A cystic fibrosis patient homozygous for the new frameshift mutation 936delTA: description and clinical data

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Abstract

We report the identification of a new frameshift mutation (936delTA) in exon 6b of the CFTR gene. In the screening of 486 unrelated Spanish CF patients we found a patient homozygous for 936delTA (with consanguineous parents) and a patient heterozygous for ΔF508 and 936delTA. Genotype-phenotype correlation studies showed that 936delTA is associated with pancreatic insufficiency and chronic pulmonary colonisation.

(J Med Genet 1994;31:369-370)

Cystic fibrosis (CF) is the most common severe autosomal recessive disease in the white population, affecting about 1 in 2500 persons.¹ The CF transmembrane regulator (CFTR) gene is specifically expressed in secretory epithelial cells, from pancreas, colon, sweat ducts, lung epithelium, and genital ducts.² Mutations in the CFTR gene alter chloride secretion across the apical membrane of epithelial cells, resulting in aberrant secretions in the lungs and pancreas, and leading to chronic obstructive lung disease and digestive disorders.¹

The majority of the CF mutations identified up to now (more than 350, Cystic Fibrosis Genetic Analysis Consortium data, September 1993) are associated with severe clinical presentation. Owing to the fact that the majority of CF mutations are uncommon, it is difficult to obtain reasonable genotype-phenotype correlations. Homozygosity for a given mutation provides an accurate correlation, as well as giving an indication of the severity of the mutation. However, patients homozygous for an uncommon mutation are rare.³ We report a new frameshift mutation (936delTA) in exon 6b of the CFTR gene and provide clinical data of a patient homozygous for 936delTA and a patient heterozygous for ΔF508 and 936delTA.

Materials and methods

A total of 486 unrelated Spanish families, with at least one affected child with a confirmed diagnosis of CF, was investigated. All patients had a minimum of two positive chloride sweat tests (Cl⁻ > 80 mmol/l). Genomic DNA was isolated from peripheral blood lymphocytes according to standard protocols.

To identify mutations responsible for CF in

Spanish CF patients, SSCP analysis was performed on a large series of CF samples.⁴ PCR conditions were: denaturation at 95°C for 30 seconds, annealing at 56°C for 30 seconds and extension at 74°C for 30 seconds for 35 cycles. The reaction mix contained 0.1 μl of [α -³²P]dCTP (3000 Ci/mmol), 1 μl of PCR buffer (Perkin Elmer Cetus N808-0006), 60 μmol/l of each dNTP, 10 pmol of each primer, 100 ng of DNA, and 1 unit of *Taq* DNA polymerase, in a final volume of 10 μl. The primers used were 16bD1 (5' AATAATGCCCATCTGTTGA-ATAA 3') and 6bi-3.⁵

After PCR, DNA products of exon 6b were digested with *AluI*. SSCP electrophoresis was run at 4°C on a 6% non-denaturing polyacrylamide gel without glycerol, at 30 W for five hours. One abnormal band was detected in the samples analysed. DNA from the sample with the abnormal band was PCR amplified and then purified on a Strategene PrimeErase Quik column (400705). Direct automatic sequencing was performed with 3.5 pmol of sequencing primer using a *Taq* DyeDeoxy™ Terminator Cycle Sequencing Kit (ABI) (401113), according to the manufacturer's recommendations. Sequencing conditions were denaturation at 96°C for 30 seconds, annealing at 50°C for 30 seconds, and extension at 60°C for four minutes, for 28 cycles.

Results and discussion

In the analysis of exon 6b we detected one sample (89-468) with an abnormal SSCP migration pattern (figure A). Direct sequencing of the sample, using primers 16bD1 and 6bi-3, showed a two base pair deletion at nucleotide 936 (mutation 936delTA) (figure B). This causes a shift in the reading frame with a stop codon at the new codon 272 which should lead to truncation of the CFTR protein.

The 936delTA mutation was found associated with haplotype 22-22-17 for IVS8CA-IVS17BTA-IVS17BCA⁶ and the deletion can easily be detected by gel electrophoresis on an 8% polyacrylamide gel. A total of three 936delTA alleles were found in the screening of 972 CF Spanish chromosomes (3/972; final estimated frequency of 0.3%), two in the homozygous CF patient (with consanguineous parents) and the other allele in a CF patient heterozygous for ΔF508 and 936delTA. The grandparents of both CF patients originate from Badajoz (a province in the west of Spain), suggesting that these 936delTA alleles could have a common origin.

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Received 19 October 1993.
Accepted for publication 30 November 1993.

(A) SSCP analysis of exon 6b. Primers used were 16bD1 and 6bi-3. SSCP-PCR products were digested with AluI. Samples were electrophoresed in non-denaturing conditions, without glycerol, at 30 W and at 4°C for five hours. Sample 5 (patient 89-468) shows an abnormal migration band pattern. (B) Automatic sequencing of exon 6b of the CFTR gene using the primer 6bi-3. CF patient sequence shows a deletion of two base pairs, at nucleotides 935 and 937, when compared to the normal sequence.

Clinical data (table) were available for both CF patients bearing mutation 936delTA. The homozygous patient (89-468) is 7 years old and was diagnosed when he was 1 month old; he is pancreatic insufficient and has chronic lung colonisation by *Pseudomonas aeruginosa* (since he was 2 months old), *Haemophylus influenzae*, and *Staphylococcus aureus*. He is significantly below the average weight (3rd to 10th centile) and height (10th to 25th centile) for his age. Owing to his young age FEV₁ and FVC studies were not performed. Two brothers of this patient (89-468) died when they were children of unknown causes; his parents are first cousins. The heterozygous patient (91-113) is 11 years old and was diagnosed when she was 19 months old; she is pancreatic insufficient

Clinical and laboratory features of persons with 936delTA

	Patients	
	89-468	91-113
Genotype	936delTA	936delTA
Present age	7 y	11 y
Age at diagnosis	1 mth	1 y 7 mth
Sex	M	F
Sweat chloride (mEq/l)	120	110
Meconium ileus	No	No
Dehydration	No	—
Height (cm) (centile)	115 (10-25)	127 (3)
Weight (kg) (centile)	18 (3-10)	21 (3)
Chrispin-Norman*	2	12
Lung colonisation with bacterial pathogens	Yes	Yes
Pancreatic insufficiency	Yes	Yes
Shwachman-Kulczycki†	75	76
FEV ₁ (%) predicted	—	60
FVC(%) predicted	—	60
Other clinical features	No	No

* Chest radiograph score of Chrispin-Norman, 0 being the best score and 36 the worst.⁷
† Clinical score system of Shwachman-Kulczycki, of which 100 is the best clinical score.⁸ Forced expiratory volume in 1 second (FEV₁)% predicted; forced vital capacity (FVC)% predicted.

and has had lung colonisations by *Pseudomonas aeruginosa* since she was 5 years old. She is also below the average weight (3rd centile) and height (3rd centile). The CF mutation on the other chromosome is ΔF508. The 936delTA homozygote and the 936delTA/ΔF508 patients have allowed us to assess the clinical features of this mutation, establishing a good genotype-phenotype correlation. The clinical presentation of patients bearing 936delTA suggests its association with pancreatic insufficiency and chronic lung colonisation. This CF phenotype would be the result of the shift in the reading frame, which would produce a truncated and non-functional CFTR protein.

We thank H Kruyer and M Lench for help with the manuscript, and Dr G Antiñolo for contributing clinical data. This work was supported by the Fondo de Investigaciones Sanitarias de la Seguridad Social (93/0202) and the Institut Català de la Salut. MC received a fellowship from the Ministerio Español de Educación y Ciencia.

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