MUTATION IN BRIEF

Novel Missense Mutation (G314R) in a Cystic Fibrosis Patient With Hepatic Failure

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INTRODUCTION

Cystic fibrosis is caused by mutations in the gene encoding the cystic fibrosis transmembrane conductance regulator (CFTR) (Kerem et al., 1989; Riordan et al., 1989; Rommens et al., 1989). The clinical manifestations of cystic fibrosis are thought to be related to abnormal chloride conductance due to defective synthesis, processing, or function of CFTR chloride channels (Welsh, 1990; Drumm et al., 1991; Collins, 1992; Welsh et al., 1992; Riordan, 1993). We have identified a novel missense mutation in a native-American/Caucasian patient in exon 7 of the CFTR, by chemical mismatch cleavage of cDNA obtained through reverse transcription of mRNA from nasal scraping.

MATERIALS AND METHODS

The subject is a 13-year-old female. Her ethnic background includes Dutch/French from the paternal side and native-American (Cherokee)/Caucasian from the maternal side. She was diagnosed with CF at 3 weeks of age, with a sweat chloride concentration of 107 mEq/liter. She is pancreatic insufficient. Her height is 142 cm (4% for age) and weight is 36 kg (25% for age). Pulmonary function testing has revealed FVC of 1.83 liters (81% of predicted), and FEV1 of 1.47 liters (73% of predicted). She recently developed intrahepatic biliary cirrhosis and liver failure and is awaiting a liver transplant.

Detection of AF508 Mutation by Reversed Dot-Blot Assay was done (Saiki et al., 1989). The patient considered here was identified through this technique as AF508/unknown. Detection of the unknown mutation was done by analysis of CFTR mRNA. Nasal epithelial tissue was obtained from the patient and a normal subject by nasal scrape as previously described (Strong et al., 1992). RNA was extracted from nasal epithelial cells using RNAzol B (Tel Test), according to manufacturer’s specifications. PCR amplification spanning the codon region of CFTR cDNA was performed through amplification of five overlapping Polymerase Chain Reactions (PCR) using CFTR primers (Table 1). The patient was found to be heterozygous for ΔF508 at the RNA level. Standard conditions were used for all PCRs (Sambrook et al., 1989) RT/PCR products were visualized in a 1% agarose gel. Chemical mismatch cleavage (CMC) was done as previously described (Strong et al., 1991) based on a modification of the published technique (Cotton et al., 1988; Grompe et al., 1989).

The cDNA fragment determined to have a mutation through the chemical mismatch cleavage was amplified, gel purified, and directly sequenced based on the method of Kretz et al. (1989) using the Sequenase 2.0 Kit (U.S. Biochemical Corp). CFTR exon 7 was amplified from genomic DNA from the patient, her parents, 25 normal chromosomes, and 25 CF chromosomes by PCR with exon 7 specific primers: 5’AGACCATGCTCAGATCTCCCAT 3’ and 5’GATCAGTTCTAATGACTTGGCA 3’. The PCR product was digested with Ddel and visualized on a 12% nondenaturing polyacrylamide gel.

The effect of the G314R mutation on Cl channel function was investigated by expressing the altered protein in Xenopus oocytes. The methodology has

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been described in detail (Drumm et al., 1991; Smit et al., 1993). Expression assays were conducted under conditions previously shown to give rise to robust expression of CFTR-mediated Cl-currents in oocytes injected with mRNA transcribed from wild-type CFTR or mutants such as ΔF508.

RESULTS

A mismatch was detected in the region of exons 1–7 (data not shown). The 1130-bp fragment was sequenced, and the substitution of C for G was detected at 1072 bp. This substitution resulted in the change of arginine for glycine at position 314 (G314R), which, according to the predicted topology of CFTR, lies in the 5th putative membrane spanning segment. The substitution of arginine for glycine at 314 results in elimination of a Ddel site. Restriction digest analysis revealed that the patient is heterozygous for this mutation. To determine if this substitution was found in additional normal chromosomes or CF chromosomes, DNA from our panel of CF parents was amplified and restriction analysis was performed. Of 25 normal chromosomes and 25 CF chromosomes screened, none were identified as having this mutation. The purpose of the screening was to detect if this mutation occurs in high frequency. To our knowledge, this mutation has not been identified in other CF patients.

To determine if this mutation is maternal (native-American (Cherokee)/Caucasian) or paternal (Caucasian) in origin, DNA samples from both parents were obtained. Exon 7 of the patient and her parents' DNA was amplified and restriction digest analysis with Ddel was performed. The G314R mutation was identified as maternal (Fig. 1). The ΔF508 mutation was shown to be paternal (data not shown). It would have been of interest to obtain DNA samples from the maternal grandparents to track further the ethnic background of the mutation; however, both of them are deceased.

A total of 35 oocytes from 10 different frogs were assayed in 10 different rounds of injection with G314R CFTR. Of these only two exhibited a detectable response (80 nA as compared to >800 nA for wt) to the application of forskolin and IBMX. The response of one of these to forskolin and IBMX is shown in Figure 2. In contrast, oocytes that were injected with mRNA transcribed from the wt construct exhibited robust responses to the application of 10 μM forskolin and 5 mM IBMX. Because the results with G314R were largely negative, we also compared cAMP-induced Cl currents in oocytes expressing CFTRs in which more conservative substitutions were made at the same position. CFTR constructs bearing either the G314A or G314E substitution were associated with readily discernable cAMP-induced Cl currents. The G314E substitution has been associated with cystic fibrosis (Golla et al., 1994).

DISCUSSION

Cystic fibrosis is characterized by viscous secretions in the tracheobronchial tree and pancreas leading to chronic pulmonary diseases and pancreatic insufficiency in most cases. Diagnosis is made by abnormal sweat electrolytes in most cases (Boat et al., 1989).

In this report we describe a patient with a nucleotide substitution in exon 7 in one CFTR allele and ΔF508 in the other allele. This mutation was not found in 25 normal chromosomes and 25 CF
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chromosomes. The substitution of G to C results in a glycine to arginine substitution at amino acid 314 (G314R). This mutation is in the fifth putative hydrophobic transmembrane segment and results in the addition of a positive charge and a bulky side chain. The mutation is associated with severe disease and liver failure in our patient. The mutation, being maternal in origin, could be specific to the Cherokee CF population.

Expression of CFTR constructs bearing the
G341R mutation in Xenopus oocytes provided evidence that this substitution severely compromises the CI channel function of CFTR. Cyclic AMP-activated CI currents were only barely detectable with this construct, whereas wt and ΔF508 CFTR, as well as variants bearing more conservative substitutions at the same site (G314A and G314E), were associated with the expression of significant CI channel function.

Reduced expression of CFTR CI channel function for G341R could arise in at least two ways that are not mutually exclusive. The mutation could result in defective processing of the protein so that the amount of CFTR reaching the plasma membrane is reduced (Cheng et al., 1990). The mutation could also compromise the channel function by altering either the conduction and/or the gating properties of the CFTR CI channels (Drumm et al., 1991; Sheppard et al., 1993). The data presented here do not distinguish between these alternatives, but it is noteworthy that ΔF508 CFTR, which is not delivered to the plasma membrane of mammalian cells at 37°C, nevertheless gives rise to robust CI channel activity in Xenopus oocytes (Drumm et al., 1991).

The location of the mutation in the predicted membrane spanning domain and the fact that it adds a net positive charge raises the possibility that the conduction properties of the channel could be compromised as shown previously for other mutations in this domain (Drumm et al., 1991; Sheppard et al., 1993).

Regardless of the mechanism, the combination of genetic and functional data presented here leaves little doubt that the G341R mutation is the cause of near total absence of CFTR CI function and severe clinical disease in this patient.

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REFERENCES


