



*Short Communication*

## Novel truncating mutations in the *CFTR* gene causing a severe form of cystic fibrosis in Italian patients

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**ABSTRACT.** Cystic fibrosis (CF) is a common recessive genetic disease caused by mutations in the gene encoding for the cystic fibrosis transmembrane conductance regulator (CFTR) protein. More than 1800 different mutations have been described to date. Here, we report 3 novel mutations in *CFTR* in 3 Italian CF patients. To detect and identify 36 frequent mutations in Caucasians, we used the INNO-LiPA CFTR19 and INNO-LiPA CFTR17+Tn Update kits (Innogenetics; Ghent, Belgium). Our first analysis did not reveal both of the responsible mutations; thus, direct sequencing of the *CFTR* gene coding region was performed. The 3 patients were compound heterozygous. In one allele, the F508del (c.1521\_1523delCTT, p.PHE508del) mutation in exon 11 was observed in each case. For the second allele, in patient No.1, direct sequencing revealed an 11-base pair deletion (GAGGCGATACT) in exon 14 (c.2236\_2246del; pGlu746Alafs\*29). In patient No. 2,

direct sequencing revealed a nonsense mutation at nucleotide 3892 (c.3892G>T) in exon 24. In patient No. 3, direct sequencing revealed a deletion of cytosine in exon 27 (c.4296delC; p.Asn1432Lysfs\*16). These 3 novel mutations indicate the production of a truncated protein, which consequently results in a non-functional polypeptide.

**Key words:** Cystic fibrosis; Novel mutation; Pancreatic insufficiency; Severe mutations

## INTRODUCTION

Cystic fibrosis (CF) is the most common recessive genetic disease in the Caucasian population with a prevalence of 1 in 2500 newborns (Davis et al., 1996). It is a multi-organ disease caused by mutations in the gene encoding for the cystic fibrosis transmembrane conductance regulator (CFTR) protein. CFTR is a chloride channel that is predominately localized on the lumen-facing (apical) membranes of epithelial cells that participate in regulated transport of salt and water across epithelial tissues (Kirk, 2000).

More than 1800 mutations have been described in *CFTR*, which have been classified into 5 classes based on molecular alterations at the protein level (Bienvenu, 1997). The altered protein causes dense mucous epithelial secretions and thus clinical expression involving the gastrointestinal and pulmonary systems, pancreas, liver, and reproductive tract.

In this study, we identified 3 novel mutations in 3 Italian CF patients.

## MATERIAL AND METHODS

CF patients were enrolled at the Cystic Fibrosis Regional Center of the Institute for Maternal and Child Health, IRCCS “Burlo Garofolo”, Trieste.

Diagnosis was made after 2 positive sweat tests were performed according to Gibson and Cooke (1959).

### Patients

#### *Patient 1*

The first patient (female) was diagnosed at 4 months of age. She presented characteristic symptoms of CF, including pancreatic insufficiency, gastrointestinal and respiratory disorders, poor growth, and high levels of chloride (71 mM) in her sweat. Her chloride/sodium ratio was higher than 1.0 (1.3).

#### *Patient 2*

The second patient (female) was diagnosed at birth. She presented pancreatic insufficiency, gastrointestinal disorder, poor growth, and high levels of chloride (94 mM) in her sweat. Her chloride/sodium ratio was 1.2.

### ***Patient 3***

The third patient (female) was diagnosed at 6 months of age. She presented electrolyte depletion (hypochloremia, hyponatraemia) and dehydration, pancreatic insufficiency, gastrointestinal disorder, poor growth, and borderline chloride levels (65 mM). Her chloride/sodium ratio was 1.5.

#### **DNA extraction**

Genomic DNA samples of the patients were extracted from peripheral blood using the QIAamp DNA Blood Mini Kit (Qiagen; Hilden, Germany).

#### ***CFTR* genotyping**

##### ***First-level analysis***

To detect and identify 36 frequent mutations in Caucasians, we used the INNO-LiPA CFTR19 and INNO-LiPA CFTR17+Tn Update kits (Innogenetics; Ghent, Belgium). This is a multiparameter line probe assay based on the reverse hybridization principle for the simultaneous detection and identification of 36 CF-related mutations and their wild-type sequences in human whole blood.

##### ***Second-level analysis***

Because the first level of analysis did not reveal both mutations responsible for CF disease, direct sequencing of the *CFTR* gene coding region (including the 27 exons and the exon-intron boundaries) was performed.

Sequencing analysis was carried out using the Big Dye Terminator cycle sequencing kit (Applied Biosystems; Foster City, CA, USA) and an ABI PRISM 3130 (Applied Biosystems) genetic analyzer.

Mutation nomenclature was defined according to international recommendations ([www.hgvs.org/mutnomen](http://www.hgvs.org/mutnomen)). Nucleotide (cDNA) numbering used +1 as the A in the ATG codon for the reference sequence GenBank NM\_000492.2.

## **RESULTS**

### **First-level analysis**

All 3 patients were heterozygous for an F508del (c.1521\_1523delCTT, p.PHE508del) mutation in exon 11, but the second mutation was not detected.

### **Direct sequencing**

#### ***Patient No. 1***

Direct sequencing of all exons and their flanking regions revealed an 11-base pair deletion (GAGGCGATACT) in exon 14 (c.2236\_2246del; pGlu746Alafs\*29). This deletion

introduced a stop codon (TAA) 29 amino acids downstream. The new mutation was of paternal origin (Figure 1).

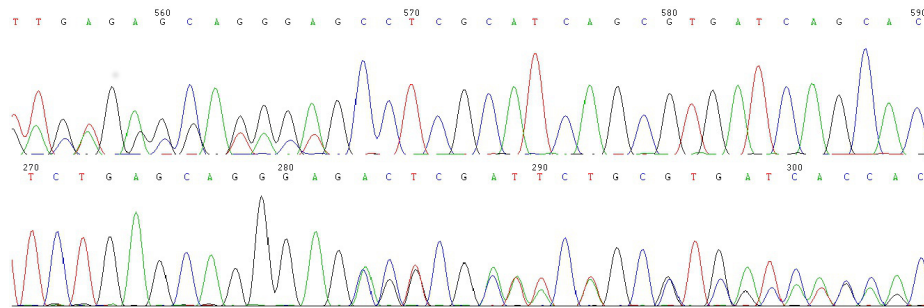


Figure 1. Electropherogram of the mutation in Patient No. 1.

**Patient No. 2**

Direct sequencing of all exons and their flanking regions revealed a nonsense mutation at nucleotide 3892 (c.3892G>T) in exon 24, which changed a glycine residue to a stop codon (TGA) at amino acid 1298 (Gly1298\*). The new mutation was of maternal origin (Figure 2).

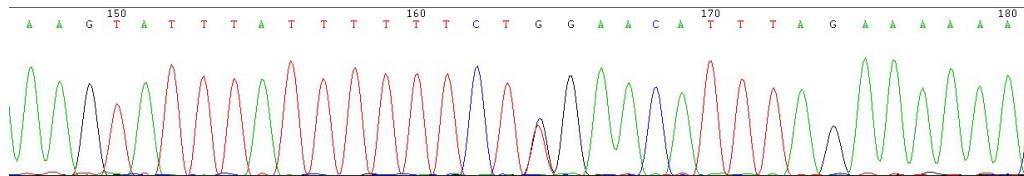


Figure 2. Electropherogram of the mutation in Patient No. 2.

**Patient No. 3**

Direct sequencing of all exons and their flanking regions revealed a deletion of a cytosine in exon 27 (c.4296delC; p.Asn1432Lysfs\*16). This deletion predicted a new stop codon (TGA) 16 amino acids downstream. The new mutation was of paternal origin (Figure 3).

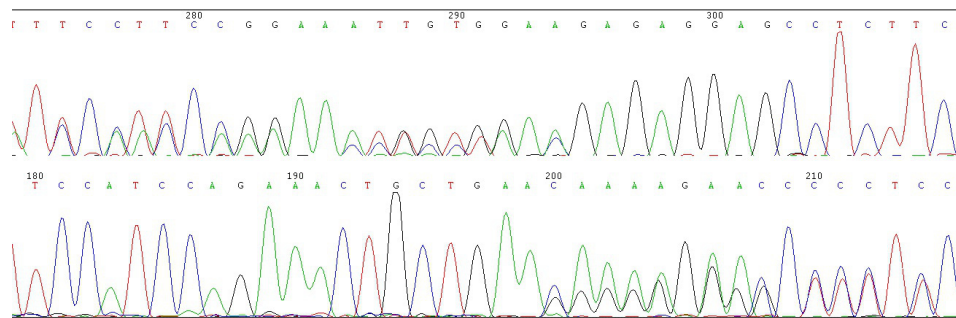


Figure 3. Electropherogram of the mutation in Patient No. 3.

## DISCUSSION

CFTR is a chloride channel encoded by a gene that is defective in CF patients. CFTR is predominately localized on the lumen-facing (apical) membranes of epithelial cells, participating in regulated transport of salt and water across epithelial tissues (Gibson and Cooke, 1959).

The *CFTR* gene is located on the long (q) arm of chromosome 7 at position 31.2.

*CFTR* mutations can be divided into 5 classes based on molecular alteration at the protein level: the first class includes mutations such as the introduction of a premature stop codon, causing a defect in the production of functional CFTR protein. These are responsible for the lack of synthesis of the protein. Second-class mutations include those causing a block in protein processing. The third and fourth classes include mutations that cause either a block in the regulation or an altered conductance of the CFTR protein. Finally, the fifth class of mutations causes reduced protein synthesis (Naren and Kirk, 2000).

The c.2236\_2246delGAGGCGATACT mutation in the CFTR R-domain predicts a new stop codon (TAA) 29 amino acids downstream. The deletion lies in a region of a deletion hotspot. Interestingly, 2 other deletions have been independently described in two previous reports, including 2372del8 (c.2240\_2247delCGATACTG; p.Ile748Serfs\*28) (Zielenski and Tsui, 1995) and 2380\_2387del (c.2248\_2255del; p.Pro750Glnfs\*26) (Chevalier-Porst et al., 1993), which are located in the same DNA tract. These deletion events may be sustained by the presence of a short inverted repeat that causes either replication or recombination errors.

The c.3892G>T (p.Gly1298\*) mutation is a nonsense mutation that introduces a new stop codon at the amino acid 1298. This mutation is located in CFTR nucleotide binding domain 2 and causes a severe form of CF as synthesis of the wild-type protein is suppressed.

The c.4296delC mutation creates a stop codon 16 amino acids downstream, leading to deletion of 124 C-terminus amino acids from the CFTR protein, including the TRL-COOH domain. As described for the c.4296\_4297insGA and p.Ser1435Glyfs\*14 mutations (Claustres et al., 2008), this highly conserved domain matches the binding consensus domain of the Na<sup>+</sup>/H<sup>+</sup> exchanger regulatory factor. This cytoplasmic phosphoprotein may play an important regulatory role in CFTR function (Jézéquel et al., 2000). Previous studies (Wang et al., 1998; Mickle et al., 1998; Moyer et al., 1999) found that a nonsense mutation in exon 24 encoded a truncated version of the CFTR protein, which was missing the last 26 amino acids and became mispolarized to the lateral membrane of the epithelia.

These 3 novel mutations predicted a truncated protein and, as a consequence, a non-functional polypeptide that can be considered as first-class mutations. The mutations identified in this study were severe mutations associated with pancreatic insufficiency.

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