



## Acquired Cystic Fibrosis Transmembrane Conductance Regulator Dysfunction in the Lower Airways in COPD

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**Background:** Cigarette smoke and smoking-induced inflammation decrease cystic fibrosis transmembrane conductance regulator (CFTR) activity and mucociliary transport in the nasal airway and cultured bronchial epithelial cells. This raises the possibility that lower airway CFTR dysfunction may contribute to the pathophysiology of COPD. We compared lower airway CFTR activity in current and former smokers with COPD, current smokers without COPD, and lifelong nonsmokers to examine the relationships between clinical characteristics and CFTR expression and function.

**Methods:** Demographic, spirometry, and symptom questionnaire data were collected. CFTR activity was determined by nasal potential difference (NPD) and lower airway potential difference (LAPD) assays. The primary measure of CFTR function was the total change in chloride transport ( $\Delta$ chloride-free isoproterenol). CFTR protein expression in endobronchial biopsy specimens was measured by Western blot.

**Results:** Compared with healthy nonsmokers ( $n = 11$ ), current smokers ( $n = 17$ ) showed a significant reduction in LAPD CFTR activity ( $\Delta$ chloride-free isoproterenol,  $-8.70$  mV vs  $-15.9$  mV;  $P = .003$ ). Similar reductions were observed in smokers with and without COPD. Former smokers with COPD ( $n = 7$ ) showed a nonsignificant reduction in chloride conductance ( $-12.7$  mV). A similar pattern was observed for CFTR protein expression. Univariate analysis demonstrated correlations between LAPD CFTR activity and current smoking, the presence of chronic bronchitis, and dyspnea scores.

**Conclusions:** Smokers with and without COPD have reduced lower airway CFTR activity compared with healthy nonsmokers, and this finding correlates with disease phenotype. Acquired CFTR dysfunction may contribute to COPD pathogenesis. *CHEST 2013; 144(2):498–506*

**Abbreviations:** BCSS = Breathlessness, Cough, and Sputum Scale; CF = cystic fibrosis; CFTR = cystic fibrosis transmembrane conductance regulator; HNE = human neutrophil elastase; LAPD = lower airway potential difference; NPD = nasal potential difference

The public health impact of COPD continues to increase, now accounting for  $>$ \$40 billion in annual health-care costs<sup>1</sup> and surpassing stroke as the third leading cause of death in the United States.<sup>2</sup> At present, there are no effective therapies for the mucus stasis characteristic of the disease, although its presence has been associated with accelerated loss of lung function and mortality.<sup>3,4</sup> This is due to multiple complex mechanisms and the absence of a potent and bioavailable drug to target these pathways.<sup>5</sup>

Small airway mucus obstruction in COPD leads to a pathologic cascade involving bacterial colonization,

chronic neutrophilic inflammation, and recurrent exacerbations that are reminiscent of cystic fibrosis (CF) lung disease.<sup>6–8</sup> The phenotypic similarity between COPD and CF is most pronounced in those with symptoms of chronic bronchitis in whom a relatively high incidence of bronchiectasis has been reported<sup>9</sup>; however, the overproduction, poor clearance, and accumulation of hyperviscous mucus within the small airways is also present in patients with an emphysema-dominant phenotype.<sup>3,4</sup>

An improved understanding of the role of CF transmembrane conductance regulator (CFTR) in the

maintenance of normal epithelial function has revealed that mild and variable *CFTR* mutations play a causative role in a number of diseases not classically associated with CF.<sup>10</sup> With the clinical validation of potent modulators of *CFTR* ion channel activity,<sup>11</sup> there is considerable interest in examining the role of *CFTR* dysfunction in the pathogenesis of other diseases not previously believed to result from reduced epithelial ion transport, including COPD.

Our laboratory and others have reported that cigarette smoke exposure reduces *CFTR* expression, function, and mucociliary transport<sup>12-15</sup> in vitro and that smokers with and without COPD exhibit reduced *CFTR*-dependent nasal potential difference (NPD) in the upper airway.<sup>15</sup> However, *CFTR* activity has not been previously measured in the lungs of individuals with COPD. We hypothesized that *CFTR* dysfunction would be present in the lower airway of individuals with COPD and that the abnormality would correlate with the presence of chronic bronchitis and markers of disease severity.

## MATERIALS AND METHODS

### *Study Subjects and Study Protocol*

The study was approved by the University of Alabama at Birmingham Institutional Review Board (approval number F090626003), and all patients provided written informed consent. Patients aged 40 to 80 years were categorized into four subgroups by smoking status and the presence or absence of airflow obstruction ( $FEV_1/FVC$  below the lower limit of normal for age, race, sex, and height). A minimum of 10 pack-years tobacco use was required for patients with COPD, and abstinence was confirmed with serum cotinine levels. Former smokers were abstinent for  $\geq 1$  year. Exclusion criteria were asthma, bronchiectasis, or other lung disease and any change in respiratory medications or respiratory illness in the month prior to enrollment.

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### *Study Assessments*

**Spirometry and Exercise Testing:** Postbronchodilator spirometry and 6-min walk tests were performed according to American Thoracic Society standards.<sup>16,17</sup>

**Questionnaires:** Dyspnea was assessed with the Modified Medical Research Council scale.<sup>18</sup> Subjects were queried about the presence of chronic bronchitis on the basis of the American Thoracic Society/Medical Research Council definition of cough and sputum present most days of 3 months in 2 consecutive years. The severity of bronchitis symptoms was assessed with the Breathlessness, Cough, and Sputum Scale (BCSS).<sup>19</sup>

**NPD Measurements:** NPD studies were performed according to the description by Solomon et al<sup>20</sup> with Electronic Data Capture (ADInstruments), KCl calomel electrodes (Thermo Fisher Scientific Inc), and 3% agar nasal catheter and reference bridges.<sup>15,21</sup>

**Lower Airway Potential Difference Measurements:** During bronchoscopy under conscious sedation with limited topical anesthesia (1% lidocaine to the vocal cords and right mainstem bronchus), lower airway potential difference (LAPD) measurements were performed to quantify *CFTR* activity in the lower airway (Fig 1). The equipment and conditions for measuring bioelectric changes across the airway mucosa were based on the standard NPD protocol adapted to the bronchoscopic technique, including use of agar-filled PE90 tubing for the probing electrode and limiting perfusion solutions to Ringer, Ringer plus amiloride (100  $\mu$ M), and chloride-free gluconate with amiloride plus isoproterenol (100  $\mu$ M).<sup>22</sup> A stable potential with the mean value of a 10-s scoring interval after perfusion of each solution was recorded by a blinded investigator. All measures were made at the lingula outlet after airway inspection.

**BAL:** BAL (125 mL saline) was performed immediately after LAPD measurements in the lingula.

***CFTR* Protein Expression in Endobronchial Biopsy Specimens:** After LAPD measurements and BAL, six endobronchial biopsy specimens were obtained from second- through fifth-order bifurcations in the lower lobes and were flash frozen in liquid nitrogen. *CFTR* protein expression was estimated by Western blot with a 1:1 mixture of 570 and 596 monoclonal anti-*CFTR* antibodies.<sup>23-25</sup>

**Human Neutrophil Elastase Assay:** To estimate the activity of human neutrophil elastase (HNE) in BAL supernatants, InnoZyme

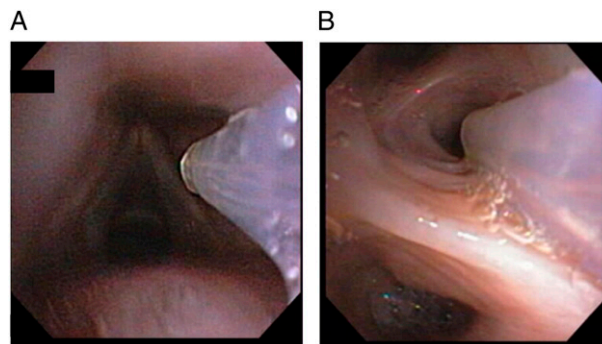


FIGURE 1. Bronchoscopic placement of the lower airway potential difference catheter. A, Supraglottic view. B, View from position in the lingula outlet.

Human Neutrophil Elastase Immunocapture Activity assay kits (Calbiochem-EMD Millipore) were used.<sup>26</sup>

**Inflammatory Cytokine Assay:** BAL supernatants were analyzed for the presence of inflammatory mediators by MILLIPLEX MAP Human Cytokine/Chemokine Premixed 42 Plex assay kits (EMD Millipore).

**Genetic Analysis:** CFTR genetic testing was performed at a commercially accredited facility (Medical Genetics Laboratories at Baylor College of Medicine, Houston, Texas) and included the 23 mutations recommended by the American College of Medical Genetics. Patients with any CF-causing allele were excluded.

### Statistical Analysis

All measures of CFTR activity and expression were analyzed with descriptive statistics. Results between groups were compared by analysis of variance, and  $P < .05$  was considered significant. Sample size calculations were based on prior experience with NPD and indicated that enrollment of eight patients per group would provide 80% power to detect a 5-mV difference in LAPD ( $\Delta$ chloride-free plus isoproterenol) compared with healthy nonsmokers. Pearson correlations were performed to examine the relationship between clinical characteristics and LAPD-measured CFTR activity as well as between NPD and LAPD. Univariate linear regression was used to determine whether clinical parameters or inflammatory markers were associated with LAPD.

## RESULTS

### Study Subjects

We enrolled 48 patients of whom eight did not meet spirometric inclusion criteria or were excluded because of comorbid illnesses that increased the risk of bronchoscopy complications (e-Fig 1). Of the remaining 40 patients, 35 had acceptable LAPD tracings. Tracings were not available for five patients because of technical issues with the perfusion catheter ( $n = 3$ ), leakage of perfusion fluids ( $n = 1$ ), or patient intolerance of bronchoscopy ( $n = 1$ ). Three patients (two COPD smokers and one healthy non-

smoker) underwent repeat testing to assess the within-subject reproducibility of LAPD; the mean values of the two independent measures were used in these cases. There were no instances of clinically significant bronchospasm, hypoxemia, or bleeding. Subject groups were well matched for age, although there were numerically more women among healthy nonsmokers and fewer African Americans among COPD smokers (Table 1). A range of lung function was observed in patients with COPD ( $FEV_1$ , 35%-82% predicted), and active smokers were more likely to have chronic bronchitis ( $P < .001$ ).

### Measures of CFTR Activity in the Lower Airway

Representative LAPD tracings are shown in Figure 2 and demonstrated the expected change in potential difference in normal control patients with sequential perfusion of Ringer solution, Ringer solution plus amiloride, and chloride-free isoproterenol. Healthy smokers and COPD smokers showed a reduced response to chloride-free isoproterenol, whereas COPD former smokers showed an intermediate response. A significant difference in CFTR-dependent chloride transport ( $\Delta$ chloride-free isoproterenol) was observed among the four patient groups (Fig 3A). Healthy smokers showed a significant reduction in  $\Delta$ chloride-free isoproterenol compared with normal control patients ( $-8.35 \pm 3.10$  mV vs  $-15.90 \pm 5.73$  mV;  $P < .002$ ), and a similar decrement was observed in smokers with COPD ( $-9.19 \pm 7.21$  mV;  $P = .054$ ). Former smokers with COPD showed a modest reduction in chloride transport, although this finding was not statistically significant ( $-12.70 \pm 7.88$  mV). All patients with COPD showed a trend toward reduced chloride transport compared with healthy nonsmokers ( $-10.90 \pm 7.75$  mV;  $P = .09$ ), an effect mediated by greater variability among COPD former smokers.

**Table 1—Patient Characteristics**

| Characteristic               | Healthy Nonsmokers<br>(n = 11) | Healthy Smokers<br>(n = 10) | Smokers With<br>COPD (n = 7) | Former Smokers<br>With COPD (n = 7) |
|------------------------------|--------------------------------|-----------------------------|------------------------------|-------------------------------------|
| Age, y                       | 58 (41-74)                     | 50 (45-62)                  | 54 (52-63)                   | 66 (59-70)                          |
| Female sex                   | 8 (73)                         | 3 (30)                      | 1 (14)                       | 2 (29)                              |
| African American race        | 6 (55)                         | 7 (70)                      | 1 (14)                       | 5 (71)                              |
| BMI, kg/m <sup>2</sup>       | 31 (22-43)                     | 30 (24-38)                  | 25 (21-41)                   | 29 (21-54)                          |
| FEV <sub>1</sub> % predicted | 97 (90-109)                    | 90 (85-128)                 | 48 (36-82)                   | 65 (43-73)                          |
| FEV <sub>1</sub> /FVC        | 0.79 (0.72-0.86)               | 0.78 (0.71-0.88)            | 0.43 (0.36-0.68)             | 0.53 (0.41-0.63)                    |
| Pack-y                       | 0 (0-0)                        | 33 (12-54)                  | 52.5 (35-147)                | 45 (39-80)                          |
| Chronic bronchitis           | 0 (0)                          | 5 (50)                      | 5 (71)                       | 1 (14)                              |
| BCSS                         | 0 (0-0)                        | 0.5 (0-4)                   | 5 (2-9)                      | 1 (0-7)                             |
| MMRC dyspnea score           | 0 (0-1)                        | 1 (0-2)                     | 1 (0-3)                      | 2 (0-3)                             |
| Exacerbation in prior year   | 0 (0)                          | 1 (10)                      | 1 (14)                       | 2 (29)                              |
| 6-min walk, m                | 417 (349-457)                  | 372 (146-503)               | 303 (253-480)                | 330 (137-388)                       |
| BODE index                   | N/A                            | N/A                         | 3 (1-4)                      | 2 (1-5)                             |

Data are presented as median (range) or No. (%). BCSS = Breathlessness, Cough, and Sputum Scale; BODE = BMI, obstruction, dyspnea, exercise tolerance; MMRC = Modified Medical Research Council; N/A = not applicable.

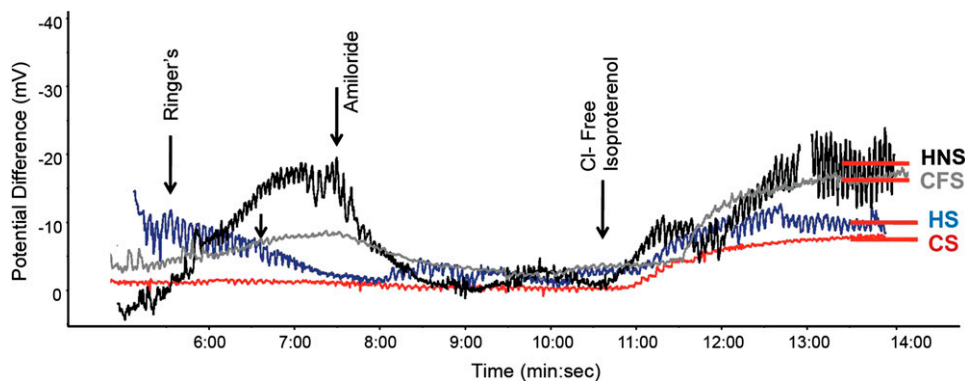


FIGURE 2. Lower airway potential difference in smokers with and without COPD. Representative lower airway potential difference tracings for each disease group. The start of each perfusion solution is marked with an arrow. Stable potential difference achieved after chloride-free isoproterenol perfusion is denoted with a red bar. High-frequency (0.2-0.5 Hz) noise most visible at the end of the tracing represents artifact caused by respiration. CFS = COPD former smoker; CS = COPD smoker; HNS = healthy nonsmoker; HS = healthy smoker.

Two smokers with COPD and one healthy nonsmoker underwent two bronchoscopic LAPD measurements during procedures occurring > 1 month apart. The mean difference in  $\Delta$ chloride-free isoproterenol between the two measurements was  $1.43 \pm 3.59$  mV, which compared favorably to prior NPD studies.<sup>20,23</sup> LAPD also revealed abnormalities in estimates of sodium transport, including reduced baseline (Fig 3B) and amiloride-sensitive (Fig 3C) potential differences in healthy smokers and COPD smokers compared with healthy nonsmokers.

#### CFTR Protein Expression

The reduction in CFTR activity in smokers with and without COPD was supported by suppressed CFTR protein expression in endobronchial biopsy specimens (Fig 4). Concordant with functional studies, COPD former smokers had intermediate expression.

#### Relationship Between CFTR Activity in the Lower Airway and Clinical Status

Univariate analysis of likely contributors to reduced CFTR activity in the lower airway revealed statistically significant associations between chloride transport measured by LAPD and current smoking, the presence of chronic bronchitis, and dyspnea (Table 2). Trends between CFTR activity and BCSS score, lung function, and smoking intensity (pack-years) were also observed. Both BCSS and chronic bronchitis were correlated with current smoking ( $r = 0.57$  [ $P < .001$ ] and  $r = 0.39$  [ $P = .02$ ], respectively).

#### Correlation Between CFTR Function in the Nose and Lower Airway

No significant correlation was observed between CFTR-dependent chloride transport measured by

NPD and that measured by LAPD ( $r = 0.17$ ,  $P = .34$ ) (Fig 5A). LAPD did offer additional information regarding CFTR function, particularly in the COPD former smoker group. As shown in Figure 5B, three of seven former smokers with COPD had an LAPD chloride conductance of less than normal, despite having an NPD that was greater than normal. This pattern was observed in only one of 10 healthy smokers, one of 11 healthy nonsmokers, none of seven COPD smokers, and two of 28 in the other disease groups combined ( $P < .05$ ).

#### Relationship Between LAPD and the Inflammatory Microenvironment of the Lung

As demonstrated in Figure 6A, HNE activity was significantly increased in smokers with and without COPD compared with healthy nonsmokers ( $P < .05$ ); activity was also increased in former smokers with COPD, although this difference was not statistically significant. Correlation of HNE levels with CFTR-dependent ion transport as determined by LAPD did not demonstrate a significant relationship between the two (Fig 6B). As summarized in e-Table 1, differences in other BAL inflammatory mediators were observed between groups, although none exhibited a consistent finding that could relate lung inflammation to CFTR activity.

## DISCUSSION

The results demonstrate that smokers with and without COPD have reduced chloride conductance in the lower airway and that this ion transport abnormality is associated with the presence of chronic bronchitis and dyspnea. These results extend previous findings, suggesting that cigarette smoke has deleterious

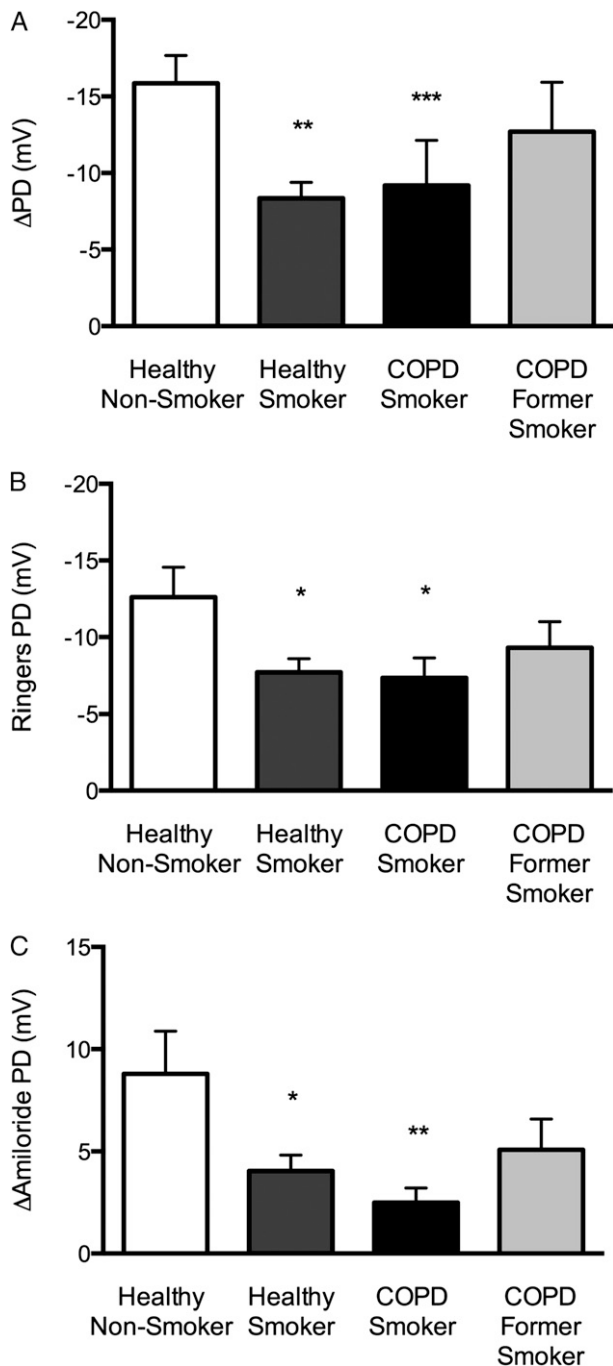


FIGURE 3. Ion transport activity in smokers with and without COPD. A, Cystic fibrosis transmembrane conductance regulator (CFTR) activity estimated in the lower airway by the change in PD following perfusion with chloride-free isoproterenol.  $**P < .005$ ;  $***P = .054$ . B, Baseline potential difference as determined by the stable PD following perfusion with Ringer solution. Baseline PD was lower in both healthy smokers ( $-7.71 \pm 0.88$ ) and COPD smokers ( $-7.33 \pm 1.30$ ) than in healthy nonsmokers ( $-12.61 \pm 1.94$ ).  $*P < .05$ . C, Amiloride-sensitive PD determined by the change in PD with amiloride perfusion. Amiloride-sensitive PD was lower in healthy smokers ( $4.04 \pm 0.78$  mV) and COPD smokers ( $2.49 \pm 0.72$  mV) than in healthy nonsmokers ( $8.79 \pm 2.09$  mV); COPD former smokers again were intermediate but not statistically different from healthy control patients ( $5.08 \pm 1.50$ ).  $*P < .05$ ;  $**P < .005$ . PD = potential difference.

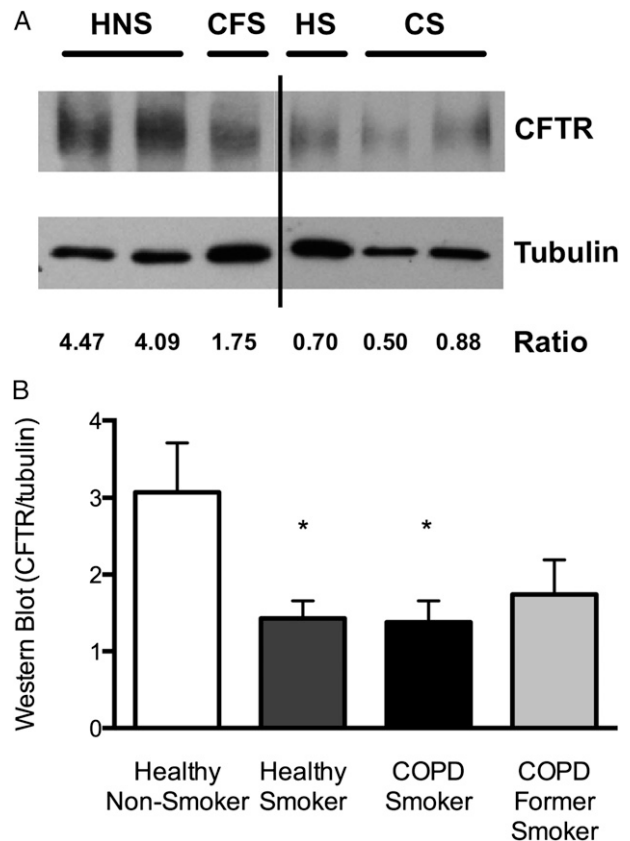


FIGURE 4. CFTR expression in the lower airway as determined by Western blot analysis. A, Representative blot demonstrating CFTR C-band expression compared with tubulin loading control. The densitometry ratio for each respective lane is shown. B, Summary data of that shown in A.  $*P < .05$ . See Figure 2 and 3 legends for expansion of abbreviations.

effects on CFTR activity and epithelial function,<sup>12-15,27</sup> and establish a link between CFTR dysfunction in the lung and clinically relevant COPD symptoms. Detection of this ion transport abnormality was facilitated by the development of a reliable technique for measuring transepithelial LAPD during bronchoscopy

Table 2—Univariate Analysis Between Clinical Characteristics and Percent Normal LAPD

| Parameter                    | $\beta$ (95% CI)       | P Value |
|------------------------------|------------------------|---------|
| Age                          | 1.08 (−0.73 to 2.88)   | .23     |
| Female sex                   | 14.8 (−15.6 to 45.3)   | .33     |
| African American race        | −3.91 (−34.3 to 26.5)  | .80     |
| Current smoking              | −37.4 (−64.7 to −10.2) | .01     |
| Smoking intensity            | −0.43 (−0.91 to 0.04)  | .07     |
| BMI                          | 0.96 (−1.15 to 3.06)   | .36     |
| FEV <sub>1</sub> % predicted | 48.6 (−12.2 to 109)    | .11     |
| FEV <sub>1</sub> /FVC        | 22.1 (−15.0 to 59.1)   | .23     |
| Chronic bronchitis           | −35.2 (−65.3 to −5.04) | .02     |
| BCSS                         | −4.73 (−10.4 to 0.89)  | .10     |
| MMRC dyspnea score           | −18.4 (−33.0 to −3.88) | .01     |
| Exacerbation in prior year   | 9.85 (−37.6 to 57.3)   | .68     |
| 6-min walk                   | 0.05 (−0.12 to 0.23)   | .53     |

See Table 1 legend for expansion of abbreviations.

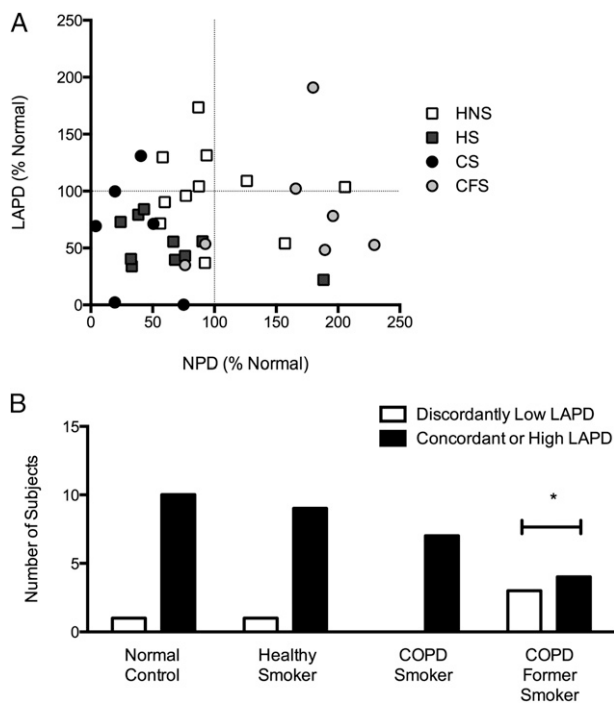


FIGURE 5. Correlation of CFTR function as estimated by NPD and LAPD. Each parameter was calculated as the  $\Delta$ chloride-free isoproterenol perfusion compared with the respective value in the normal control population. A, Correlation between NPD and LAPD. No significant correlation was observed between CFTR-dependent chloride transport measured by NPD compared with LAPD in the overall population ( $r = 0.17$ ,  $P = .34$ ) or in healthy nonsmokers ( $r = -0.13$ ,  $P = .70$ ). B, Number of patients with a discordantly low LAPD compared with NPD vs the number of patients with a concordant or high LAPD. Discordantly low LAPD was defined as an LAPD below the mean (eg,  $< 100\%$  of normal) when the NPD was equal to or above the mean (eg,  $\geq 100\%$  of normal). \* $P < .05$  by Fisher exact test. LAPD = lower airway potential difference; NPD = nasal potential difference. See Figure 2 and 3 legends for expansion of other abbreviations.

under conscious sedation, a method suitable for future research applications, including use in clinical trials to monitor the effect of CFTR modulators.

This study is the first to demonstrate, to our knowledge, that abnormal chloride transport is present in the lungs of smokers with and without COPD and that this defect is associated with clinical phenotype, specifically the presence of chronic bronchitis and the severity of dyspnea, and is consistent with prior NPD data in this population.<sup>15</sup> We were unable to demonstrate an independent association between CFTR activity in the lower airway and the severity of bronchitic symptoms because the sample size was relatively small and because there was a strong correlation between each of these parameters and active smoking. Regardless, the results suggest that the pathophysiologic similarities between CF and COPD<sup>5</sup> may be partially related to the presence of smoking-induced CFTR dysfunction and that this abnormality may contribute to phenotypic expression in susceptible individuals.

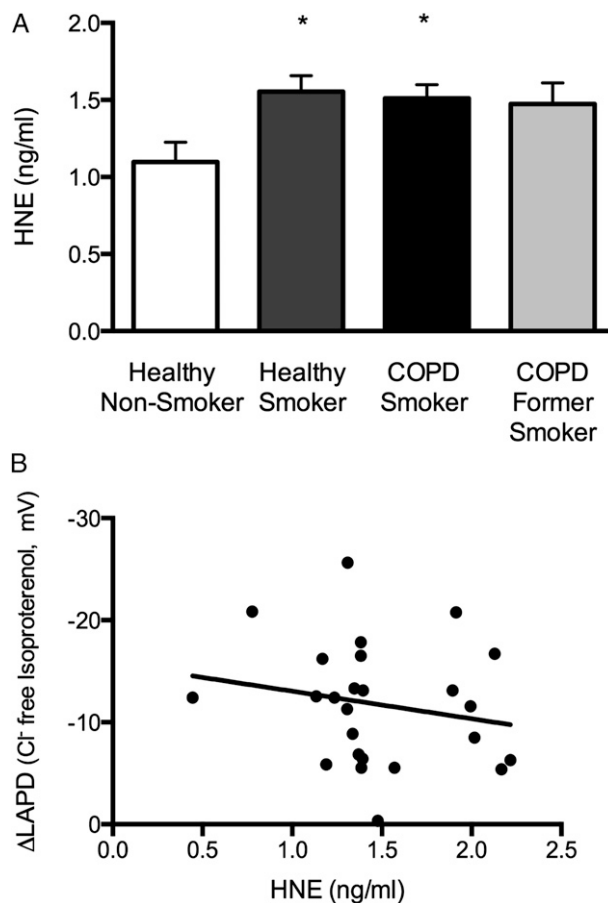


FIGURE 6. HNE levels in BAL samples. A, Mean HNE concentration in BAL samples (three 50- $\mu$ L aliquots/sample) measured by fluorogenic substrate cleavage assay compared with purified substrate. \* $P < .05$ . B, Correlation between CFTR activity measured by LAPD ( $\Delta$ chloride-free plus isoproterenol perfusion) and BAL HNE concentration. The regression line was not statistically significant. HNE = human neutrophil elastase. See Figure 3 and 5 legends for expansion of other abbreviations.

Patients with COPD historically have been grouped into two categories: those exhibiting emphysema (characterized by alveolar destruction and abnormally increased lung compliance) and those with chronic bronchitis (defined by chronic mucus production and characterized by goblet cell hyperplasia and mucus hypersecretion).<sup>25</sup> More data have established additional clinical and radiologic features that may aid in the identification of specific COPD subphenotypes with discrete molecular characteristics and, thus, lead to the development of targeted therapies.<sup>29,30</sup> The present results suggest that acquired CFTR dysfunction may define a specific subphenotype and contribute to COPD pathogenesis in affected individuals. This concept is supported by the study by Koblizek et al,<sup>31</sup> who demonstrated that reduced nasal mucociliary transport is associated with cigarette smoking and correlates with chronic bronchitis symptoms, and suggesting that CFTR dysfunction may underlie these effects.

We have previously reported that subjects with COPD who no longer smoke have normal chloride transport as measured by NPD, suggesting that CFTR function in the upper airway can recover after smoking cessation perhaps because of rapid epithelial cell turnover or other mechanisms.<sup>15</sup> The kinetics of the recovery of CFTR activity after smoking cessation have not yet been described but may be different in the lung compared with the nasal airway because of differences in their microenvironments. Given that small airway mucoobstruction and chronic neutrophilic inflammation both persist in the lower airway of patients with COPD, even after smoking cessation,<sup>32</sup> the present data indicate that it may be important to directly measure LAPD rather than to rely on NPD measurements as a proxy for CFTR function. As we show, the correlation between NPD and LAPD measures is poor, particularly among former smokers with COPD in whom a partial chloride transport defect may persist specifically in the lung, suggesting that local factors unique to the lower airway could contribute. This partial defect could be explained by reduced epithelial CFTR expression as a result of its continued degradation by HNE, a mechanism recently established by Le Gars et al<sup>33</sup> in mice and supported by the overall pattern of the present Western blot analysis and BAL data. Other local pulmonary factors, including hypoxia<sup>34</sup> and the unfolded protein response,<sup>35</sup> may also affect CFTR activity in the lower airway. Further studies are warranted to determine the relative importance of these pathways and to identify the patient phenotype most at risk for persistent CFTR abnormality despite smoking cessation.

This study has several important limitations, including the relatively small sample size, that did not allow us to determine whether CFTR dysfunction persists in former smokers with COPD or the clinical characteristics independently associated with its presence. In this regard, larger studies; the recruitment of a more homogeneous population in terms of disease severity and expression; and more detailed clinical phenotyping, such as with sputum volume, rheology, or CT scan, to quantify and characterize bronchitis severity are needed.<sup>36</sup> Although the study focused on CFTR-dependent chloride transport, we also report significant changes in traditional measures of sodium transport in smokers with and without COPD. This finding contrasts with the typical hyperpolarized baseline potential characteristic of CF<sup>22</sup> but is compatible with prior reports of abnormal sodium conductance in the nasal airways of smokers<sup>12,15</sup> and may indicate the importance of anion transport as opposed to excess sodium resorption in mediating mucus stasis in COPD. We cannot exclude the possibility that the observed reductions in chloride transport may be partially due to the presence of smoking-induced squa-

mous metaplasia and a consequent reduction in surface CFTR. However, the defect in chloride transport that we report is similar to that found in smoke exposure experiments that used single CFTR channels and human bronchial epithelial cells, and prior studies of CFTR-independent anion transport by NPD in smokers with and without COPD established that disrupted baseline potential difference is not likely a result of nonspecific epithelial injury from smoke exposure.<sup>15</sup> Although lidocaine has been reported to affect epithelial sodium transport,<sup>37</sup> it is unlikely that this had an impact on the present findings because the dose was minimal, not delivered to the site of LAPD measurement, and was used in all patients, including healthy control patients who showed normal ion transport.

In conclusion, the data establish that acquired CFTR dysfunction is present in the lower airways of smokers with and without COPD and that its presence is associated with clinical symptoms in a subpopulation of affected individuals. Additional studies are needed to determine whether CFTR dysfunction persists in former smokers with COPD and to investigate the relationship between CFTR function in the lower airway and measures of downstream epithelial physiology, including mucociliary transport and whole-lung mucociliary clearance, as well as proof-of-concept trials to examine whether CFTR modulators play a therapeutic role in COPD.

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**Author contributions:** Drs Dransfield and Rowe had full access to all of the data in the study and take responsibility for the integrity of the data and the accuracy of the data analysis.

*Dr Dransfield:* contributed to the study design, study procedures, data analysis, manuscript writing, and manuscript editing.

*Dr Wilhelm:* contributed to the study procedures, data analysis, and manuscript editing.

*Dr Flanagan:* contributed to the study procedures, data analysis, and manuscript editing.

*Dr Courville:* contributed to the study procedures, data analysis, and manuscript editing.

*Ms Tidwell:* contributed to the study procedures, data analysis, and manuscript editing.

*Dr Raju:* contributed to the study design, study procedures, data analysis, manuscript writing, and manuscript editing.

*Dr Gagar:* contributed to the study design and manuscript editing.

*Dr Steele:* contributed to the data analysis and manuscript editing.

*Dr Tang:* contributed to the study procedures, data analysis, and manuscript editing.

*Dr Liu:* contributed to the data analysis and manuscript editing.

*Dr Rowe:* contributed to the study design, study procedures, data analysis, manuscript writing, and manuscript editing.

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**Additional information:** The e-Figure and e-Table can be found in the "Supplemental Materials" area of the online article.

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