A serine to proline substitution (S1255P) in the second nucleotide binding fold of the cystic fibrosis gene

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The gene defective in cystic fibrosis (CF) has been identified in 1989 and is predicted to encode a protein of 1480 amino acids (1, 2, 3). In this protein, called the cystic fibrosis transmembrane conductance regulator (CFTR), a deletion of 3 base pair (bp) resulting in the loss of a phenylalanine at amino acid position 508 (ΔF508), was found in 68% of CF chromosomes (4, 5). This major mutation is in exon 10 of the gene, which is part of one of two potential ATP-binding sites in the CFTR. These sites, termed nucleotide (ATP)-binding folds (NBFs), are important in the provision of energy for the functioning of the protein (6, 7).

In order to identify unknown mutations in 37 non-ΔF508 Belgian CF chromosomes, we decided to sequence exon 20 (second NBF) of the CFTR, after polymerase chain reaction (PCR) of this exon and flanking genomic sequences (8). In one patient, carrying a ΔF508 mutation on his maternal CF allele, a T to C substitution was found at the first position of codon 1255, nucleotide 3895, of the CFTR (Figure 1). This mutation predicts a serine to proline replacement (S1255P). The T to C substitution creates a third MaeIII site in the 473 bp PCR product. Digestion of normal product with this enzyme produces fragments of 58, 75 and 340 bp. In the presence of the S1255P mutation, the additional site reduces the largest fragment to fragments of 76 and 264 bp (Figure 2). In this way, it could be demonstrated that S1255P segregates with the paternal and grand-paternal CF chromosome (haplotype B, 5), as predicted by RFLP analysis (data not shown).

The patient (LT) is a boy born after an uneventful pregnancy at 40 weeks (December 1987). The birthweight was 3.510 kg. The diagnosis of CF was made by a routine trypsin test on a blood sample collected at day 5. Pilocarpine sweat test at the age of 3.5 months confirmed this diagnosis. No gastrointestinal problems were noticed during the first year. Pancreatic enzymes were added to the diet from the age of diagnosis and classical treatment of physiotherapy and mucolytic aerosol were instored. Pulmonary infections occurred on 3 occasions and were treated successfully by intravenous antibiotherapy. At the age of 4 years, physical examination is normal with weight on percentile 25 and length on percentile 50. ENT evaluation showed nasopharyngeal adenoid (hypertrophy and nasal polypsy stage 1). Adenoids were removed. The perfusion lung scintigraphy showed exclusion of basal segments in the left lung and defects of the right middle lobe. Gastrointestinal tract is normal under pancreas supplementation. Biological and nutritional status showed a light hypovitaminosis D.

S1255P could still represent a rare polymorphism segregating with the CF allele in this family. However, it was absent in 50 normal and 75 ΔF508 chromosomes. Moreover, the serine at position 1255 is conserved in the CFTR protein of mouse (9, 10), Xenopus laevis (11) and dogfish (12), but not in bovine (13). Finally, functional analysis of S1255P mutant CFTR has shown that it has an altered response to ATP (M.J.Welsh, University of Iowa, USA, personal communication). This defect is in accordance with the position of the S1255P mutation in the second NBF, a region dependent on ATP for the activation of the transport process of the CFTR (14). All this evidence indicates that S1255P is causative of CF in this family.

The frequency of S1255P seems to be very low or S1255P may even be unique to this family. However, the identification of mutations is necessary for precise genetic counseling in CF families and each mutation could provide information on the functional important sites of the CFTR. Direct sequencing of whole exons after PCR amplification or the application of related techniques (15, 16) are very useful in the search for less frequent mutations.

Figure 1. Direct sequencing of exon 20 after PCR amplification. Only that part of exon 20, containing the T to C substitution (arrow), is shown. Left: normal, right: S1255P patient.
Figure 2. Detection of the S1255P mutation on a 3% agarose gel after digestion of exon 20 PCR product with Maelll. Lanes 1 and 2, undigested and digested PCR product of the S1255P patient, lane 3 mother of patient (ΔF508 carrier), lane 4 and 5 father and paternal grandfather of patient, lane 6 brother of patient (ΔF508 carrier), M: DIGest II molecular weight standard (Pharmacia, Sweden).

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