Mutation Report

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1283 del A: A Novel Mutation in Exon 8 of the Cystic Fibrosis Gene

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Description of the Mutation

We report here a novel frameshift mutation in exon 8 of the cystic fibrosis (CF) trans-membrane conductance regulator (CFTR) gene. The mutation is a 1-base (A) deletion at position 1283.

Source of Material

A couple of French origin with a history of 2 children with meconium ileus (born in 1956 and 1957, at a time when no information on sweat test, immunoreactive trypsin assay, or albumin meconium test was available) who died within months of birth was referred for genetic counselling for the benefit of a healthy daughter. Parental DNA samples were subjected to CFTR gene analysis. The mother was $\Delta F508$ heterozygous. The father and his daughter were heterozygous for a new frameshift mutation in exon 8 of the CFTR gene. The two CF patients were probably compound heterozygous: $\Delta F508/1283 \text{ delA}$ (fig. 1). DNA of these two patients was not collected forty years ago.

Method

In our screening strategy for mutations in the CFTR gene, we have developed conditions for denaturing gradient gel electrophoresis (DGGE) analysis. DGGE was performed using chemical clamps instead of a long GC sequence attached to the 5' end of one of the primers. PCR was performed in a volume of 50 μl containing 50 mM Tris-HCl pH 8.3, 2.5 mM $MgCl_2$, 1 mM of all four deoxynucleotides together, 0.5 mM each of the primers (upstream 8i5 5'-ATTAATGC-TATTCTGATTCT3' and downstream primer DG8i3 5'-psoralen-CAGTTAGGTGTTTAGAGCAAAC3'; Appligène Oncor), 2.5 U Taq polymerase (Perkin-Elmer Cetus) and 200 ng of genomic DNA. Samples were heated at 94°C for 7 min and at

55 °C for 1 min. Afterwards, forty cycles were performed with denaturation at 94°C for 30 s, annealing at 55°C for 30s, and extension at 72°C for 30 s. DNA synthesis step at 72°C of the final cycle was extended to 5 min. The amplified DNA was irradiated for 10 min at room temperature using a UV source (365 nm) composed of two 6-watt lamps 1 cm above the sample (approximately 1 J/cm²). After irradiation, one fifth of the amplified product was loaded on a 6% polyacryl-amide gel with a linearly increasing parallel gradient

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Mutant TAT AAG ACA TTG GAT ATA ACT TAA

Tyr Lys Thr Leu Asp Ile Thr Stop

H H ACN NACC

^a 1/8 j j j j j y 1/8 j

Wild-type TAT AAG ACA TTG GAA TAT AAC TTA ACG Tyr Lys Thr Leu Glu Tyr Asn
Leu Thr

Fig. 1. Fluorescence sequence analysis of exon 8 of the CFTR gene. A Sequence of exon 8 of the CFTR gene from the subject showing one base deletion at position 1283. The arrow indicates the position of the mutation (heterozygous individual). B Wild-type sequence of exon 8 of the CFTR gene.

from 10 to 60% denaturant under 180 V 6.5 h [1]. PCR products were sequenced with Taq polymerase and Dye Terminators using protocols supplied by the manufacturer on an Applied Biosystems 377 DNA sequencer.

Frequency

This mutation was found once in 200 non-ΔF508 CF chromosomes from unrelated French CF patients and not at all in 100 normal chromosomes from parents and spouses of CF patients screened. In the French popu-

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lation, the identified mutations account for approximately 92% of all CF chromosomes [2].

Comments

Since the identification of the CFTR gene, more than 600 mutations have been reported to the Cystic Fibrosis Genetic Analysis Consortium in addition to the major mutation (ΔF508).

Only one mutation in exon 8 has been published: W401X (G→A at 1335) [3]. In the analysis of exon 8 and flanking regions,

we detected one sample with an abnormal DGGE migration pattern. Direct sequencing showed a deletion of A at position 1283 (fig. 1). The sequence alteration results in shifting of the reading frame, and introduces a stop codon (UAA) at position 387. This single-base deletion does not occur in a known restriction site.

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