groups. The majority (83%) were of European origin, but 10 were from North Africa, 3 were from the Middle East, 4 were from Pakistan, 3 were from Turkey, 1 was from South Africa, and 4 were of unknown origin.

Haplotype Analysis, Allelic Association, and Dating of the Mutation

Genomic DNA was amplified with fluorescently labeled primers corresponding to the microsatellite loci *D17S1528, D17S1798, D17S2167, D17S829, D17S1828,* and *D17S1876*. Primer sequences, PCR amplification, gel electrophoresis, and data analysis were done as described elsewhere (The Cystinosis Collaborative Research Group 1995; Dib et al. 1996; McDowell et al. 1996). Statistical comparison of allele frequencies between deleted and control chromosomes was made on the basis of a χ^2 test for a 2 × 2 table, with alleles classified into two groups, one for an allele potentially associated with the deletion and one for all others combined into a single group. The age of the deletion was estimated by use of a simple approach that looked at only a single mutation and that reconstructed likely ancestral recombinants (Risch et al. 1995). Assuming that *g* generations have elapsed since the deletion occurred on a sole chromosome with an original associated allele at a locus close to the deletion locus, then $1 - pD = [1 - (1 - \theta)^g]\ (1 - pN)$, where θ is the recombination fraction between the two loci, pD the frequency of the associated allele on deleted chromosomes, and pN the frequency for that allele on control chromosomes (Risch et al. 1995); hence, *g* may be estimated.

Southern Blot Analysis

Genomic DNA from affected individuals was digested by *Hin*dIII and hybridized with a *CTNS* cDNA probe spanning the whole coding sequence as well as 286 bp and 1134 bp of the 5′ and 3′ untranslated regions, respectively. Southern blotting and hybridizations were performed as described elsewhere (Antignac et al. 1992).

PCR Amplification and Direct Sequencing

Primer sequences and PCR conditions were selected with the OLIGO 5.0 program (NBI). The primers, spanning the breakpoints of the ~65-kb deletion (65A–65A.1 and 65A–65A.2), and the P11 deletion (P11.1–P11.2) were as follows: 65A, 5′-CCGGAGTCTACAGGGCACAG-3′; 65A.1: 5′-GTCCCGGCTCACCCTCTTCC-3′, 65.A2: 5′-GGCCATGTAGCTCTCACCTC-3′; P11.1: 5′-AAGGGCAGAATGAGGTTGGA-3′; P11.2: 5′-AAGGGCAGCAAGGCACTCGT-3′. After denaturation at 94°C for 5 min, amplification was done with 30 cycles of 1 min at 94°C, 1 min at the annealing temperature of 58°C, and 1 min at 72°C, with a final extension step of 5 min at 72°C. PCR products were purified by means of the Wizard PCR Preps DNA-purification System (Promega) and were directly sequenced by use of an Applied Biosystems DNA sequencer (model 373A) and the BigDye™ terminator cycle sequencing kit (Perkin-Elmer), according to the manufacturer's protocol. All sequences were analyzed with BLAST programs (Altschul et al. 1990) after identification and masking of repetitive sequences with the program RepeatMasker (Smit 1996).

Results

Haplotypes

Initially, DNA from 41 unrelated patients and their families was typed with six microsatellite loci, which span the cystinosis locus and cover a 5-cM interval of the short arm of chromosome 17. Strong linkage disequilibrium with a cystinosis mutation was not observed for any haplotype across this region, and linkage disequilibrium was not found with the flanking marker *D17S2167*. However, in the European families, a 215-bp allele of the other flanking marker *D17S1828* was observed more frequently on chromosomes carrying the ~65-kb deletion than on chromosomes from unaffected family members. We therefore typed an additional 25 patients and their families with the *D17S1828* marker. To establish haplotypes and to observe the frequency of recombination across the *CTNS* region, we also typed these 25 families with *D17S2167* and with *D17S829,* which is intragenic. The results with marker *D17S1828* are shown (table 1) for a total of 117 chromosomes carrying known *CTNS* mutations. Of these, 98 were from European individuals and 19 were from individuals from other ethnic groups. The control (noncystinotic) chromosomes were those without *CTNS* mutations from the carrier parents. The 215-bp allele of marker *D17S1828* was detected on 26.7% of our European control chromosomes (23 of 86) and on 21.8% of 261 unrelated CEPH chromosomes. The frequency of this allele on chromosomes with the large *CTNS* deletion was significantly higher (46.3%), giving a *P* value of .0004.

Haplotypes from cystinosis families, on the basis of either three or six microsatellite markers, gave 325 informative meioses for the region, which spans markers *D17S829* and *D17S1828*. For these families, a recombination frequency of .0186 (6/325) between the two markers was observed. Substitution of these data into the formula $1 - pD = [1 - (1 - \theta)^g]\ (1 - pN)$ gives an estimate of ~63.5 generations (95% confidence interval [CI] 29–73), since the deletion first arose on the chromosome carrying the 215-bp allele and, assuming 25 years per generation, this suggests the deletion arose on this background ~1,580 years ago (95% CI 725–4, 325).

Detection of Junction Fragments

To determine the deletion breakpoints, we studied DNA from our first cohort of patients, whose STS content had been analyzed elsewhere. Southern blots containing *Hin*dIII-digested DNA were hybridized with the *CTNS* cDNA probe (see Subjects and Methods). DNA from unaffected controls produced a band pattern consistent with the known map of the cystinosis region, including a doublet of 2.8 kb, which contains both the 3′ end of exon 12 and exon 4 (fig. 1*A*). In the DNA from the 20 patients shown to be homozygous for the large deletion by PCR analysis, the 9.9-kb and 11-kb fragments corresponding to exons 1–3 and to exons 5–11 plus the 5′ end of exon 12, respectively, were absent, whereas a 2.8-kb band was present. This band is likely to consist of the 3′ part of exon 12, whereas the exon 4 part is probably missing. By contrast, a novel 6.6-kb fragment was detected in all cases. The detection of this abnormal band in addition to the normal fragments, in 19 other patients, confirmed that they were heterozygously deleted, as indicated by haplotype analysis. Considering the 27 patients for whom deletions had not been detected by PCR and haplotype analysis, all displayed the normal hybridization pattern. Finally, hybridization of the *CTNS* probe to a Southern blot containing the *Hin*dIII-digested DNA of patient P11 detected the normal 2.8- and 11-kb fragments as well as the 6.6-kb junction fragment, whereas the normal 9.9-kb fragment was absent. This pattern is in agreement with a large deletion on one allele and a smaller one, encompassing only exons 1–3, on the other allele (fig. 1*B*), indicating that this patient was compound heterozygous for two different deletions and not homozygous for the small deletion, as suggested by STS content analysis.

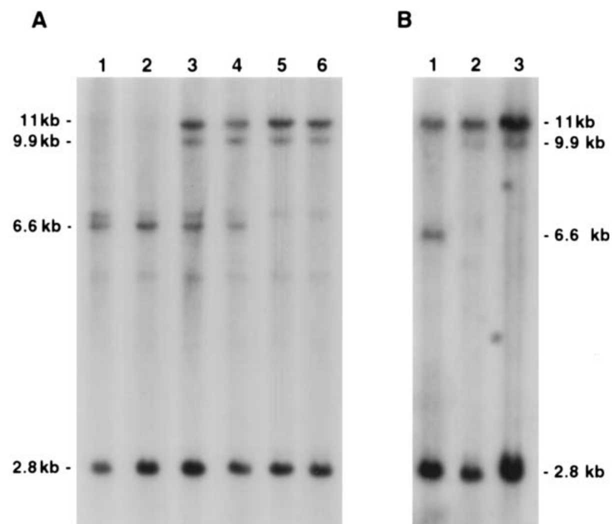

Figure 1 Southern blot analysis of *Hin*dIII-digested DNA hybridized with a full-length *CTNS* cDNA probe. *A,* DNA from individuals with homozygous (lanes 1 and 2) and heterozygous (lanes 3 and 4) deletions for the ~65-kb deletion and from control individuals (lanes 5 and 6). *B*, DNA from patient P11 (lane 1) and control individuals (lanes 2 and 3).

Identification of the Proximal and Distal Deletion Breakpoints

By PCR amplification and long-range sequencing across the breakpoints of the major *CTNS* deletions, a

Table 1

Frequency of the 215-bp Allele of Marker *D17S1828*

Chromosome Origin and Allele	No. of Cystinotic Chromosomes: With ~65-kb Deletion	No. of Cystinotic Chromosomes: Without ~65-kb Deletion	No. of Non-cystinotic Chromosomes
Chromosomes of Europeans:			
Allele 215	26	10	23
Non-215 allele	30	32	63
Chromosomes of non-Europeans:			
Allele 215	NA	6	5
Non-215 allele	NA	13	16

Note.—NA = not available.

fine map of the region could be constructed as shown in fig. 2*A*.

Large Deletion.—By studying the STS content of the regions spanned by the deletions, we previously mapped the 5′ breakpoint of the large deletion between the end of cosmid clone C11155 (marker C11155L) and the end of P1 artificial chromosome (PAC) K03130 subclone 2 (Town et al. 1998). Both of these genomic clones contain expressed sequence tags (ESTs) (AA099495 and T85505, respectively), which, in turn, contain sequences homologous to Alu repeats. We have amplified by PCR and sequenced the intervening 1764 bp between the two ESTs. This region contains two Alu repeats, at nucleotide positions 299–580 and 1590–1763, and part of an L2 sequence of the LINE family at position 744–1338. The 3′ breakpoint was mapped elsewhere between exons 10 and 11. PCR amplification and sequencing showed that the *CTNS* intron 10 is 1681 bp in length and contains a repeat sequence of the MIR family at nucleotide position 1168–1275. We designed primers located in nonrepeated regions (primers 65A–65A.1), outside the deletion boundaries, which amplify a 1.25-kb bridging fragment in patients with homozygous deletions but which do not give a PCR product with control DNA. The 1.25-kb product of five patients was sequenced in its entirety and was compared with the sequence of the respective 5′ and 3′ normal segments. This comparison showed that the 5′ deletion breakpoint is located in the L2 repeat, 994/995 bp downstream of the EST clone AA099495, whereas the 3′ deletion breakpoint is actually located within an exon, 4–5 bp before the 3′ end of exon 10. No sequence homology was detected between the 5′ and 3′ sequences flanking the breakpoints, and each breakpoint junction showed only a 1-bp overlap, suggesting an end-joining mechanism (fig. 2*B*).

P11 Deletion.—For the small deletion found in family P11, the 5′ breakpoint was mapped to the 5-kb PAC K03130 subclone 5, whereas the 3′ breakpoint was mapped to PAC K03130 subclone 49, which contains the microsatellite *D17S829*. A 2.15-kb PCR product spanning the breakpoint junction was amplified from patient P11's DNA, with the use of two primers located in the nondeleted regions of PAC K03130 subclones 5 and 49. Direct sequencing of the PCR product and comparison with the normal sequences showed that the 3′ deletion breakpoint is located within intron 3, 1832 bp downstream of the end of exon 3, in the stretch of GT repeats of marker *D17S829*. This marker is framed by

A

B

~65 kb deletion

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CACAAGCCCCCTCCCCCCGGCATAACCCACAATGGGCTCCTGTTCCCTCCACATTCACTCCCCTCCCGCTCCCTGCAGCA   normal sequence (between AA099595 and T85505 )
CACAAGCCCCCTCCCCCCGGCATAACCCACAATGGGCTCACAGGTACCTCCAGGGCCCTGTTCACATGGCCGGTGGCAGG   mutant allele
CTACATCAAGCTCGCAGTCACGCTGGTCAAGTATTTTCCACAGGTACCTCCAGGGCCCTGTTCACATGGCCGGTGGCAGG   normal sequence (CTNS intron 10)
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P11 deletion

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CCGCCCCTGGAAGCAAAAGAGAACACATGGGCAGGGGCAGACACACAGGCAGGGGGAGGGGCGCAGGCCTCCCGGGATGT   normal sequence (subclone 5)
CCGCCCCTGGAAGCAAAAGAGAACACATGGGCAGTGTTCTCTTTTCTGGGTGTGTGTCTGCGCGCATGTATGTGTGCCCG   mutant allele
TGTGTGTGTGTGTGTGTGTGTGTGTGCCTGGGCGTGTGTGTGTGTGCCTGGGTGTGTGTCTGCGCGCATGTATGTGTGCCCG   normal sequence (D17S829)
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Figure 2 Deletion breakpoint junctions. *A,* Restriction enzyme map of PAC clone K03130 (thin horizontal bar). *Hin*dIII (above) and *Bam*HI (below) sites are indicated (vertical lines). Random subclones derived from this PAC and used as a source of genomic sequence are shown below (thick numbered lines). ESTs (AA099495, T85505, AA313583, and U90913) are indicated by arrows. The 12 *CTNS* exons are indicated by blackened boxes with their respective number above each box. The regions containing the ~65-kb and the P11 deletion breakpoints are enlarged in triangles above and below, respectively, the map. The breakpoint positions are indicated by arrowheads. Alu, MIR, and L2 repeats are indicated by dotted, blackened, and hatched arrows, respectively. *B,* Nucleotide sequences of normal and deleted alleles. The asterisks (*) indicate the nucleotide matches. The one base of overlap (C) at the ~65-kb deletion junction is indicated in bold. The short direct repeats are underlined, and the 11-bp insertion, as well as the homologous sequence in the normal sequence, are shaded.

several repeat sequences, one MIR repeat and one Alu repeat, which are, respectively, 103–246 bp and 314–618 bp downstream of the breakpoint, and an Alu repeat 726–417 bp upstream of the breakpoint. There is also a single Alu repeat 202–496 bp downstream of the 5′ breakpoint. No sequence homology around the junction was detected. An 11-bp insertion was found at the breakpoint junction of the deletion. This sequence is homologous to the sequence 20–10 bp upstream of the 5′ breakpoint but is located in the reverse orientation. Additionally, two copies of a short direct GGGT repeat were found 3 and 1 nucleotides away from the insertion (fig. 2*B*). Given the location of the breakpoints and the knowledge of the precise size of the PAC K03130 subclones from sequencing data, we were able to estimate the size of the P11 deletion to 13 kb.

PCR Deletion Test

To provide a more robust PCR deletion test, we used the genomic sequence around the ~65-kb deletion breakpoint junction to design another set of primers (65A–65A.2) that amplify a smaller, 360-bp bridging fragment. Similarly, we also designed a set of primers, P11.1–P11.2, that amplify a 755-bp fragment containing the breakpoint of the small deletion.

All the 67 unrelated cystinotic individuals of our first group were screened by PCR with the primers of the marker *D17S829* and the three sets of breakpoint primers (65A–65A1 and 65A–65A2 for the large deletion and P11.1–P11.2 for the small deletion). In all cases except that of patient P11, the DNA of patients with homozygous or heterozygous deletions for the ~65 kb produced an amplification product with primers 65A–65A1 and 65A–65A2, but not with the primers P11.1–P11.2. The amplification of a fragment from all three sets of breakpoint primers with the DNA of patient P11 confirmed the presence of both deletions in this individual. No fragment was obtained, either with the primer sets 65A–65A.1 and 65A–65A.2 or with P11.1–P11.2, by amplification of the DNA of the 27 patients without deletions. To prove the robustness of the PCR test with primers 65A–65A.2 and to demonstrate that the ~65-kb deletion was present on both alleles of the patients with homozygous deletions, we tested the segregation of the deletion in 10 families (61 unaffected individuals) for which the heterozygous carrier status had been determined for 34 individuals by leukocyte cystine content measurement and haplotype analysis (Jean et al. 1996). As expected, the 360-bp deletion fragment was present in each patient and in every heterozygous carrier but was not found in the 27 unaffected, noncarrier relatives. For family P11, amplification products were obtained with the sets of primers 65A–65A.1 and 65A–65A.2 in the maternal carriers and with P11.1–P11.2 in the paternal carriers (fig. 3).

We then used the PCR assay to test DNA from our second cohort of 80 patients who had not been previously analyzed. DNA was tested by PCR with the primer sets 65A–65A.2 and P11.1–P11.2 and with the *D17S829* primers. No PCR product was obtained with the primers P11.1–P11.2 in any case. In 35 affected individuals, no PCR product was obtained with the *D17S829* primers, whereas a 360-bp fragment was obtained with the primers 65A–65A.2, indicating that these individuals had homozygous deletions. Twenty-one displayed amplification products with both sets of primers (*D17S829* and 65A–65A2), indicating that they had heterozygous deletions. Twenty-four gave a PCR fragment with *D17S829,* whereas no PCR fragment was detected for the primers 65A–65A.2, indicating the absence of the ~65-kb deletion. To confirm that there was no heterozygous deletion that could have been missed by PCR, we verified by Southern blot hybridization that all 24 patients did not display the 6.6-kb junction fragment. When results were taken from both cohorts of patients, among the 147 unrelated patients, 55 (37.4%) were ho-

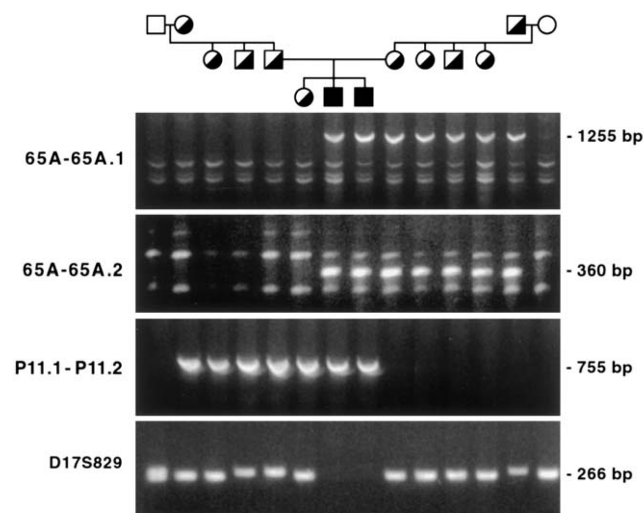

Figure 3 Segregation of the ~65-kb deletion and the small P11 deletion in family P11. The two affected sibs (blackened squares) display an amplification product with the primer sets 65A–65A.1 and 65A–65A.2, both of which detect the ~65-kb deletion, and with the P11 primers, which detect the smaller deletion but do not display any amplification product with *D17S829,* which is located within both deletions. Their carrier sister as well as the cystinosis carriers of the paternal branch display an amplification product with the P11 primers, indicating that they are heterozygous for the small deletion, whereas all the cystinosis carriers of the maternal branch display amplification products with the primers 65A–65A.1 and 65A–65A.2, indicating that they are heterozygous for the ~65-kb deletion. Heterozygous carriers and normal individuals have been determined by the measurement of leukocyte cystine content and haplotype analysis (Jean et al. 1996).

mozygous and 41 (27.9%) were heterozygous for the large ∼65-kb deletion. Of the 24 patients of non-European origin, only two, from North Africa, carried the deletion. This raises to 76% the percentage of cystinotic patients homozygous or heterozygous for the ∼65-kb deletion in the European population.

Discussion

We have shown that deletions of the *CTNS* gene are the most frequent mutations underlying nephropathic cystinosis, and we have detected two types of deletions on the basis of STS content analysis—a large deletion (∼65 kb) found in all families but one and a smaller one (9.5–16 kb) found in only one family (Town et al. 1998). These results have been confirmed by Shotelersuk et al. (1998), in an American-based population of cystinotic patients. The authors found that 50 of 108 patients, all of European descent, had homozygous deletions, with 48 of them probably bearing the ∼65-kb deletion, as deduced by STS content. The present study reports the characterization of the breakpoints of the two deletions that we identified elsewhere. The breakpoints occur in regions containing several repetitive elements either from Alu, MIR, or LINE2 families (Smit 1996) but do not involve unequal recombination between two of them. The small deletion junction contains an 11-bp insertion homologous to the sequence 20–10 bp upstream of the 5′ breakpoint but located in the reverse orientation, suggesting complex reorganization at the deletion junction. The large ∼65-kb deletion appears to represent a clean breakage and reunion event with one nucleotide homology at the junction. These two types of deletion junctions—insertional junctions and junctions exhibiting limited amounts of homology (one to six nucleotides)—have already been found in various hereditary diseases (Henthorn et al. 1990; Woods-Samuels et al. 1991), indicating that nonhomologous recombination events underlie the mechanism of the deletions associated with cystinosis. Contrary to the recombination between homologous regions giving rise to recurrent rearrangements (Lakich et al. 1993), this mechanism favors the possibility of a founder effect for the mutation. This hypothesis is strengthened by the demonstration of an identical breakpoint in all cases. Actually, we have shown that the breakpoint sequence is strictly identical in the five unrelated patients for whom we sequenced the breakpoint and that the PCR product spanning the breakpoint has the same size in all patients and in all available parent DNA of children with homozygous deletions, ruling out the possibility of a larger deletion in one allele. Our data support the recent work of Anikster et al. (1999), in which an identical breakpoint sequence was confirmed in 24 different chromosomes of cystinotic patients. All the patients bearing the mutation, to date, are white; hence, a founder effect probably occurred in a white individual. To test this hypothesis, we looked for a common haplotype flanking the *CTNS* locus. Although we were not able to detect strong linkage disequilibrium between the *CTNS* deletion and any haplotype across the region, we did observe that one allele of marker *D17S1828* was overrepresented in the deleted chromosomes, as compared with the control chromosomes. This allowed us to date the mutation, although the low number of patients (mainly affecting precise evaluation of the recombination distance between *D17S829* and *D17S1828*) leads to a broad 95% CI. It can be inferred, however, from our data, that the deletion event probably took place in the middle of the first millenium AD. This is in agreement with the estimation provided by Shotelersuk et al. (1998), who proposed, on the basis of the family history of their cystinotic patients of European descent, that the deletion may have initially occurred in Germany prior to 700 A.D. The hypothesis of the deletion having arisen in northern Europe and having spread throughout Europe toward northern Africa is consistent with a north to south gradient as found for the ΔF508 cystic fibrosis mutation (Estivill et al. 1997) or α1-antitrypsin deficiency (Cox 1995). However, the existence of such a gradient is difficult to assess from our relatively small sample of patients from southern Europe and northern Africa and will be confirmed only when a larger cohort of patients from various countries is tested.

We have described here a useful and simple test for the diagnosis of cystinosis. A similar study (Anikster et al. 1999) has been done that determined the breakpoint sequence of the large deletion. This study suggested the use of a multiplex PCR-based assay as a diagnostic test for patients carrying the large deletion. However, the potential problems of unequal amplification of different fragments, inherent in multiplex PCR, were recognized by the authors themselves. To amplify the deletion breakpoint junction, we successively designed two sets of primers, amplifying fragments of 1.25 kb and 360 bp, respectively, and have shown, by comparison with Southern blot and haplotype analysis, that both of them are reliable, since they give equivalent results. Therefore, we propose the use of the primers amplifying the 360-bp fragment (65A–65A.2) for the detection of the breakpoint deletion. Thus, by combining data from just two PCRs, one using the primers corresponding to the *D17S829* marker located within the deletion and the other a set of primers spanning the ∼65-kb deletion breakpoint junction, we were able to detect both homozygous and heterozygous deletions in individuals with nephropathic cystinosis. A high proportion (76%) of the European patients carry a large deletion, and we anticipate that this will be true of the general European population. Therefore, multiplex or simplex PCR-based

methodology will provide the basis for a rapid molecular diagnostic test for cystinosis in Europe and for patients of European descent in other countries. In particular, it will be very useful for prenatal diagnosis and for detection of carrier individuals in affected families, as well as for diagnosis of new cases.

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Electronic-Database Information

Accession numbers and URLs for data in this article are as follows:

Online Mendelian Inheritance in Man (OMIM), http://www.ncbi.nlm.nih.gov/Omim (for nephropathic cystinosis [MIM 219800])

Program RepeatMasker, http://ftp.genome.washington.edu/cgi-bin/RepeatMasker (for identification and masking of repetitive sequences)

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