0031-3998/92/3205-0600$03.00/0
PEDIATRIC RESEARCH

Vol. 32, No. 5, 1992
Printed in U.S.A.

Six New Mutations in the Ornithine Transcarbamylase Gene Detected by Single-Strand Conformational Polymorphism

MENDEL TUCHMAN, ROBERT A. HOLZKNECHT, AMELIA B. GUERON, SUSAN A. BERRY, AND MICHAEL Y. TSAI

The Biochemical Genetics Laboratory, Division of Genetics and Metabolism, Departments of Pediatrics and Laboratory Medicine and Pathology, University of Minnesota, Minneapolis, Minnesota 55455

ABSTRACT. We describe six new mutations in the ornithine transcarbamylase (OTC) gene found in patients with OTC deficiency. These mutations were detected by single-strand conformational polymorphism analysis of amplified genomic DNA and characterized by direct sequencing of double-stranded DNA. Three of the mutations were found in males who had neonatal onset of hyperammonemia. The mutations are a single base deletion (guanine) in exon 5 at nucleotide 403 causing a frame-shift error, a guanine to adenine substitution at the 3′ end of intron 2 nucleotide 217 (−1) causing an acceptor splicing site error, and a guanine to adenine substitution at base 236 changing the code from glycine to glutamic acid at position 47 of the mature enzyme. Two different mutations were found in two males whose onset of clinical problems occurred after the neonatal period. One patient had a guanine to cytosine transversion in the sense strand of exon 3 at nucleotide 281 resulting in a substitution of threonine for arginine in position 62 of the mature OTC protein. This substitution changes the composition of the putative active site for carbamyl phosphate from Ser-Thr-Arg-Thr-Arg to Ser-Thr-Arg-Thr-Thr. The second patient has a guanine to thymine substitution at nucleotide 912 of the sense strand of exon 9, changing the code for leucine to phenylalanine in position 272 of the mature OTC protein. This changes a conserved domain of the gene likely to be the ornithine binding site from Phe-Leu-His-Cys-Leu-Pro to Phe-Leu-His-Cys-Phe-Pro. One female with OTC deficiency had a thymine to guanine substitution at nucleotide 1033, changing the code from tyrosine to aspartic acid at position 313 of the mature enzyme. We also confirmed an apparently common polymorphism in exon 8. An adenine or guanine in base 809 determines the code for a glutamine or an arginine, respectively, in position 238 of the mature OTC protein. (*Pediatr Res* 32: 600–604, 1992)

Abbreviations

OTC, ornithine transcarbamylase
PCR, polymerase chain reaction
SSCP, single-strand conformational polymorphism

OTC (EC 2.1.3.3.), the second enzyme of the urea cycle, catalyzes the formation of citrulline from carbamyl phosphate and ornithine in the liver and intestine. OTC deficiency, the most prevalent genetic defect of ureagenesis (1, 2) is inherited as an X-linked trait. At the molecular level, this disorder can be caused by various deletions and mutations affecting the OTC gene (3–8). Deletions affecting all or part of the OTC gene account for about 15–20% of patients, and point mutations account for the rest. Thus far, 15 different mutations have been reported in the OTC gene, all but one causing severe neonatal hyperammonemia (3–8). There are rare recurrent mutation sites with the exception of mutations in exon 5 affecting the code for arginine in position 109 of the mature protein, eliminating a *Taq*I recognition site (sequence TCGA) (3). Mutations in this codon occur in about 10% of males with acute neonatal disease (3, 4, 8). Thus, the molecular defect can be readily defined by southern blotting and *Taq*I restriction studies in only a small percentage of patients. Because there is evidence that many different mutations cause OTC deficiency, it may seem impractical to look for the deleterious mutations in patients who are found not to have a deletion or a point mutation affecting a *Taq*I recognition site. We feel, however, that it is both important and feasible to characterize mutations in most patients as such data may add to our understanding of the genotype-phenotype correlation. Moreover, defining the deleterious mutation in a family affected with OTC deficiency may provide the only reliable tool for determination of carrier status in the mother and her extended family and make prenatal diagnosis simple. We have had the opportunity to study the OTC gene in patients whose samples were referred to us from other centers as well as in patients cared for at the University of Minnesota. We show that by using PCR amplification, SSCP analysis of amplified exons, and direct sequencing of the relatively short PCR products it is both feasible and practical to define mutations in individual patients with OTC deficiency.

In this study, we used the above methodology to define six new mutations in the OTC gene. Three of the mutations were found in three patients with "neonatal onset" of hyperammonemia, one consisting of a single base deletion, one point mutation causing an mRNA splicing error, and another point mutation causing an amino acid substitution. Two other point mutations causing amino acid substitutions in the binding sites of carbamyl phosphate and ornithine were found in two patients with "late onset disease," and one point mutation was found in an OTC-deficient heterozygous female. This approach of SSCP analysis followed by direct sequencing of the abnormal exon could become routine for investigation of families with OTC deficiency who are in need of accurate genetic counseling and prenatal diagnosis.

Received for rapid publication June 8, 1992; accepted July 16, 1992.
Correspondence and reprint requests: Mendel Tuchman, M.D., Department of Pediatrics, University of Minnesota, Box 400, Mayo Memorial Building, 420 Delaware St. S.E., Minneapolis, MN 55455.

PATIENTS

Patient no. 29. Patient no. 29 was a male born after normal pregnancy, labor, and delivery. On the 4th d of life, he became

lethargic and then comatose. His blood ammonia rose rapidly to above 3000 μmol/L and he died at 1 wk of age. The diagnosis of OTC deficiency was confirmed by enzymatic assays of liver tissue showing undetectable OTC activity.

Patient no. 32. Patient no. 32 was a male born after normal pregnancy, labor, and delivery. The family history was significant in that the mother and maternal grandmother were vegetarian. At the age of 72 h, he became lethargic and then comatose. His blood ammonia level rose to above 3000 μmol/L and he died 10 h afterward. The diagnosis of OTC deficiency was established by enzymatic assay of liver tissue showing undetectable OTC activity.

Patient no. 35. The patient, a 7-y-old boy, never had major health problems until 1 wk before his demise. After a minor car accident, he developed protracted vomiting. He was admitted to the local hospital, where he became rapidly combative and then comatose. His blood ammonia level was 233 μg/dL (normal, <70), and the presumptive diagnosis of Reye's syndrome was made. His hyperammonemia reached a level of 780 μmol/L. He had a metabolic acidosis, which worsened, and he required bicarbonate to maintain normal blood pH.

Laboratory studies on admission demonstrated low plasma arginine, absent citrulline, and a mildly elevated level of glutamine (731 μmol/L). Urinary orotic acid was 3300 μg/mg creatinine (upper limit of normal, 7 μg/mg creatinine). The presumptive diagnosis of OTC deficiency was made.

Therapy with infusion of glucose, sodium benzoate, sodium phenylacetate, and arginine and administration of lactulose and neomycin as well as peritoneal dialysis did not prevent his death. Enzyme analysis of the patient's liver indicated an *in vitro* OTC activity that was 0.7 μmol/g wet wt/min (normal = 82.5 ± 18.7, mean ± SD). Western blot analysis showed a markedly reduced amount of OTC protein in liver tissue (data not shown). At an ornithine concentration of 5 mM (saturating) and varying carbamyl phosphate concentrations of 5, 20, 50, and 100 mM, the patient's liver OTC activity did not change (0.9 μmol/g wet wt/min).

Patient no. 36. Patient no. 36 is a male born after uneventful pregnancy, labor, and delivery who was healthy until the age of 7 mo, when he developed hyperammonemia in the range of 200–400 μmol/L (normal, <50). His plasma glutamine level was elevated and he had orotic aciduria. An older sister also had mild hyperammonemia, and both the sister and mother showed spontaneous orotic aciduria. After this first illness, he had numerous recurrent hyperammonemic episodes and developed severe developmental delay and disruptive behavior. The enzymatic diagnosis of OTC deficiency was established in our laboratory by enzymatic assays of liver tissue; three different sections of liver were analyzed and showed 2.4, 2.5, and 3.0 μmol/g/min of citrulline production. Western blot analysis showed undetectable OTC protein (data not shown).

Patient no. 85. Patient no. 85 is a male born after a pregnancy complicated by preeclampsia. He fed well for the first 3 d of life but was sleepy. On the 5th d of life, he became unresponsive and was brought to the hospital. His blood ammonia level was 827 μmol/L, rose to 1300 μmol/L, and fell rapidly when hemodialysis was instituted. Plasma amino acid analysis was normal except for elevated glutamine level, whereas urinary orotic acid level was elevated. OTC enzymatic activity was undetectable in his liver. He developed seizures but subsequently recovered and was discharged from the hospital. He underwent successful liver transplantation at the age of 5 mo, but he is still developmentally delayed and is having feeding problems.

Patient no. 45. Patient no. 45 was a 33-mo-old girl who was admitted to the hospital with seizures, coma, and elevated serum levels of liver enzymes. She was in good health until 3 mo before her admission, when she developed episodic vomiting. On the day of admission, she presented with myoclonic jerks and progressed to coma. Her blood ammonia was 525 μmol/L, and elevated levels of orotic acid were found in her urine. She died 5 d later. The diagnosis of OTC deficiency was established by assays of her liver tissue. Ten different sections of the liver biopsy material were assayed for OTC activity and were found to range between 3 and 16% of normal. The amounts of OTC protein detected on Western blots correlated qualitatively with the residual enzymatic activity (data not shown).

MATERIALS AND METHODS

Liver tissue. Biopsy or autopsy tissue was obtained from patients and flash frozen in liquid nitrogen. The tissue was stored at −80°C until used for enzyme studies or DNA extraction. Assays of liver OTC activity were performed by a radiochromatographic method described previously (9).

DNA isolation. Genomic DNA was isolated from liver tissue homogenized in 10 mM Tris, pH 8.2, 0.1 M EDTA. After treatment with 0.5% SDS, RNAase and proteinase K, the DNA was extracted using phenol and chloroform and precipitated with cold ethanol according to standard procedures.

Amplification of genomic DNA. Amplification of all 10 exons of the OTC gene was performed by PCR. The amplification conditions and the sequences of the synthetic primers were reported previously (8). Some of the primers overlapped with intron sequences only, so that a few flanking intron bases of the patients' DNA were also amplified. Several primers extended a few bases into the exons, overlapping six of the donor splicing sites (GT), six of the acceptor splicing sites (AG), and 31 codons. Therefore, mutations at these sites cannot be detected by these primers. Each exon was amplified separately, except exons 7 and 8 were amplified as one DNA segment containing the short (80-bp) intron 7. Visualization of the amplified exons was performed on a 4% 3:1 Nu Sieve (FMC Bioproducts, Rockland, ME) agarose gel containing ethidium bromide.

SSCP analysis. The PCR products of all 10 exons were screened for mutations or polymorphisms on a nondenaturing gel containing 6% acrylamide (1% cross-linking) and 5 to 10% glycerol modified from the method of Orita *et al.* (10, 11). A Bio-Rad Mini-Protean II (Bio-Rad Laboratories, Richmond, CA) vertical gel assembly was used with 1.0-mm gel spacers. The plates' dimensions were 7.2 cm (height) × 10.2 cm (width). Samples loaded onto the gel consisted of 3 μL of the PCR product, 3 μL of formamide, and 2 μL of the loading dye containing 30% glycerol. Before loading on the gel, the samples were denatured for 5 min at 95°C, followed by rapid cooling in a 4°C bath. Because the amplified OTC exons ranged in size from 98 to 329 bp, it was necessary to run separate exons at different voltages while varying total run time. All exons were electrophoresed in a standard Tris-borate-EDTA buffer inside a 4°C cold room for 1.5 to 3 h at a constant voltage ranging from 100 to 200 V. Visualization of single-stranded DNA on the gels was accomplished by silver staining using a kit purchased from Bio-Rad Labs.

Purification and direct sequencing of double-stranded PCR product. The PCR products of exons showing abnormal SSCP patterns were first purified on a 2% low melting point agarose gel. The bands were excised from the gel and further purification was accomplished using the Mermaid kit (Bio 101, La Jolla, CA). We used 20 μL of the "glassfog" product to purify the amplified DNA of the abnormal exons of a size that ranged between 129 and 196 bp. The elution volume, to recover the DNA from the glassfog, was 40 μL. Each exon was sequenced in the forward and reverse directions using the sense and antisense PCR primers, respectively. The purified double-stranded DNA was used as a template for "Sequenase" reactions (United States Biochemical, Cleveland, OH) according to the manufacturer's instructions with the following modifications. Dimethyl sulfoxide was added (1 μL) to the tubes containing template DNA and one of the PCR primers, and the mixture was heated at 95°C for 5 min, followed by rapid freezing in a methanol/dry ice bath. Subsequently, 6.5 μL of "labeling mix," including $MnCl_2$ and [α-^{35}S]

deoxyATP, as per instructions in the kit, were added to the frozen primer-template mixture. The mixture was hand thawed, vortexed, and centrifuged briefly. Immediately afterwards, aliquots of the above solution were added to the previously prepared "termination" reaction tubes containing either dideoxy-guanosine triphosphate, dideoxyATP, dideoxy-thymidine triphosphate, or dideoxy-cytidine triphosphate followed by incubation and completion according to the instructions. A 6% polyacrylamide-urea gel was used to separate [α-^{35}S]deoxyATP-labeled products before autoradiography.

RESULTS

The SSCP analysis of the OTC exons in the six patients revealed in each case only one exon with an abnormal migration pattern of one or both of the single strands of DNA. The abnormal exons were patient no. 29, exon 5; patient no. 32, exon 3; patient no. 35, exon 3; patient no. 36, exon 9; patient no. 85, exon 3; and patient no. 45, exon 10. Figures 1 and 2 show the SSCP analyses of the abnormal exons in each patient along with the respective exons of other patients with normal migration patterns. The female patient (no. 45) showed four single-strand bands of exon 10, indicating that she was heterozygous for a mutation in that exon.

Having identified the abnormal exons in these patients as the suspected locations of mutations, we proceeded with direct sequencing of the respective PCR products to define the defects. Figure 3 shows a portion of the DNA sequences from each patient. The six mutations are summarized in Table 1. The single base deletion (guanine) in exon 5 at nucleotide 403 found in patient no. 29 should cause a frame-shift error resulting in a premature termination codon in exon 6. The mutation in patient no. 32, a guanine to adenine substitution at the 3′ end of intron 2, nucleotide 217 (−1), causes an error in the AG acceptor splice site. This should result in an abnormally spliced mRNA and a nonfunctional enzyme. Patient no. 85 has a point mutation consisting of a guanine to adenine substitution at base 236, changing the code from glycine to glutamic acid at position 47 of the mature enzyme. The significance of this mutation is discussed below. Patient no. 35 had a guanine to cytosine transversion in the sense strand of exon 3 at nucleotide 281 resulting in a substitution of threonine for arginine in position 62 of the mature OTC protein. This substitution, which is likely to be deleterious, changes the composition of the putative active site for carbamyl phosphate from Ser-Thr-Arg-Thr-Arg to Ser-Thr-Arg-Thr-Thr. Patient no. 36 has a point mutation consisting of a guanine to thymine substitution at nucleotide 912 of the sense

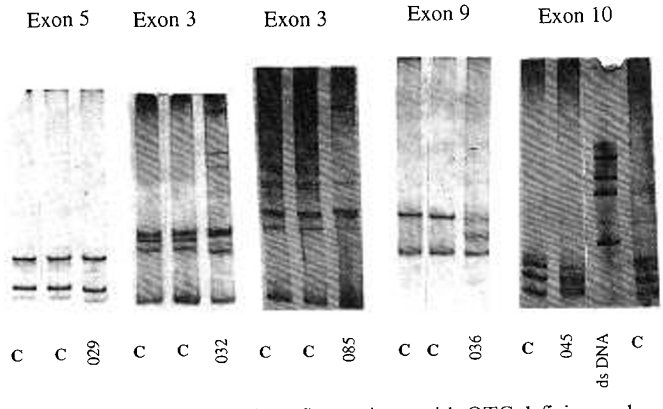

Fig. 1. SSCP analysis of the five patients with OTC deficiency showing abnormal migrations of single strands of amplified DNA derived from various exons of the OTC gene. Shown also is DNA from patients with normal migration patterns (*c*). Patient no. 45, who was a female heterozygous for OTC deficiency, has four single-strand bands. DNA samples were denatured and electrophoresed on a polyacrylamide gel containing glycerol. Visualization was achieved by silver staining.

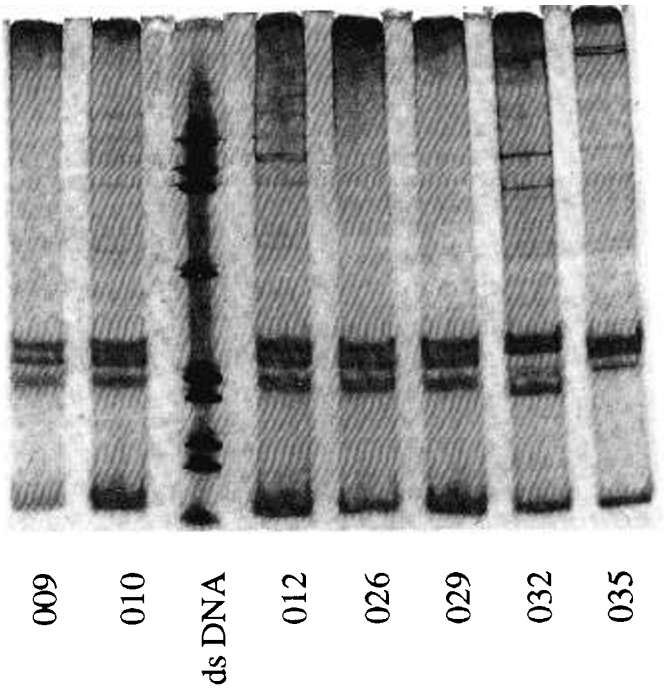

Fig. 2. SSCP analysis showing exon 3 of the OTC gene amplified from genomic DNA of seven male OTC-deficient patients. Patient no. 32 and patient no. 35 show different migration patterns compared to the other five patients whose migration patterns are considered to be normal. The "double-stranded DNA" control is nondenatured Phi-X 174 DNA.

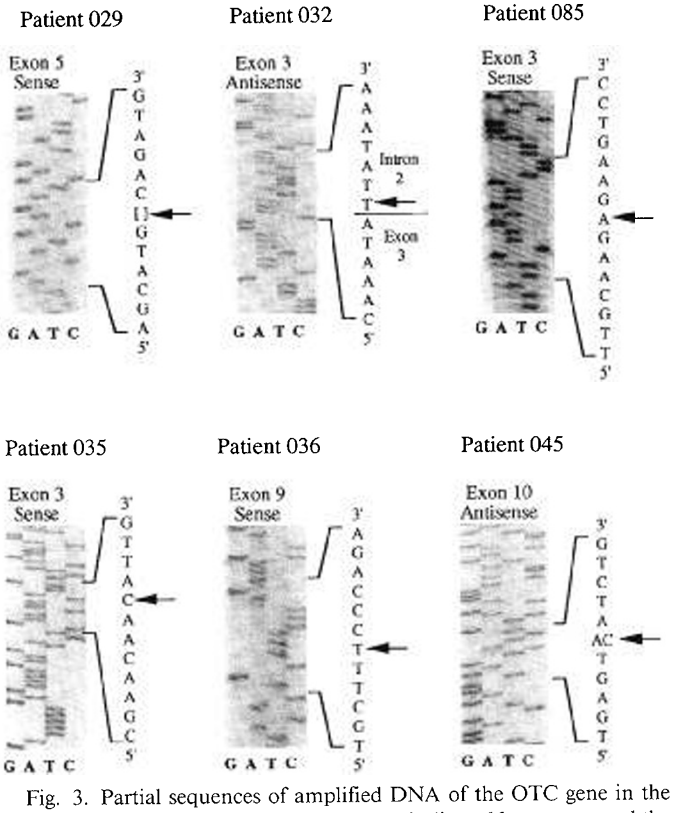

Fig. 3. Partial sequences of amplified DNA of the OTC gene in the six patients described herein. Mutations are indicated by *arrows*, and the *empty brackets* indicate a single base deletion in patient no. 29. Sequencing was performed by the dideoxy termination method using denatured double-stranded DNA and one of the PCR primers.

Table 1. *Mutations identified in six patients with OTC deficiency*

Patient no.	Exon no.	Base no. and mutation	Change
29	5	403, deletion of G	Frame-shift error
32	Intron 2	217 (−1), G to A	Acceptor splice site error
35	3	281, G to C	Arginine 62 to threonine
36	9	912, G to T	Leucine 272 to phenylalanine
85	3	236, G to A	Glycine 47 to glutamic acid
45	10	1033, T to G	Tyrosine 313 to aspartic acid

strand, changing the code for leucine to phenylalanine in position 272 of the mature OTC enzyme. This mutation is also likely to be deleterious because it changes a conserved domain of the gene that probably encodes the ornithine binding site, altering the amino acid sequence Phe-Leu-His-Cys-Leu-Pro to Phe-Leu-His-Cys-Phe-Pro. Patient no. 45 also had a point mutation consisting of a thymine to guanine substitution at nucleotide 1033, changing the code from tyrosine to aspartic acid at position 313 of the enzyme.

Not shown in the figures is an apparently common polymorphism detected in exon 8 of other patients with OTC deficiency that we have studied. An adenine or guanine in base 809 determines the code for a glutamine or an arginine in position 238 of the mature OTC protein. In the human cDNA sequence published by Horwich *et al.* (12), a CGA sequence codes for arginine, whereas Hata *et al.* (13) report a CAA sequence coding for glutamine. We sequenced exon 8 in two patients and found each to contain one of these polymorphisms.

DISCUSSION

Six new mutations in patients with OTC deficiency are described here. Two of the three mutations found in patients with acute neonatal presentation readily explain the lack of OTC activity in their livers. In patient no. 29, a single base deletion in exon 5 would cause a shift in the reading frame resulting in a premature termination of translation. To the best of our knowledge, this is the only single base deletion in the OTC gene reported. Patient no. 32 had a mutation in the acceptor splice site at the 3′ end of intron 2 altering the AG dinucleotide. This is likely to result in an mRNA missing all or part of the exon 3 sequence. It may therefore result in enhanced degradation of the mRNA or would encode an abnormal OTC protein if translation takes place. We were fortunate to find this particular mutation because the sequences of several of the primers used for amplification of other exons cover splicing recognition sites. Carstens *et al.* (7) reported one deletion and two mutations affecting mRNA splicing in patients with OTC deficiency. One of the mutations at the AG 3′ splice acceptor site of intron 4 resulted from an adenine to thymine substitution. Their postulation that abnormal RNA splicing may be one of the mechanisms causing OTC deficiency is strengthened by a similar mutation affecting an adjacent guanine in patient no. 32.

In contrast to the above two mutations, where causality of OTC deficiency can be established almost with certainty based on the underlying mutation, the mutation found in patient no. 85 may or may not be the deleterious mutation. The amino acid change affected by this mutation involves the substitution of a neutral amino acid, glycine, in position 47 of the mature human enzyme, with the acidic amino acid, glutamic acid. If such a substitution occurs in a crucial domain of the enzyme, it will be deleterious. The glycine in this position is conserved in rat and in *Aspergillus nidulans* but not in *Escherichia coli*, according to one report (14), whereas a second report (15) lists this glycine as conserved also in *E. coli*. The sensitivity of SSCP analysis in detecting abnormal DNA sequences is variable depending on the structure of the strand and the mutation involved; therefore, an undetectable mutation may exist in addition to a polymorphism. In the absence of other OTC-deficient patients with an identical mutation, expression studies of this mutated gene will need to be performed to establish its effect on the function of the OTC protein and rule out a polymorphism at this site.

Patient no. 35 had a mutation consisting of a guanine to cytosine transversion in the sense strand, resulting in arginine replacement by threonine at amino acid 62 of the mature protein. This changes the highly conserved motif of Ser-Thr-Arg-Thr-Arg (16) to Ser-Thr-Arg-Thr-Thr. It is very likely that this region of the protein is part of the active site responsible for binding the phosphate moiety of carbamyl phosphate. Mammalian and bacterial aspartate transcarbamylase, a key enzyme of *de novo* pyrimidine synthesis that also uses carbamyl phosphate as a substrate, shares an identical conserved gene domain (17). Our studies in this patient show that this mutation greatly reduces but does not abolish enzymatic activity. It is not known if this mutation confers instability to the formation of the active homotrimer enzyme complex or if the enzyme is more susceptible to protein degradation as a result of the mutation. From the Western blot data published previously (18), it seems that one or both are likely.

Patient no. 36 has OTC deficiency with onset of clinical problems after the neonatal period. This is associated with the presence of OTC residual enzymatic activity of about 3% of normal in his liver. The mutation in this patient is a guanine to thymine substitution in nucleotide 912 of the sense strand, changing the code for leucine to phenylalanine in position 272 of the mature OTC protein. This changes a motif conserved across several species (16), including yeast, *Aspergillus*, *E. coli*, and rat (14), that is likely to be the ornithine binding site from Phe-Leu-His-Cys-Leu-Pro to Phe-Leu-His-Cys-Phe-Pro. The adjacent cysteine residue within this motif is rare in the mature OTC enzyme and is probably important for ornithine binding (19). Thus, we found two patients with late onset disease to have mutations altering the binding sites for ornithine and carbamylphosphate, respectively. In light of the very low *in vitro* enzymatic activities in both patients, it seems remarkable that the patients did not exhibit a more severe degree of clinical symptoms earlier in life. We have studied the enzymatic activity in liver samples from males with late onset OTC deficiency and found very low residual activity levels (20). At this time, it is not known what *in vivo* levels of OTC activity are necessary for adequate removal of ammonia during different catabolic states.

Patient no. 45 was heterozygous for a new mutation changing a tyrosine in position 313 to aspartic acid. It is uncertain whether this mutation is the deleterious one responsible for that patient's disease. This tyrosine residue is conserved in the rat but not in bacteria (14, 15). In families such as this, where mutations are found and polymorphisms need to be ruled out, finding that the mutation occurred spontaneously in an affected family would improve the chance that the mutation is in fact deleterious.

In this report, we describe a simple and effective way for defining unknown mutations in patients with OTC deficiency. Though SSCP analysis is not 100% sensitive for detecting base changes in DNA (21), the procedure makes it possible to locate candidate exons for direct sequencing. SSCP also makes it possible to test family members of patients with OTC deficiency whose mutation was defined by the method described here. Most female carriers of OTC deficiency are completely asymptomatic unless they are unfavorably lyonized. Biochemical carrier testing, including the allopurinol challenge test, is not reliable enough to make decisions involving prenatal diagnosis. Therefore, it is important to define the deleterious mutation causing OTC deficiency in every family in which restriction fragment length polymorphism analysis is not relevant (only one affected individual and unclear carrier status) or that is uninformative for restriction fragment length polymorphism analysis. We believe that the approach described in this report makes it possible to

improve molecular diagnosis in families of which a member has OTC deficiency.

Acknowledgment. The authors thank Dr. Marshall Summar, Dr. Joel Charrow, Dr. Ellen Roback, and Dr. Mark Luquette for sending samples of their patients to our laboratory.

REFERENCES

1. Brusilow SW, Horwich AL 1989 Urea cycle enzymes. In: Scriver CR, Beaudet AL, Sly WS, Valle D (eds) The Metabolic Basis of Inherited Disease. McGraw-Hill, New York, pp 629–663
2. Nagata N, Matsuda I, Oyanagi K 1991 Estimated frequency of urea cycle enzymopathies in Japan. J Med Genet 39:228–229
3. Maddalena A, Edward SJ, O'Brien WE, Nussbaum RL 1988 Characterization of point mutations in the same arginine codon in three unrelated patients with ornithine transcarbamylase deficiency. J Clin Invest 82:1353–1358
4. Grompe M, Caskey CT, Fenwick RG 1991 Improved molecular diagnostics for ornithine transcarbamylase deficiency. Am J Hum Genet 48:212–222
5. Hentzen D, Pelet A, Feldman D, Rabier D, Berthelot J, Munnich A 1991 Fatal hyperammonemia resulting from a C to T mutation at a *Msp*I site of the ornithine transcarbamylase gene. Hum Genet 88:153–156
6. Finkelstein JE, Francomano CA, Brusilow SW, Traystman MD 1990 Use of denaturing gradient gel electrophoresis for detection of mutation and prospective diagnosis in late onset ornithine transcarbamylase deficiency. Genomics 7:167–172
7. Carstens RP, Fenton WA, Rosenberg LR 1991 Identification of RNA splicing errors resulting in human ornithine transcarbamylase deficiency. Am J Hum Genet 48:1105–1114
8. Suess PJ, Tsai MY, Holzknecht RA, Horowitz M, Tuchman M 1992 Screening for gene deletions and known mutations in 13 patients with ornithine transcarbamylase deficiency. Biochem Med Metab Biol 47:250–259
9. Tuchman M, Tsai MY, Holzknecht RA, Brusilow S 1989 Carbamyl phosphate synthetase and ornithine transcarbamylase activities in enzyme-deficient human liver measured by radiochromatography and correlated with outcome. Pediatr Res 26:77–82
10. Orita M, Iwahana H, Kanazawa H, Hayashi K, Sekiya T 1989 Detection of polymorphisms of human DNA by gel electrophoresis as single-strand conformation polymorphisms. Proc Natl Acad Sci USA 86:2766–2770
11. Orita M, Suzuki Y, Sekiya T, Hayashi K 1989 Rapid and sensitive detection of point mutations and DNA polymorphisms using the polymerase chain reaction. Genomics 5:874–879
12. Horwich AL, Fenton WA, Williams KR, Kalousek F, Kraus JP, Doolittle RF, Konigsberg W, Rosenberg LE 1984 Structure and expression of a complementary DNA for the nuclear coded precursor of human mitochondrial ornithine transcarbamylase. Science 224:1068–1074
13. Hata A, Tsuzuki T, Shimada K, Takiguchi M, Mori M, Matsuda I 1988 Structure of the human ornithine transcarbamylase gene. J Biochem 103:302–308
14. Huygen R, Crabeel M, Glansdorff N 1987 Nucleotide sequence of the ARG3 gene of the yeast *Saccharomyces cerevisiae* encoding ornithine carbamoyltransferase: comparison with other carbamoyltransferases. Eur J Biochem 166:371–377
15. McIntyre P, Graf L, Mercer JFB, Wake SA, Hudson P, Hoogenraad N 1985 The primary structure of the imported mitochondrial protein, ornithine transcarbamylase from rat liver: mRNA levels during ontogeny. DNA 4:147–156
16. Kraus JP, Hodges PE, Williamson CL, Horwich AL, Kalousek F, Williams KR, Rosenberg LE 1985 A cDNA clone for the precursor of rat mitochondrial ornithine transcarbamylase: comparison of the rat and human leader sequences and conservation of catalytic sites. Nucleic Acid Res 13:943–952
17. Krause KL, Volz KW, Lipscomb WN 1987 2.5 Å structure of aspartate carbamoyltransferase complexed with bisubstrate analog N-(phosphonacetyl)-L-aspartate. J Mol Biol 193:527–553
18. Zhang W, Holzknecht RA, Tuchman M 1990 Immunochemical analysis of carbamyl phosphate synthetase I and ornithine transcarbamylase deficient livers: elevated N-acetylglutamate level in CRM negative carbamyl phosphate synthetase I deficiency. Clin Invest Med 13:183–188
19. Marshall M, Cohen PP 1980 The essential sulfhydryl group of ornithine transcarbamylases. Reaction with anionic, aromatic disulfides and properties of its cyano derivative. J Biol Chem 225:7291–7295
20. Tuchman M, Holzknecht RA 1991 Heterogeneity of patients with late onset ornithine transcarbamylase deficiency. Clin Invest Med 14:320–324
21. Hayashi K 1991 PCR-SSCP: a simple and sensitive method for detection of mutations in the genomic DNA. PCR Methods and Applications 1:34–38