Supplementary Methods

Rectal biopsies procedure

Colon preparation (cleaning) was done by applying an enema of saline solution (0.9% NaCl) or 12% glycerine solution or by oral mannitol. Superficial 5-6 rectal mucosa specimens (3-4 mm in diameter) were obtained with or without sedation (depending on individuals' will or collaboration) by colon forceps (Endoflex® 3.4mm, Voerde, Germany) with visual examination, avoiding the risk of bleeding or of collecting damaged tissue, and immediately stored in ice-cold RPMI1640 with 5% (v/v) Fetal Bovine Serum (FBS).

Ussing chamber measurements

Rectal biopsy specimens were mounted and analysed in modified micro-Ussing chambers as previously described under open-circuit conditions [1–3]. Values for the transepithelial voltage (V_{te}) were referred to the serosal surface of the epithelium. Transepithelial resistance (R_{te}) was determined by applying intermittent (1s) current pulses (0.5 μA). The equivalent short-circuit current (I_{sc}) was calculated according to Ohm's law ($I_{sc} = V_{te} / R_{te}$), after appropriate correction for fluid resistance. Briefly, the luminal and basolateral surfaces of the epithelium were continuously perfused (5 ml/min) with Ringer solution of the following composition (mmol/l): NaCl 145, KH_2PO_4 0.4, K_2HPO_4 1.6, D-glucose 5, $MgCl_2$ 1, Ca-gluconate 1.3, pH 7.40, at 37° C. $HCO3^-$ free buffer solutions were used to exclude a possible contribution of CFTR-independent electrogenic $HCO3^-$ secretion, which would be indistinguishable from electrogenic Cl^- secretion and thus may mimic residual Cl^- channel function in CF colonic epithelia [4].

Tissues were equilibrated in the micro-Ussing chambers for 30 min in perfused Ringer solution before the above experimental protocol [5]. Values for basal transepithelial resistance (R_{te}) were similar for all groups of patients: $R_{te\ (Control)}$ = 18.63 ± 0.85 Ω.cm^2 (n = 18); $R_{te\ (non\text{-}CF)}$ = 19.12 ± 0.12 Ω.cm^2 (n = 28); $R_{te\ (Classic\ CF)}$ = 19.60 ± 1.16 Ω.cm^2 (n = 55); and $R_{te\ (Non\text{-}Classic\ CF)}$ = 21.68 ± 2.80 Ω.cm^2 (n=12). Amiloride (Amil, 20 µM, luminal) was added to block electrogenic sodium (Na^+) absorption through the epithelial Na^+ channel (ENaC) and Indomethacin (Indo, 10 µM, basolateral) was applied for 40-60 min to inhibit endogenous cAMP formation through prostaglandins [1–5]. As before [3,5,6], Na^+ absorption was significantly augmented in CF rectal tissues in comparison to control (p=0.041) or non-CF (p=0.014) rectal tissues: $I_{sc\text{-}Amil\ (Classic\ CF)}$ = 86.75 ± 17.03 µA/cm^2; $I_{sc\text{-}Amil\ (Non\text{-}Classic\ CF)}$ = 64 . 39 ± 17.79 µA/cm^2; $I_{sc\text{-}Amil\ (Control)}$ = 38.56 ± 5.90 µA/cm^2; and $I_{sc\text{-}Amil\ (Non\text{-}CF)}$ = 37.63 ± 6.34 µA/cm^2. As reported [1,2] cAMP-dependent and cholinergic Cl^- secretion in human rectal tissues relies on functional CFTR. Thus, we used 3-isobutyl-1-methylxantine (IBMX, 100 µM, basolateral) and forskolin (2 µM, basolateral) to activate cAMP-dependent Cl^- secretion and carbachol (CCH, 100 µM, basolateral) for cholinergic co-activation [1–3]. Thus, percentage of CFTR function was calculated for maximal CFTR activation ($\Delta I_{sc\text{-}IBMX/Fsk}$ + $\Delta I_{sc\text{-}CCH(IBMX/Fsk)}$) and normalized to the correspondent mean value (-217.45 µA/cm^2) for the reference non-CF control group.

CFTR Genotyping

To detect CFTR mutations that were not identified by screening the 6 most common CFTR mutations (F508del, G551D, G542X, R1162X, N1303K, R553X), an extended CFTR

mutation search was done consisting in two-step automatic DNA sequencing of all 27 exons and the respective flanking intronic regions of the *CFTR* gene. The first step in genotyping included detection of mutations for 15 exons (exons 3, 6a, 7, 9, 10, 11, 12, 13, 16, 17b, 18, 19, 20, 21 and 24), which show a detection frequency of 95.14%. Such test method with a mutation detection rate of 95%, gives us a 90% probability of finding two abnormal alleles, 10% probability of finding one abnormal allele and 0% probability of finding no abnormal alleles [7,8]. To detect CFTR mutations that were not identified by the above described screening method, we performed DNA sequencing of the remaining 12 exons of the *CFTR* gene for CF patients. The mutations were classified according to the European Consensus [9] as: a) mutations that cause CF disease; b) mutations that result in a CFTR-related disorder; c) mutations with no known clinical consequence; and d) mutations of unproven or uncertain clinical relevance (Table S2).

Statistics

Pearson coefficients (*r)* were used to find correlations and partial correlations between clinical outcomes and CFTR function. As previously described [10,11], a mixed model regression analysis was chosen to determine the rate of decline in FEV_1 *vs. Age* among the established groups; and Kruskal-Wallis test for independent samples was used to find differences between the distribution of FEV_1 *vs. Age* across those groups. For Crosstabs, Pearson Chi-Square Tests were used to determinate independence between the variables analysed. Monte Carlo estimates of the exact p-value are provided

whenever the data are too sparse or unbalanced for the asymptotic results to be reliable.

A stepwise Discriminant Analysis with Wilks' Λ method was used to identify which variable or variables in study are able to discriminate with highest accuracy the established groups in this study. The assumptions of normality and homogeneity of variance-covariance matrices of each group were tested with Shapiro-Wilk (since one of the groups was small) and Box M tests, respectively. A Classification Analysis was also performed to obtain Fisher's linear classification functions that could predict in which group new cases would be classified.

Histological preparations

Rectal biopsies collected simultaneously with the ones for bioelectrical measurements were fixed in 4% formaldehyde, embed in paraffin and cut in thin sections (2-3 μM). These sections were then deparaffinize in xylene (2 times, 10 minutes each), re-hydrate in 2 changes of absolute alcohol (5 minutes each), 95% alcohol for 2 minutes, 70% alcohol for 2 minutes and briefly in distilled water. Hematoxylin-eosin (HE) and Tricome's Masson stainings were done as previously[6]. All slides were mounted with xylene based mounting medium. In HE stained sections we observe nuclei in blue and cytoplasm in pink to red. For Tricome's Masson we observe collagen in blue, nuclei in black, and muscle and cytoplasm in red (blood cells in bright red).

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