A novel mutation in exon 3 of the CFTR gene

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Received: 20 December 1991 / Revised: 28 July 1992

Abstract. We have screened the 27 exons of the cystic fibrosis transmembrane conductance regulator gene in 87 non-AF508 chromosomes of Breton origin using the combined techniques of denaturing gradient gel electrophoresis and direct sequencing. By this process, we have detected a new missense mutation, G91R, which results in an arginine for glycine at codon 91. Three affected patients with a ΔF508/G91R genotype are pancreatic sufficient. Such observations could facilitate a better understanding of the functional importance of different regions of the encoded product and of the pathogenesis of the disease.

Introduction

The cystic fibrosis (CF) gene was cloned and sequenced 3 years ago. Its 27 exons span 230 kb on the long arm of chromosome 7. The entire coding sequence with the immediate flanking regions have been determined (Kerem et al. 1989; Riordan et al. 1989; Rommens et al. 1989; Zielenski et al. 1991a). Comparative studies of the encoded 1480 amino acid protein, the cystic fibrosis transmembrane regulator (CFTR), have shown that it shares a striking homology with the P glycoproteins, a family of proteins involved in multidrug resistance. CFTR consists of five domains: two transmembrane domains and two ATP-binding domains (nucleotide binding folds; NBFs) that are separated by a regulatory domain (R domain). It now seems evident that CFTR is a chloride channel regulated by cAMP (Gregory et al. 1990; Cheng et al. 1991; Kartner et al. 1991; Rommens et al. 1991).

A deletion of phenylalanine at position 508 of the CFTR gene accounts for about 70% of the CF chromosomes so far tested, this percentage varying slightly with geographic location between ethnic groups (Worldwide Survey 1990). Over 250 different mutations have been identified in the CFTR gene by the Cystic Fibrosis Genetic Analysis Consortium. Most of these mutations, including missense, nonsense, frameshift or mutations affecting splicing, are located in the two NBFs of the CFTR (Cutting et al. 1990; Dean et al. 1990; Férec et al. 1992; Fanen et al. 1992; Guillermit et al. 1990; Kerem et al. 1990; Vidaud et al. 1990; Zielenski et al. 1991b). We have undertaken a systematic analysis of our CF chromosomes using denaturing gradient gel electrophoresis (DGGE) (Lerman and Silverstein 1987; Myers et al. 1987; Sheffield et al. 1989) followed by direct sequencing (Sanger et al. 1977) of the exons of interest. This process has led us to identify a novel mutation in exon 3 of the CFTR gene of three patients manifesting pancreatic sufficiency.

Materials and methods

We initiated a systematic analysis of the CF chromosomes carrying an unidentified mutation in our cohort of CF families from Brittany (Celtic origin). We screened the 27 exons and the corresponding intron/exon boundaries of each of these chromosomes. Initially, all our CF chromosomes were analysed for the presence or absence of the AF508 deletion using polymerase chain reaction (PCR) amplification of exon 10 followed by electrophoresis using a polyacrylamide gel analysis technique (Rommens et al. 1990). CF patients carrying at least one non-ΔF508 chromosome were screened for mutations in the 27 exons of the gene. A two-step analysis was carried out: (1) DGGE, which allowed us to detect PCR products with altered mobilities in the gel (Lerman and Silverstein 1987; Myers et al. 1987; Sheffield et al. 1989); (2) direct sequencing of these products to identify any mutations (Sanger et al. 1977).

DGGE

DNA amplification was performed as previously described (Guillermit et al. 1990). Briefly, genomic DNA (500 ng–1 μg) was subjected to amplification (Saiki et al. 1988) in a 100-μl reaction mixture containing 50 mM KCl, 10 mM TRIS-HCl (pH 8.3), 1.5 mM MgCl2, 200 μM of each dNTPs, 50 pmoles of each primer and 1U Taq polymerase. The primer amplification sequences were 3'5': ATT CAC CAG ATT TCG TAG TC 3' and 3'5': CGC CCG CCG CGC CCC GCG CCC GTC CCG CCG CCC CCG CCT TGG GTT AAT CTC CTT CGA 3'. Thirty cycles were performed (1-min denaturation at 94°C, 30-s annealing at 55°C and 2-min extension at 72°C). Samples were then loaded onto a 6.5% polyacrylamide gel with a linearly increasing gradient from 0% to 50% [100% denaturant = 7 M urea, 40% formamide, acrylamide/bisacrylamide 40:2] at 75 V for 7 h. After electrophoresis, the gels were stained with ethidium bromide and photographed under UV transillumination. PCR fragments that displayed altered mobilities in the gel were subsequently sequenced.
**Direct sequencing of amplified DNA**

Single-stranded DNA was obtained by asymmetric PCR by using the unbalanced method of Gyllensten and Erlich (1988); this technique generates a single-stranded product suitable for sequencing. One of the two primers used for the initial amplification was reduced 50-fold and 30 cycles were performed. The single-stranded product was then concentrated using Centricon 30 (Amicon, Danvers, Mass.) and 7μl of the reaction product was used for sequencing by the dideoxy nucleotide chain termination method (Sanger et al. 1977). The 3′ primer was used as sequencing primer, with the Sequence enzyme (Tabor and Richardson 1987).

**Results**

By screening exon 3 in 87 non-ΔF508 chromosomes, we have detected two fragments with altered patterns of migration on the gel. The two homoduplexes and heteroduplexes are clearly separated (Fig. 1). The homoduplex of the mutated allele is situated at a higher position in the denaturing gel. Direct sequencing of these PCR products shows that a G has been replaced by an A at nucleotide position 403, thereby producing an arginine instead of a glycine at codon 91 (G91R) (Fig. 2). This result is consistent with the DGGE pattern of migration, because a less stable homoduplex is present in the mutated sequence, viz. the nucleotide at position 403 is an A (2 hydrogen bonds) in the mutated sequence, instead of a G (3 hydrogen bonds) as found in the normal sequence.

The missense point mutation occurs in an exon encoding part of the first transmembrane domain of the CFTR molecule. G91R was found on two chromosomes among the 87 analysed and was associated with haplotype C in both cases (KM19 = 1; XV2c = 2). Three of our patients displayed this genotype, as one of the above cases had an affected sibling. These three individuals are compound heterozygotes bearing ΔF508 on the other chromosome. A genealogical study of these two families (Chaventré et al. 1991) has allowed us to identify a common ancestral chromosome 7 generations earlier. This is consistent with haplotype data that indicates that the chromosomes of the two families are identical by descent. The two brothers of 4 years and 1 year old, respectively, displayed pancreatic sufficiencies with steatorrhea values of 2.2g/24h and 3.1g/24h, respectively. The other affected child was 12 years old. He was also pancreatic sufficient, but unfortunately recent steatorrhea values were not available. Neither meconium ileus at birth nor cirrhosis were detected in these three patients.

**Discussion**

The major mutation of the CFTR gene, the deletion of phenylalanine at position 508, is present in about 70% of the CF chromosomes analysed throughout the world. For the remaining 30%, an increasing number of mutations are being identified. The most striking observation arising from studies on CF-associated mutations is the high frequency with which they are in the first NBF of the molecule (Tabor 1991). Different types of mutations have been reported, i.e. missense, nonsense, frameshift, insertion, repetition or deletion, but most of these are rare.

To understand the clinical consequence of the mutations, Kerem et al. (1989) have proposed a classification that divides patients into two classes: pancreatic insufficient and pancreatic sufficient. Patients with two severe alleles are expected to be pancreatic insufficient, whereas patients with one or two mild alleles are classified as pancreatic sufficient (Kerem et al. 1989). This assumption has recently been confirmed using a large number of patients in which the majority of the mutations had previously been characterized (Kristidis et al. 1991).

In this study, we have reported a new mutation located in exon 3 (G91R) of three patients, two of whom are siblings. We have shown these three chromosomes to be identical by descent by a genealogical study (Chaventré et al. 1991). All these patients carry the same genotype, are compound heterozygotes ΔF508/G91R and are pancreatic sufficient.

Most of the missense mutations so far reported in the first NBF (G551D, V520F, R560T, etc.) result in a severe phenotype. These mutations are assumed to lead to a more severe phenotype than mutations situated in the second NBF. The missense mutation G85E (Zielenski et al. 1991b), which results in a glutamic acid for a glycine at position 85 of the first transmembrane domain produces very mild symptoms of the disease, despite the radical change in amino acid sequence (Chalkley and Harris 1991). Other missense mutations reported by Kristidis et al. (1991) as being located in the transmembrane domain, i.e. R117H (exon 4), R334W (exon 7), R347P (exon 7), A455E (exon 9) and P574H (exon 12), are associated with pancreatic sufficiency; these observations are consistent with the genetic hypothesis that pancreatic sufficiency is a