Intestinal current measurement versus nasal potential difference measurements for diagnosis of cystic fibrosis: a case–control study

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Abstract

Background: Nasal potential difference (NPD) and intestinal current measurement (ICM) are functional CFTR tests that are used as adjunctive diagnostic tools for cystic fibrosis (CF). Smoking has a systemic negative impact on CFTR function. A diagnostic comparison between NPD and ICM and the impact of smoking on both CFTR tests has not been done.

Methods: The sweat chloride test, NPD, and ICM were performed in 18 patients with CF (sweat chloride >60 mmol/l), including 6 pancreatic sufficient (PS) patients, and 13 healthy controls, including 8 smokers. The NPD CFTR response to Cl-free and isoproterenol perfusion ($\Delta$OCl$^+$ + Iso) was compared to the ICM CFTR response to forskolin/IBMX, carbachol, and histamine ($\Delta$Isc, forskolin/IBMX+ carbachol+histamine$^-$).

Results: The mean NPD CFTR response and ICM CFTR response between patients with CF and healthy controls was significantly different (p <0.001), but not between patients with CF who were PS and those who were pancreatic insufficient (PI). Smokers have a decreased CFTR response measured by NPD (p = 0.049). For ICM there is a trend towards decreased CFTR response (NS). Three healthy control smokers had NPD responses within the CF-range. In contrast to NPD, there was no overlap of the ICM response between patients with CF and controls.

Conclusions: ICM is superior to NPD in distinguishing between patients with CF who have a sweat chloride > 60 mmol/l and healthy controls, including smokers. Neither NPD nor ICM differentiated between patients with CF who were PS from those who were PI. Smoking has a negative impact on CFTR function in healthy controls measured by NPD and challenges the diagnostic interpretation of NPD, but not ICM.

Keywords: (3–10): Cystic fibrosis, Nasal potential difference, Intestinal current measurement, Sweat chloride, Sweat test, Diagnosis, Smoking

Background

Cystic fibrosis (CF) is diagnosed based on a defined clinical phenotype and confirmation of cystic fibrosis transmembrane regulator (CFTR) dysfunction, commonly demonstrated by a sweat chloride value of $\geq$ 60 mmol/l and/or detection of two CF-causing mutations [1,2]. A small but increasing number of patients present with clinical symptoms characteristic of CF, an intermediate (30–60 mmol/l) or negative ($\leq$ 29 mmol/l) sweat test, and less than two CF-causing mutations [3-6]. For these query CF patients, a specific and sensitive CFTR functional test to exclude or confirm a CFTR functional defect characteristic of CF is needed [1,6]. CFTR modulating and correcting drugs have improved CFTR-function in cell cultures [7]. To test their effect in CF-patients, especially those with rare mutations, CFTR-functional tests with low variability and high reproducibility are needed [7].

In addition to sweat testing, two additional CFTR functional tests have been developed over the past 30 years; nasal potential difference (NPD) measurement [8] and intestinal current measurement (ICM) [9]. CFTR function is measured in vivo in the respiratory epithelium by NPD and ex vivo in superficial rectal biopsies by ICM. International standard operating procedures (SOPs) have been
established for both tests [8,10]. Both tests block epithelial sodium channels by amiloride and stimulate cAMP-mediated CFTR-mediated chloride transport in chloride-free solution and isoproterenol (NPD) or forskolin and IBMX (ICM). In addition, cholinergic chloride transport is tested by carbachol with ICM. The change after chloride-free and isoproterenol perfusion ($\Delta_0Cl^{-} + \text{Iso}$) with NPD [8] and the sum of the responses after carbachol, forskolin/IBMX ([11]) plus histamine ($\Delta_{\text{isc}}$, forskolin/IBMX + carbachol + histamine) with ICM [12] has been proposed to be the best parameter of CFTR function. Both techniques can discriminate CF patients from healthy controls (NPD [13,14]; ICM [11,12,15,16]), but comparative clinical trials are lacking. Smoking has a systemic negative impact on CFTR-function [17,18], but the impact on the diagnostic aspects of NPD and ICM have not been investigated.

We performed NPD and ICM measurement in CF-patients and healthy controls to determine (1) the ability of these measurements to differentiate CF patients from healthy controls, and (2) the influence of smoking on CFTR function in healthy controls.

**Methods**

Between October 2012 and February 2013, 18 patients with CF and 13 healthy controls were recruited at the Justus-Liebig-University, Giessen, Germany. For this study, the diagnosis of CF was based on at least one clinical manifestation of CF, sweat chloride ≥60 mmol/l and the presence of two CF-causing mutations [1]. Pancreatic sufficiency (PS) was defined as fecal elastase >100 μg/g stool. Healthy controls had no clinical manifestation of CF and a sweat chloride value <60 mmol/l (Non-CF). Smoking was defined as any active or passive exposure to tobacco smoke. Exclusion criteria were participation in another medical clinical trial during the past 30 days, acute respiratory symptoms, intake of ivacaftor, known hemorrhoids, or bleeding diathesis. The ethics committee of the Justus-Liebig-Universität Giessen approved the protocol (AZ109/12). The study was performed in accordance with the declaration of Helsinki. Written informed consent was obtained from each participant aged 18 years and older. For participants younger than 18 years of age, written informed consent was obtained from each participant’s parents or legal guardian, and age-appropriate consent was obtained from each participant. The sweat test, NPD, and ICM were performed on the same day for each subject.

The sweat test was performed according to Clinical and Laboratory Standards Institute guidelines [19]. For sweat stimulation and collection, the Macroduct® system (Wescor, Table 1 Characteristics and CFTR response of pancreatic insufficient (CF-PI) and pancreatic sufficient (CF-PS) patients with CF and controls

|                          | CF-PI (n = 12) | CF-PS (n = 6) | CF-all (n = 18) | Controls (n = 13) |
|--------------------------|---------------|---------------|-----------------|------------------|
| **Age, years**           | 24.0 ± 6.1    | 23.3 ± 11.8   | 22.8 ± 8.0      | 30.6 ± 10.4      |
|                          | 22.0 (19.0 – 26.0) | 16.0 (14.5 – 30.5) | 20.5 (18.3 – 25.3) | 25.0 (23.5 – 35.5) |
| **Gender, females:males**| 3:9           | 5:1           | 8:1             | 7:6              |
| **Body mass index Z-score**| -1.18 ± 0.80  | -0.62 ± 1.41  | -0.99 ± 1.03*   | 0.00 ± 0.65*     |
|                          | -1.05 (–2.40 - 0.00) | 1.41 (–0.20 - 0.70) | -0.90 (–2.60 - 0.70) | 0.00 (–1.10 - 1.30) |
| **Sweat chloride (mmol/l)**| 110 ± 13**    | 86 ± 14**     | 102 ± 17*       | 19 ± 8*          |
|                          | 106 (92 – 140) | 90 (70 – 99)  | 104 (70 – 140)  | 19 (10 – 36)     |
| **NPD CFTR response average $\Delta_0Cl^{-} + \text{Iso}$ (mV)** | 4.6 ± 3.9 | 1.5 ± 4.1 | 3.6 ± 4.1* | -13.6 ± 8.5* |
|                          | 5.1 (–3.0 -11.9) | 1.5 (–3.2 -6.23) | 4.5 (–3.2 -11.9) | -12.7 (–26.4 - -1.92) |
| **ICM CFTR response average $\Delta_{\text{isc}}$ ($\mu$A/cm²)** | -0.3 ± 8.1 | 5.3 ± 10.9 | 1.6 ± 9.2* | 77.8 ± 34.8* |
|                          | -0.6 (–12.6 – 17.9) | 5.0 (–9.7 – 19.0) | 0.1 (–12.6 – 19.0) | 65.3 (39.6 -140.9) |
| **Genotyping**            | F508/F508 (6x) | F508/R347P (2x) | F508/R347P (2x) | F508/R347P (2x) |
|                          | F508/G551D (2x) | F508/3849 + 10 kb C -> T (2x) | F508/3849 + 10 kb C -> T (2x) | F508/3849 + 10 kb C -> T (2x) |
|                          | F508/G542X | F508/R347W | F508/R347W | F508/R347W |
|                          | F508/N1303K | F508/7 | F508/7 | F508/7 |
|                          | F508/1248 + 1G-A | F508/1248 + 1G-A | F508/1248 + 1G-A | F508/1248 + 1G-A |
|                          | F508/dele 14a,15,16,17a,17b | F508/dele 14a,15,16,17a,17b | F508/dele 14a,15,16,17a,17b | F508/dele 14a,15,16,17a,17b |

Data are shown as ratios or the mean ± standard deviation incl. median (min – max). *p <0.001 (CF-all versus controls); **p = 0.003 (CF-PI versus CF-PS).
Inc., Logan, USA) was used. Chloride was measured by chloride titration. A sweat chloride level ≥60 mmol/l was interpreted as within the CF range, 30–60 mmol/l as equivocal, and ≤29 mmol as normal [1].

NPD was performed by one operator who was accredited by the Cystic Fibrosis Foundations’ (CFF) Therapeutic Developments Network (TDN), and followed the CFF TDN SOP (version: January 2009) [8]. We used terbutaline as a substitute for isoproterenol in accordance with the SOP. The chloride-free and isoproterenol response (Δ0Cl− + Iso) (NPD CFTR response) and the Wilschanski score (defined as e(response to chloride-free and isoproterenol/response to amiloride))^2 [20] representing the CFTR response were calculated as the average or best result from both nostrils. The Δ0Cl− + Iso was interpreted as normal when < −12 mV, as in the CF range when > −7.7 mV, and as equivocal for results between −12 and −7.7 mV [6]. The Wilschanski score was interpreted as normal (<0.65), in the CF-range (>0.70), or equivocal (0.65–0.70) [20]. If the mean Δ0Cl− + Iso was > −7.7 mV in healthy controls, the NPD was repeated on a different day. Only the measurement with the highest Δ0Cl− + Iso was reported. If both measurements confirmed a Δ0Cl− + Iso in the CF range, CFTR genotyping (sequencing and multiplex ligation-dependent probe amplification) was offered as part of the participant’s clinical care and reported as part of the baseline data. Genotyping of all healthy controls was not ethically approved.

The ICM followed the European Cystic Fibrosis Society-Therapeutic Development Network (ECFS-TDN) SOP (V2.7; Oct 26, 2011), which is based on the Rotterdam protocol. The tissues sliders (P2407C [1.5 mm diameter aperture slider; area 0.018 cm²] or P2407B [1.2 mm diameter aperture slider; area 0.011 cm²]; Physiologic Instruments, San Diego, USA) were mounted without tissue in the chambers (4-chamber system [EM-LV SYS-4; Physiologic Instrument, San Diego, USA]), which were filled on both sides with 2 ml Meyler buffer solution (10 mM Hepes; 0.3 mM Na2HPO4; 0.4 mM NaH2PO4; 1.0 mM MgCl2; 1.3 mM CaCl2; 4.7 mM KCl; 128 mM NaCl; 20.2 mM NaHCO3; 10 mM D-Glucose; 0.01 mM indomethacin; pH 7.4; osmolarity 300 mOsm). PowerLab (4/30; ADInstruments Ltd., Dunedin, New Zealand) and Labchart® software (release 7.2; ADInstruments Ltd., Dunedin, New Zealand) were used for data acquisition. A stable open Potential Difference (PD) was ensured and an input offset to 0 mV was performed. Fluid resistance compensation was performed by applying short current pulses.

Figure 1 Correlation of average Δ0Cl− + Iso (NPD) and sweat chloride. The normal range is indicated for values below and left of the dotted lines, and the intermediate range is shown between the solid and dotted lines.
(15 μA) by the VCC MC4S Multi-Channel Voltage Current Clamp (Physiologic Instrument, San Diego, USA) and adjusting the fluid resistance.

For ICM, at least 4 superficial rectal biopsies were obtained by suction biopsies (aspiration biopsy instrument according to Wilital (UE7605); ulrich GmbH, Ulm, Germany) without prior bowel preparation. Biopsies were immediately stored in ice-cold buffer solution (Dulbecco’s phosphate buffered saline and indomethacin, final concentration 10 μM) and mounted on adequate tissue sliders. After mounting the sliders in the heated and slightly sparged (95% O2/5% CO2) 4-chamber system, each basal resistance was measured by applying short current pulses (15 μA) and registering the corresponding change in Vt (typical range 15–30 Ohm × cm²) with the VCC MC4S Multi-Channel Voltage Current Clamp (Physiologic Instrument, San Diego, USA). After that the voltage was clamped at 0 mV and the raw short circuit current (Isc) was recorded from then on. Due to different sliders with different areas (P2407C [1.5 mm diameter aperture slider; area 0.018 cm²] or P2407B [1.2 mm diameter aperture slider; area 0.011 cm²]; Physiologic Instruments, San Diego, USA), the raw Isc was converted to Isc (μA/cm²). After applying 100 μM carbachol (which stimulates cholinergic Cl⁻ secretion by opening basolateral K⁺ channels) to the serosal compartment, an Isc response was evoked for quality control of the biopsy. After a 40-min equilibration in Meyler buffer, the basal Isc was noted and 2 μl amiloride (to block amiloride-sensitive sodium channels) was added to the mucosal compartment. After 5 min or when the Isc was stable, 10 μM forskolin and 100 μM IBMX (to stimulate cAMP-dependent CFTR-Cl⁻ transport) were added to the mucosal and serosal compartments (ΔIsc, forskolin/IBMX). After a minimum of 10 min, 10 μM genisteine (CFTR-potentiator) was added to both compartments. After a minimum of 5 min, 100 μM carbachol was added to the serosal compartment (ΔIsc, carbachol). After a minimum of 10 min, 200 μM 4,4′-Diisothiocyano-2,2′-stilbenedisulfonic acid (DIDS) (blocking non-CFTR-Cl⁻ channels) was added to the mucosal compartment. After 5 min, 500 μM histamine (to stimulate Ca²⁺ and protein kinase C-mediated CFTR Cl⁻ secretion) was added to the serosal compartment (ΔIsc, histamine). In the open circuit, the final transepithelial voltage and final resistance were measured by applying short current pulses as in the beginning. Preliminary data suggested that the average ΔIsc, forskolin/IBMX+carbachol+histamine is the best diagnostic ICM parameter for chloride secretory response (ICM CFTR response), but reference ranges have not been established [10].

![Figure 2](http://www.biomedcentral.com/1471-2466/14/156)

**Figure 2** Correlation of the average Wilschanski score (NPD) and sweat chloride. The normal range is shown below and left of the dotted lines, and the intermediate range is shown between the solid and dotted lines.
Data are presented as the mean ± standard deviation (SD) (normally distributed variables), respective the median values, and 25th and 75th percentiles (non-normally distributed variables). Group comparisons were performed using the Student’s t test or Mann–Whitney U test for normally or non-normally distributed variables, respectively. Statistical significance was defined as p < 0.05. All analyses were performed with IBM SPSS Statistics 21 (release 21; IBM, Armonk, USA).

Results

Our study included 18 patients with CF and 13 healthy controls with a median age of 20.5 and 25.0 years, respectively (NS) (Table 1). As consequence of our inclusion criteria, sweat chloride values distinguish patients with CF from healthy controls (p < 0.001). Although the mean sweat chloride differed between CF-PS and CF-PI patients (p = 0.003), an individual overlap occurs (Table 1, Figure 1). The mean NPD CFTR response significantly discriminated between CF patients and healthy controls (p < 0.001), but not between patients with CF who were PS versus PI (Table 1). Four healthy controls had an average \( \Delta 0\text{Cl}^- + \text{Iso} \) of >−7.7 mV. Three out of four controls had a repeatable average NPD CFTR response in the CF-range for \( \Delta 0\text{Cl}^- + \text{Iso} \geq −7.7 \) mV (23% of all healthy controls) (Figure 1), and two additional controls when using the Wilschanski score (15% of all healthy controls) (Figure 2). All these healthy controls were smokers. CFTR genotyping was offered to these three healthy controls as part of clinical routine and none had two CF-causing mutations (Table 1). For ICM a median of 6 (5–7) rectal biopsies were sampled per patient without severe adverse events. The mean ICM CFTR response was significantly different between CF patients and healthy controls (p < 0.001), but not between patients with CF who were PS versus those who were PI (Table 1). We could not detect any age-dependency of the response to Isoproterenol/Forskolin. In contrast to NPD, there was no overlap between CF-patients and controls (Figures 3 and 4). Using the best instead of the average NPD, the CFTR response overlap did not change (Additional file 1). Using the best instead of the average ICM, the CFTR response resulted in one overlap (Additional file 2).

In healthy controls, smoking had no influence on sweat chloride (NS), but decreased CFTR function as measured by NPD (p = 0.049) (Table 2 and Figure 5).
and resulted in intermediate or even abnormal NPD results, but not ICM or sweat chloride results. There is a trend in ICM measurements indicative that smoking might not only affect CFTR in the respiratory tract, but also in the intestine (Table 2 and Figure 6).

Discussion
ICM is superior to NPD for distinguishing between CF patients with a sweat chloride >60 mmol/l and healthy controls. Neither NPD nor ICM differentiated patients with CF who were PS from those who were PI. Smoking has a negative impact on CFTR function in healthy controls measured by NPD, and challenges the diagnostic interpretation of NPD. There is a trend in ICM indicative that smoking might not only affect CFTR in the respiratory tract, but also in the intestine, which has no impact on diagnostic interpretation.

NPD has been used as a diagnostic test for CF since the late 1980s [14,21,22]. Studies have shown 94.8–100% sensitivity and 96.5–100% specificity of Δ0Cl− + Iso for separating PI patients with CF from healthy controls [13,14,23]. Experience with a broader spectrum of patients with CF [24,25] and equivocal patients (sweat chloride <60 mmol/l and less than two CF-causing mutations) [20] described a clinically relevant overlap for ΔCl− + Iso. Even in F508del homozygous patients, a residual CFTR NPD response with [26] or without [27,28] an observed clinical difference has been described. Some centers introduced an intermediate category for ΔCl− + Iso [6], interpret the highest NPD CFTR response [29], or use a composite score that includes sodium and chloride conductance [20,30].Irrespective of the diagnostic criteria, our result showed a clinically

Table 2 Influence of smoking (active and/or passive) in healthy controls on NPD and ICM CFTR responses

|                        | Nonsmokers | Smokers | p-value |
|------------------------|------------|---------|---------|
| Sweat chloride (mmol/L)| 18 ± 8     | 20 ± 9  | NS      |
| NPD CFTR response      | −19.3 ± 7.4| −10.1 ± 7.3| 0.049  |
| Average Δ0Cl− + Iso (mV)| 97.2 ± 37.1| 65.7 ± 29.1| NS      |
| ICM CFTR response      | 97.2 ± 37.1| 65.7 ± 29.1| NS      |
| Average ΔIsc (μA/cm²)  | (forskolin/IBMX + carbachol + histamine) | All values are shown as the mean ± standard deviation.
relevant overlap between patients with CF and healthy controls. A normal average NPD CFTR response excludes CF, but an abnormal average NPD CFTR response could occur in healthy controls, especially in smokers, and can lead to a false-positive diagnosis of CF. A repeated measurement of a pathologic NPD response reduced the false-positive results in 1 out of 4 healthy controls in our cohort and should be recommended as a standard approach.

ICM was developed as a research tool for CFTR function in the 1990s [31] and has been used as a diagnostic test since the early 2000s [15,32]. Two different protocols are established; the Freiburg protocol [9] and the original [33] and adapted [34] Rotterdam protocol. We used the newest ECFS-ICM-SOP, which is an adapted Rotterdam protocol. The combination of cAMP-mediated Cl− secretion, and the carbachol and histamine (Rotterdam protocol) responses separate patients with CF from those without CF [11,12,34], but not patients with CF who are PS from those who are PI [11,12], which is in accordance with our results. The 50% loss of CFTR protein in CF heterozygotes could not be detected by ICM [35] independent of the protocol [15,32]. For the Rotterdam protocol, De Jonge postulated that the ICM response is not proportional to the CFTR amount in the apical membrane of colonocytes except at a low level (<10–15%) and could therefore only detect an 80–85% loss of CFTR expression/function [32]. Therefore, mild mutations could result in a false-negative ICM. Interestingly, Derichs reported 8 patients with a sweat chloride >60 mmol/l, fewer than two CF-causing mutations after sequencing, and a normal ICM response who were judged as CF unlikely [12]. Our results with the new ECFS-ICM SOP confirm the high predictive value and practicability of this adapted ICM Rotterdam protocol.

Our data suggest that NPD is more likely to detect CFTR dysfunction in healthy controls than the ICM or sweat test. This could be explained by tissue specific differences in CFTR expression, alternative chloride channel expression, or extrinsic factors. Kälin et al. showed identical CFTR expression in the respiratory and intestinal tract of F508del-homozygous patients and healthy controls [36]. Highly variable CFTR expression in the nose [37] and colon [38] of F508del homozygous patients has been described, varying from 0–100% [37]. Therefore, in the respiratory and intestinal tract, individual CFTR expression seems to be more relevant than tissue specific expression. Alternative chloride channels could contribute to the chloride conductance, but have not been described in the distal colon [39]. Furthermore, previous infections [40], milder trauma [14], smoking [17], increased paracellular permeability [41], and decreased CFTR expression [42] and CFTR response [43]. With the exception of smoking, these extrinsic factors are relevant only for NPD, but not for rectal biopsies [44]. Smoking causes a decreased NPD response [17], but although a decreased systemic CFTR function mediated by acrolein [18]. Raju et al. demonstrated a 65% decrease in

Figure 5 Average ΔOCi− + Iso (NPD) in healthy controls according to smoking status. The normal range is shown below the dotted line and the intermediate range is shown between the solid and dotted lines.
the ICM CFTR response in healthy smokers compared with non-smokers [18]. Our results confirm these findings. In contrast to NPD, smoking did not influence the diagnostic cut-off for ICM in our cohort. Therefore, ICM seems to be a more robust diagnostic test than NPD to distinguish primary from secondary CFTR dysfunction. This is important for the interpretation of NPD as an adjunctive diagnostic test in patients with query-CF who are exposed to smoking.

Limitations of our study include the small number of participants in each group, the lack of patients with CF with a sweat chloride value < 60 mmol/l, and patients with congenital bilateral absence of the vas deferens (CBAVD). The strength of our study is the genotyping of healthy controls with an abnormal CFTR NPD response, and the use of standardized protocols for sweat testing, NPD, and ICM.

Conclusions
From our results, a normal average NPD CFTR response excludes CF, but an intermediate or abnormal NPD CFTR response could be detected in healthy controls. NPD should be judged carefully, especially in patients with chronic rhinosinusitis and exposure to smoking. ICM combined with cAMP-mediated and cholinergic CI secretion seems to be a practicable diagnostic test with an increased specificity compared with NPD. Discordant results of both CFTR functional tests could be detected and challenge the diagnostic interpretation. Larger study groups that include smokers and patients with CBAVD or CF with a sweat chloride between 30–60 mmol/l are needed to confirm our results.

Additional files

Additional file 1: Correlation of the best and average $\Delta\Delta$Cl$^-$ (NPD). The normal range is shown below and left of the dotted lines. The intermediate range is shown between the solid and dotted lines.

Additional file 2: Correlation of the best and average $\Delta$Is$forskolin/IBMX + carbachol + histamine$ (ICM). A higher $\Delta$Is$forskolin/IBMX + carbachol + histamine$ represents a better CFTR response.

Competing interests
The authors declare that they have no competing interests.

Authors’ contributions
ABH recruited participants, performed the rectal biopsies, supported the NPD measurements, and helped draft the manuscript. SN recruited participants, performed the ICM measurements, and helped draft the manuscript. CRN supported the NPD measurements, performed the ICM measurements, and helped draft the manuscript. LN conceived the study, recruited participants, performed the rectal biopsies and statistical analysis, and drafted the manuscript. All authors read and approved the final manuscript.

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37. van Meegen MA, Terheggen-Lagro SW, Kaymans KJ, van der Ent CK, Beekman JM: Apical CFTR expression in human nasal epithelium correlates with lung disease in cystic fibrosis. *PloS One* 2013, 8(3):e57617.

38. van Barneveld A, Stanke F, Tamm S, Siebert B, Brandes G, Derichs N, Ballmann M, Junge S, Tummler B: Functional analysis of F508del CFTR in native human colon. *Biochim Biophys Acta* 2010, 1802(11):1062–1069.

39. Greger R: Role of CFTR in the colon. *Annu Rev Physiol* 2000, 62:467–491.

40. Chilvers MA, McKean M, Rutman A, Myint BS, Silverman M, O’Callaghan C: The effects of coronavirus on human nasal ciliated respiratory epithelium. *Eur Respir J* 2001, 18(6):965–970.

41. Yeo NK, Jang YJ: Rhinovirus infection-induced alteration of tight junction and adherens junction components in human nasal epithelial cells. *Laryngoscope* 2010, 120(2):346–352.

42. Brezillon S, Dupuit F, Hinnrasky J, Marchand V, Kalin N, Tummler B, Puchelle E: Decreased expression of the CFTR protein in remodeled human nasal epithelium from non-cystic fibrosis patients. *Lab Invest* 1995, 72(2):191–200.

43. Knowles M, Murray G, Shallal J, Askin F, Ranga V, Gatzy J, Boucher R: Bioelectric properties and ion flow across excised human bronchi. *J Appl Physiol* 1984, 56(4):868–877.

44. Servidoni MF, Sousa M, Vinagre AM, Cardoso SR, Ribeiro MA, Meirelles LR, de Carvalho RB, Kunzelmann K, Ribeiro AF, Ribeiro JD, Amaral MD: Rectal forceps biopsy procedure in cystic fibrosis: technical aspects and patients perspective for clinical trials feasibility. *BMC Gastroenterol* 2013, 13(1):91.