A. Hinzpeter, A. de Becdelièvre et al. Identification of a novel 5’ alternative CFTR mRNA isoform in a patient with nasal polyposis and CFTR mutations

SUPPLEMENTARY DATA

Materials and Methods

Ethical statement

Informed consent for genetic analysis and functional assays was obtained from all subjects and the local ethics committee approved the study.

Mutation nomenclature

The recommendations of the Human Genome Variation Society (http://www.hgvs.org/mutnomen/) were followed for exon numbering and mutation names, using +1 as the A of the initiation ATG codon, as well as reference sequences NM_000492.3 and NG_016465.3, also in accordance with the Cystic Fibrosis Mutation Database (http://www.genet.sickkids.on.ca/app). Reference to the traditional nomenclature is provided in brackets at the first occurrence of mutations in the text.

Genotyping

Using specific primers and the big dye terminator technology, the CFTR promoter was sequenced on the ABI3130XL capillary electrophoresis system (Applied Biosystems, Foster City, CA) and analyzed with Seqscape v2.5 software.

mRNA purification and analysis

Nasal epithelial cells were obtained by nasal brushings performed at the Otorhinolaryngology department, in the Purpan Hospital in Toulouse for the patient and his parents and in the Henri Mondor Hospital in Créteil for the controls. Cells were immediately kept in mRNAlater buffer and frozen at -20°C. mRNA were purified using RNeasy® Mini kit (Qiagen,
Courtaboeuf, France) columns and total mRNA quality was checked on Bioanalyzer 2100 (Agilent, Les Ulis, France). cDNA was obtained from 400 ng of mRNA and using the High Capacity cDNA Reverse Transcription kit (Applied Biosystems). Qualitative mRNA analysis was performed as described in (Costa et al, 2011).

CFTR quantification was performed using primers and probes designed by Applied Biosystems (TaqMan FAM/NFQ-MGB probe format, CFTR: Hs00357011_m1; KRT18: Hs01920599_gH). All reactions were run in triplicate and each sample was run in two qPCR assays. To correct for variations in the amount of input RNA and efficiency of the reverse transcription, KRT18 was quantified and results were normalized to these values. Relative amounts of CFTR mRNA were measured using the $2^{-\Delta\Delta CT}$ method. The mean of eight control samples was chosen as a calibrator, i.e., as the baseline for the comparative results. Results in the patient and his parents were controlled using GAPDH as another reference gene (Thermo Scientific; GAPDH: AX-004253-00-0400) by comparison with two normal controls.

Cell culture and transfection

BEAS-2B, Caco-2 and IB3-1 cells were purchased from ATCC (Molsheim, France). Caco-2 cells were grown at 37°C, 5% CO$_2$ in DMEM medium supplemented with 20% Fetal Calf Serum. BEAS-2B cells and IB3-1 cells were grown in LHC-8 medium supplemented with 10% FCS (all media purchased from Invitrogen). Cells were transfected with TurboFect™ in vitro Transfection Reagent (Fermentas, Thermo Fisher Scientific, Villebon-sur-Yvette, France) according to the manufacturer's instructions.

Constructs and mutagenesis

Two constructs of respectively 3581bp (c.-3628 to c.-47) and 3021bp (c.-3068 to c.-47) length of the CFTR promoter were subcloned in the pXP-1 luciferase reporter plasmid. Inserts were obtained after amplification using the following forward primers, 5’-GGCTGTTCTTACCCTTCTAG-3’ or 5’-TCATTAAATGCTTGGGCTCTTG-3’ and the
common reverse primer 5'-TCCTAATGCCAAAGACTAC-3'. The c.-2954G>A mutation was generated using the QuickChange XL site directed mutagenesis kit (Agilent).

**Promoter activity assay**

Caco-2, IB3-1 and BEAS-2B cells were seeded in 24-well plates and co-transfected with 350ng of pXP-1-\textit{Luc} containing the \textit{CFTR} 5’ UTR region and 10ng Renilla plasmid. After 24 hours, cells were lysed and both Firefly and Renilla luciferase activities were measured with the Dual-Luciferase™ Reporter Assay System (Promega, Charbonnieres, France), using a TriStar LB941 Multimode Microplate Reader (Berthold, Bad-Wildbad, Germany). Firefly luciferase activities were normalised to the level of Renilla activities and to the WT constructs. Data represent the mean +/- SE of the independent measurements.

**5’RACE and sequencing**

5’RACE procedure used the FirstChoice® RLM-RACE Kit (Life technologies) according to the manufacturer’s instructions. Briefly, the capped stable form of mRNA was selected from 10µg samples of total mRNA, from nasal cells of the index case and of one healthy control, and from primary cultures of nasal cells of two additional controls. mRNA was retrotranscribed in cDNA and amplified by PCR using the 5’RACE outer commercial primer, which hybridized with the 5’ extremity of mRNA and the 5’-GGTCATAGGAAGCTATGATTC -3’ primer located in \textit{CFTR} exon 4. A nested PCR was then performed with the 5’RACE inner primer and another primer in exon 4 (5’-GAGGCTGTACTGCTTTTGGT-3’). PCR products were separated using a 2% agarose gel before sequencing using both inner primers.

**Legend to Figure S1. Sequence of the transcripts obtained by 5’RACE analysis in the patient.**
(A) The upper band, as seen in Figure 2C, corresponds to a novel transcript containing exons 1a-L, 2 to 4, with initiation start site at c.-666. (B) The lower band, as seen in Figure 2C, corresponds to a functional transcript containing exons 1 to 4, with initiation start site at c.-71.
ABBREVIATED ABSTRACT

A novel 5’ alternative CFTR transcript was identified in a 12y-old patient presenting with isolated nasal polyposis and being heterozygous for c.2551C>T (p.Arg851*). Its characterization was made after identification of the c.-2954G>A mutation which was shown to enhance promoter activity. The novel, presumably non-functional, transcript could represent a novel molecular CFTR defect associated with CFTR-related disorders.