Cystic Fibrosis Deletion Mutation Detected by
PCR-Mediated Site-Directed Mutagenesis, Kenneth J.
Friedman, William E. Highsmith, Jr., Thomas W. Prior,
Teny R. Perry, and Lawrence M. Silverman (Dept. of
Lab. Med., Division of Molecular Pathol., 1071 Patient
Support Tower, North Carolina Memorial Hoep., Univ. of
North Carolina, Chapel Hill, NC 27599)

Cystic fibrosis (CF) is an autosomal recessive disorder
with an incidence of approximately 1 in 2000 live births
and a carrier frequency of 1 in 20 (1). The disease locus,
initially mapped to 7q31 (2), was recently identified as a
gene spanning 250 000 base pairs (bp) that codes for a
protein of 1480 amino acids (3). Approximately 70% of CF
mutations involve a deletion of 3 bp in exon 10, resulting in
the loss of a phenylalanine at position 508 in the gene
product (4). Because this mutation neither creates nor
destroy a restriction enzyme cutting site, it is currently
being detected with radiolabeled allele-specific oligonucleo-
tides (4). We report a rapid, nonisotopic technique to
detect chromosomes carrying this deletion.

Genomic DNA is extracted from leukocytes (5), and an
86-bp portion of the CF gene encompassing the deletion is
amplified by use of the polymerase chain reaction (PCR)
(6). The oligonucleotide primer sequences are 5'GCACCAT
AAGAGAATATCCT3' (forward) and 5'TCTTCACTTGGCACTGCT3' (reverse). The reverse primer is complementary to the 3'-end of exon 10. The
3'-end of the forward primer overlaps the deletion locus
and includes a single-base mismatch designed to alter the
sequence of the amplified product (7). The PCR-mediated
site-directed mutagenesis gives rise to products carrying a
novel restriction site for the enzyme MnII; this restriction site
is associated with the wild-type allele but not with the
CF mutant allele (Figure 1).

After amplification, the product is digested with MnII
and electrophoresed on polyacrylamide. Healthy, noncar-
rier individuals exhibit 56- and 30-bp fragments, whereas
affected individuals homozygous for this deletion show a
single 83-bp fragment, which lacks the MnII restriction
site. Heterozygotes for the deletion exhibit all three bands
plus a heteroduplex fragment, formed by the re-annealing
of 86- and 83-bp amplification products, migrating at ~100
bp (8). The heteroduplex band serves as a second indicator
of heterozygosity and as a control for denaturing under-
digestion (Figure 2).

About half of the individuals with CF are homozygous for
the phenylalanine deletion. Of the remaining half, 80%
will be compound heterozygotes between the phenylala-
nine deletion and less-prevalent mutations. The ability to
rapidly detect the deletion in these affected heterozygotes
will reduce the dependence on linkage analysis in family
studies, although linkage studies will still be required for
the families with affected individuals who lack the identi-
died deletion.

This test has applicability to prenatal testing, newborn
screening, and carrier detection analysis. For use in a
clinical context, the sensitivity of PCR analysis necessi-
tates rigorous adherence to procedures that will minimize

Wild-type allele: ...ATCATCTTTGGTGT... 3'
3' end of primer: ...ATCCT

PCR product: ...ATCCTTTGGTGT...

Novel MnII restriction site

CF mutant allele: ...ATCATTGGTGT...
3' end of primer: ...ATCCT

PCR product: ...ATCCTTTGGTGT...

Absence of MnII restriction site

Fig. 1. Strategy of deletion detection
Arrows indicate location of nucleotide mismatch. The horizontal bar defines the
cystic fibrosis deletion mutation. The enzyme MnII has the recognition sequence CTCCT(N)7.

Fig. 2. Use of PCR to detect the CF deletion mutation
DNA was amplified as described in text. For PCR we used 35 cycles, each
cycle consisting of 2 min of annealing at 50 °C, 1 min of extension at 72 °C, and
1 min of denaturation at 94 °C. Amplification products were digested overnight
with 4.5 U of MnII and electrophoresed on 12% polyacrylamide for 2 h at 150
V. Lane 1: blank. Family 1 (left): lane 2, CF patient homozygous for the
phenylalanine deletion; lanes 3, 4, heterozygote carriers for the deletion.
Family II (right): lane 5, affected individual heterozygous for the deletion; lane
6, carrier lacking the deletion; lane 7, heterozygote carrier for the deletion.
Lane 8: Haelll-digested φX174 DNA size marker. The faint band migrating at
45 bp is a nonspecific amplification product.
the possibility of misdiagnoses attributable to contamination (9).

References

Carbonic Anhydrase III as a Serum Marker for Diagnosis of Rhabdomyolysis, H. Syrjäla,1 J. Vuori,2 K. Huttunen,1 and H. K. Väänänen2 (Depts. of 1 Internal Medicine and 2 Anatomy, Univ. of Oulu, Kajaanintie 52, SF-90220 Oulu, Finland; 2 Deaconess Institute of Oulu, SF-90100 Oulu, Finland)

Creatine kinase (CK; EC 2.7.3.2) and myoglobin are generally used to demonstrate skeletal muscle damage in rhabdomyolysis. Carbonic anhydrase III (carbonate dehydratase; EC 4.2.1.1), a skeletal-muscle-specific protein not present in myocardium (1, 2), could be a practical and more-specific marker than CK and myoglobin for diagnosis of rhabdomyolysis.

In a prospective study we determined the serum carbonic anhydrase III (S-CA III), serum creatine kinase (S-CK), and serum myoglobin (S-myoglobin) values of 16 patients with rhabdomyolysis. S-CK was measured with Boehringer's creatine kinase UV-test kit (Boehringer GmbH, Mannheim, F.R.G.), S-myoglobin with a commercial radioimmunoassay kit (Cis, St-Quentin Yvelines Cedex, France), and CK isoenzymes were determined by a standard electrophoretic method. S-CA III was determined by a specific radioimmunoassay described previously in detail (3). All analytes were measured from the same serum sample.

The S-CA III concentration of rhabdomyolysis patients ranged from 0.17 to 13.2 mg/L, the mean (±SD) value being 2.51 ± 3.59 mg/L; in 74 healthy controls this was 0.015 ± 0.011 mg/L. Among the 16 patients with rhabdomyolysis, the mean (±SD) S-CK value was 25 200 ± 31 600 U/L, and that of S-myoglobin was 13.5 ± 16.6 mg/L. These patients had a high correlation between the S-CA III and S-CK values (r = 0.85, P <0.0001).

Pathological values for the CK-MB fraction were detected in six of the 16 patients, ranging from 40 to 1732 U/L in these six. Correlation between S-CA III and S-myoglobin is displayed in Figure 1. Changes in S-CA III and S-myoglobin were followed in twice-a-day sampling from two patients for six days after onset of muscle pain. The concentrations decreased linearly with time.

Because we have found that CA III is a more-specific marker of skeletal muscle than are myoglobin and CK (Clin Chim Acta 1990;36:635–8; this issue), we consider determination of the S-CA III concentration a practical method for demonstrating skeletal muscle damage in rhabdomyolysis patients.

References