The Association between the Anti-inflammatory Protein CC10 and Smoking Status among Participants in a Chemoprevention Trial

Jiping Chen,1,2 Stephen Lam,3 Aprilé Pilon,4 Annette McWilliams,3 James Melby,4 and Eva Szabo2

1Cancer Prevention Fellowship Program and 2Lung and Upper Aerodigestive Cancer Research Group, Division of Cancer Prevention, National Cancer Institute, NIH, Bethesda, Maryland; 3Lung Tumor Group, British Columbia Cancer Agency and the University of British Columbia, Vancouver, British Columbia, Canada; and 4Claragen, Inc., Rockville, Maryland

Abstract

CC10, the secretory product of bronchiolar Clara cells, is infrequently expressed in non-smokers. CC10 levels in bronchoalveolar lavage fluid and serum are significantly lower in current smokers than in healthy nonsmokers, but the effect of long-term smoking cessation on CC10 is unknown. We measured CC10 in baseline BAL and plasma collected from current (n = 81) and former (n = 23) smokers participating in a chemoprevention trial. Former smokers had significantly higher plasma CC10 levels compared with current smokers [mean, 62.1 ng/mL (95% CI, 43.0–81.2); range, 23.0–175.0 ng/mL for former smokers; and mean, 37.1 ng/mL (95% CI, 29.8–44.4); range, 5.0–171.0 ng/mL for current smokers; P < 0.001]. BAL CC10 levels also trended in the same direction. A significant positive correlation was found between CC10 plasma and BAL levels. After adjustment for age, sex, and pack-years of cigarette consumption, former smokers had 1.70 (95% CI, 1.23–2.36) times higher plasma CC10 levels than current smokers (P < 0.01), whereas former smokers also had nonsignificantly higher baseline BAL CC10 levels compared with current smokers [adjusted mean ratio (95% CI), 1.60 (0.92–2.80), P = 0.094 and 1.35 (0.86–2.10), P = 0.193 for the absolute and normalized BAL CC10, respectively]. These results show that sustained smoking cessation is associated with higher plasma CC10 levels, suggesting that at least some of the damage associated with tobacco smoke may be repaired by long-term smoking cessation. (Cancer Epidemiol Biomarkers Prev 2007;16(3):577–83)

Introduction

Lung cancer is a leading cause of cancer death, accounting for 12% of all new cancer cases and 29% of all cancer deaths in the United States in 2006 (1). Among the known risk factors, tobacco smoke is by far the single most important cause of lung cancer and is responsible for more than 80% to 90% of cases (2,3). Tobacco smoke not only exposes the lung to more than 60 carcinogens, including polycyclic aromatic hydrocarbons and N-nitrosamines, but also to oxidant stress and inflammatory stimuli (3). Unfortunately, even years after smoking cessation, former smokers have persistently increased lung cancer risk, and approximately half of all lung cancer cases occur in former smokers (4,5). Peto et al. (5) showed that although the cumulative risk of lung cancer in former smokers decreased dramatically with increased time since smoking cessation when compared with risk in ongoing smokers, it remained elevated when compared with the lung cancer risk in never-smokers. One potential explanation for this finding is that DNA damage, as reflected by loss of heterozygosity at multiple chromosomal loci, persists in the bronchial epithelium of former smokers years after smoking cessation (6,7).

In the current report, we address the differences between current and former smokers by focusing on the anti-inflammatory protein CC10 (Clara cell 10-kDa protein). CC10 is a 10-kDa protein predominantly secreted by nonciliated bronchiolar Clara cells that are the progenitors for both normal and neoplastic lung epithelium and are involved in lung injury repair and xenobiotic metabolism (8,9). It is one of the most abundant proteins secreted in the respiratory tract and comprises ~7% of the total protein in bronchoalveolar lavage fluid (BAL; ref. 10). CC10 is present not only in BAL but also in serum, possibly by diffusion through the bronchoalveolar-blood barrier, raising the possibility that it may be a serum biomarker for peripheral lung damage (10).

Recent studies also suggest that decreased CC10 levels may be associated with lung carcinogenesis. Serum and BAL CC10 levels are decreased significantly in lung cancer patients compared with healthy nonsmokers, and CC10 mRNA is expressed only in 5% to 25% of human NSCLCs (10,18,19). In vitro overexpression of CC10 antagonizes the neoplastic phenotype of lung cancer cell lines by inhibiting anchorage-independent growth and invasion (19). Moreover, CC10 mRNA is rapidly down-regulated when Syrian golden hamsters are exposed to the tobacco-specific nitrosamine 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanimone (NNK), before any evidence of histologic damage (19). Yang et al. (20) further showed that CC10 knock-out mice have a significantly increased incidence of airway epithelial hyperplasia and lung adenomas compared with wild-type mice after NNK exposure,
suggesting that CC10 has a protective role against NNK-induced lung tumorigenesis.

The association between CC10 levels and smoking has been studied by several investigators. CC10 levels in BAL or serum, as measured by immunoassays, are significantly lower in current smokers than nonsmokers (10, 21-27). Significantly decreased BAL CC10 levels were also detected in asymptomatic current smokers compared with nonsmokers in a recent proteomic study using surface-enhanced laser desorption ionization–mass spectrometry (28). However, the CC10 levels in former smokers and the effect of long-term smoking cessation on CC10 levels are not known. Therefore, we measured CC10 levels in current and former smokers to determine whether sustained smoking cessation is associated with an increase in CC10.

Materials and Methods

Study Population and Specimen Collection. The study population consisted of participants in a previously reported chemoprevention trial done at the British Columbia Cancer Agency that evaluated the efficacy and safety of inhaled budesonide in bronchial dysplasia regression (29). The participants were either current or former smokers aged 40 to 74 years, with a smoking history of 30 or more pack-years. Former smokers were defined as those who had quit smoking for more than 1 year by self-report, without validation with urine cotinine measurements (29). A total of 102 (98%) of the study participants (n = 104) were Caucasian. All participants had one or more sites of bronchial dysplasia (>1.2 mm in diameter) detected by autofluorescence bronchoscopy at enrollment. The study was approved by the Clinical Investigation Committee of the British Columbia Cancer Agency and the University of British Columbia.

Baseline BAL and plasma samples were collected as described previously and formed the basis for the current study (29). Briefly, autofluorescence bronchoscopy was done using the LIFE-Lung device (Xillix Technologies Corp., Richmond, British Columbia), and bronchoalveolar lavage from the right upper lobe or left upper lobe was done using five 20-ml aliquots of normal saline. Fluid from the first wash was discarded, whereas the fluid from the subsequent 20-ml washes was collected until a total of 30 mL fluid was retrieved and immediately placed at 4°C. After differential cell counts, the fluid was centrifuged, and the supernatant was stored at −83°C.

Measurement of CC10 Levels. Quantitative CC10 levels at baseline were measured in plasma and BAL using a competitive ELISA with a rabbit anti-human CC10 antibody (CC10 Sweden AB) as described previously (30, 31). Briefly, a microtiter plate was first coated with the polyclonal rabbit anti-human CC10 antibody (100 µL of 2 mg/mL stock solution) overnight at room temperature. After blocking the wells with 5% bovine serum albumin and 5% sucrose in PBS, 100 µL of mixture containing 1:1 ratio of recombinant human CC10–horseradish peroxidase (HRP) conjugate and sample (BAL or plasma) or standard were added to the wells and incubated for 1 h at room temperature. The recombinant human CC10 was made in Escherichia coli and purified, and its biological activity was assessed in a phospholipase A2 inhibition assay (CC10 Sweden AB). The wells were washed extensively and developed with standard HRP colorimetric reaction reagents (Pierce, Rockford, IL). The CC10 concentration in each sample was determined according to a CC10 standard curve generated with a set of predetermined calibrators. All samples were analyzed in duplicate. The sensitivity limit of the assay was 5 ng/mL. The coefficients of variation ranged from 10% to 20%. Duplicates that were not within a coefficient of variation of 20% were repeated.

Total protein concentration in BAL samples was measured by the bicinchoninic acid assay (Pierce). All determinations were made in duplicate. Duplicates that were not within a coefficient of variation of 20% were repeated.

Statistical Analysis. A total of 104 participants for whom specimens were available for CC10 measurements in plasma, BAL, or both were included in the analysis. Among the 104 subjects, 94 had plasma measurements available for analysis. BAL CC10 levels were analyzed both with and without normalization for the amount of protein in the BAL (ng/µg protein and ng/mL of BAL fluid, respectively). For non-normalized BAL measurements (ng/mL), 102 measurements were available for analysis. For the normalized BAL values (ng/µg protein), only 101 measurements were used for analysis because one male current smoker was excluded due to very low total protein measurement (<0.1 µg/mL) that resulted in a normalized CC10 level that was ~22 SDs higher than the average CC10 levels of current smokers.

Age and pack-years of smoking were coded as continuous variables, whereas smoking status and sex were coded as categorical variables. Study variables were described as mean ± SD, median, and range for continuous variables, or proportions for categorical variables. The distribution of the CC10 levels was strongly skewed to the right. To reduce the influence of the extreme values and improve normality, the CC10 measurements were transformed to the natural logarithmic scale (lnCC10) before statistical comparisons and regression analysis.

Baseline characteristics between current smokers and former smokers were compared by the use of either a two-sample Student’s t test for continuous variables or a Pearson χ² test for categorical variables. The comparisons of lnCC10 levels between current and former smokers or between males and females were made with the two-sample Student’s t test.

Univariate linear regression models were used to access the unadjusted association between smoking status and other factors with lnCC10. Multivariate linear regression models were used to further explore whether smoking status predicted lnCC10 levels after adjustment for other potential confounders. The associations between lnCC10 levels and smoking status were estimated by calculating the mean differences in lnCC10 with 95% confidence intervals (95% CI) between current and former smokers. The geometric mean ratio of CC10 (former versus current smokers) was obtained by exponentiating the mean difference of lnCC10. Variables that were significant in the univariate analyses or were considered to be of potential biological significance were included in the final model. Regression diagnostic assessing the residues, leverage, and influence of the model was done after regression analysis. Observations with the highest influence values were excluded before the model was refitted. To evaluate the amount of correlation between BAL and plasma lnCC10 levels, Spearman rank correlation and/or Pearson correlation coefficients were used.

Consistency of the association between CC10 levels and smoking status was examined by including interaction terms between smoking status and age or smoking status and sex in the regression models.

All statistical analyses were done using Stata statistical software version 8.2 (Stata Corporation, College Station, TX). All P values were two sided, and the level of statistical significance was set at P < 0.05.

Results

Characteristics of Current and Former Smokers. The present study was based on a cancer prevention trial that enrolled 112 participants for 6 months of treatment with budesonide, an inhaled corticosteroid, or placebo to assess the
Plasma and BAL CC10 Levels by Smoking Status or by Sex. The plasma and BAL CC10 concentrations are shown in Table 2. Former smokers had statistically significant higher plasma CC10 levels compared with current smokers (mean ± SD: 62.1 ± 42.0 ng/mL for former smokers and 37.1 ± 31.3 ng/mL for current smokers; P < 0.001). The BAL CC10 levels were, on average, ~60 and 80 times higher than plasma CC10 levels in former and current smokers, respectively. In the analysis of absolute CC10 BAL levels not adjusted for BAL protein concentration, the mean CC10 concentration was 3,831.1 ± 2,738.5 ng/mL for former smokers and 3,161.2 ± 3,822.0 ng/mL for current smokers. The mean difference in lnCC10 between the two groups was statistically significant (P < 0.05). When the BAL CC10 levels were normalized for the total protein content, the difference was no longer statistically significant (44.3 ± 38.1 ng/µg protein and 42.6 ± 64.3 ng/µg protein for former and current smokers, respectively, P = 0.167; Table 2).

Comparison of CC10 levels in men and women showed no significant differences in plasma CC10 levels or absolute BAL CC10 levels (P = 0.089 and 0.514, respectively; data not shown). However, when the CC10 levels in BAL were normalized to total protein, men had statistically significant higher BAL CC10 levels than women (mean 49.1 ± 66.8 ng/µg protein for men compared with 25.3 ± 22.0 ng/µg protein for women; P < 0.05).

**Table 1. Baseline characteristics of the study population by smoking status**

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Current smoker (N = 81)</th>
<th>Former smoker (N = 23)</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (y)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mean ± SD</td>
<td>56.2 ± 7.9</td>
<td>57.6 ± 7.1</td>
<td>0.44</td>
</tr>
<tr>
<td>95% CI</td>
<td>54.4, 57.9</td>
<td>54.5, 60.7</td>
<td></td>
</tr>
<tr>
<td>Median</td>
<td>56.2</td>
<td>55.0</td>
<td></td>
</tr>
<tr>
<td>Range</td>
<td>41.4, 73.9</td>
<td>46.4, 73.7</td>
<td></td>
</tr>
<tr>
<td>Sex, n (%)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Female</td>
<td>21 (25.9)</td>
<td>6 (26.1)</td>
<td></td>
</tr>
<tr>
<td>Male</td>
<td>60 (74.1)</td>
<td>17 (73.9)</td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>81 (100.0)</td>
<td>23 (100.0)</td>
<td>0.09</td>
</tr>
<tr>
<td>Pack-years of smoking</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mean ± SD</td>
<td>51.3 ± 18.2</td>
<td>46.9 ± 14.5</td>
<td>0.29</td>
</tr>
<tr>
<td>95% CI</td>
<td>47.3, 55.3</td>
<td>40.6, 53.2</td>
<td></td>
</tr>
<tr>
<td>Median</td>
<td>45.4</td>
<td>41.0</td>
<td></td>
</tr>
<tr>
<td>Range</td>
<td>27.0, 114.8</td>
<td>30.0, 82.0</td>
<td></td>
</tr>
<tr>
<td>Years since smoke cessation</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mean ± SD</td>
<td>N/A</td>
<td>7.4 ± 5.3</td>
<td></td>
</tr>
<tr>
<td>95% CI</td>
<td>N/A</td>
<td>5.1, 9.7</td>
<td></td>
</tr>
<tr>
<td>Median</td>
<td>N/A</td>
<td>6.0</td>
<td></td>
</tr>
<tr>
<td>Range</td>
<td>N/A</td>
<td>1.0, 18.0</td>
<td></td>
</tr>
</tbody>
</table>

Abbreviation: N/A, nonapplicable.

*The P value was calculated with the use of two-sample Student’s t test with equal variances.

†The P value was calculated with the use of the Pearson χ² test.

‡Time since quitting smoking was available in 22 former smokers.

Effects on the regression of bronchial dysplasia (29). Pretreatment samples of plasma or BAL or both were available for CC10 measurement from 104 participants. Of the 104 subjects analyzed, 81 were current smokers, and 23 were former smokers. Table 1 shows the baseline characteristics of the study population according to smoking status. The average age for current smokers was 56.2 ± 7.9 years, whereas the average age for former smokers was 57.6 ± 7.1 years. An equal proportion of both current and former smokers was male (74%). The average pack-years of smoking for current and former smokers were 51.3 ± 18.2 and 46.9 ± 14.5, respectively. For former smokers, the mean time since smoking cessation was 7.4 years. There were no statistically significant differences between current and former smokers with respect to age, sex, and pack-years of smoking.

**Table 2. CC10 levels in current and former smokers**

<table>
<thead>
<tr>
<th>CC10 levels</th>
<th>Current smoker</th>
<th>Former smoker</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Plasma CC10 levels</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Number of observations*</td>
<td>73</td>
<td>21</td>
<td>&lt;0.001†</td>
</tr>
<tr>
<td>Mean ± SD</td>
<td>37.1 ± 31.3</td>
<td>62.1 ± 42.0</td>
<td></td>
</tr>
<tr>
<td>95% CI</td>
<td>29.8, 44.4</td>
<td>43.0, 81.2</td>
<td></td>
</tr>
<tr>
<td>Median</td>
<td>29.0</td>
<td>49.0</td>
<td></td>
</tr>
<tr>
<td>Range</td>
<td>5.0, 171.0</td>
<td>23.0, 175.0</td>
<td></td>
</tr>
<tr>
<td>BAL CC10 levels</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Number of observations†</td>
<td>80</td>
<td>22</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>Mean ± SD</td>
<td>3,161.2 ± 3,822.0</td>
<td>3,831.1 ± 2,738.5</td>
<td></td>
</tr>
<tr>
<td>95% CI</td>
<td>2,310.6, 4,011.7</td>
<td>2,617.0, 5,045.5</td>
<td></td>
</tr>
<tr>
<td>Median</td>
<td>2,114.0</td>
<td>2,983.0</td>
<td></td>
</tr>
<tr>
<td>Range</td>
<td>39.0, 23,016.0</td>
<td>503.0, 11,675.0</td>
<td></td>
</tr>
<tr>
<td>BAL CC10 levels (ng/µg protein)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Number of observations‡</td>
<td>79</td>
<td>22</td>
<td>0.167†</td>
</tr>
<tr>
<td>Mean ± SD</td>
<td>42.6 ± 64.3</td>
<td>44.3 ± 38.1</td>
<td></td>
</tr>
<tr>
<td>95% CI</td>
<td>28.2, 57.0</td>
<td>27.4, 61.2</td>
<td></td>
</tr>
<tr>
<td>Median</td>
<td>23.1</td>
<td>31.1</td>
<td></td>
</tr>
<tr>
<td>Range</td>
<td>2.9, 391.0</td>
<td>9.5, 176.6</td>
<td></td>
</tr>
</tbody>
</table>

*CC10 measurements were available in 94 subjects and was not equal to total population.

†The P value was calculated by the use of two-sample Student’s t test with equal variances using the natural log-transformed CC10 levels.

‡CC10 measurements were available in 102 subjects and was not equal to total population.

The P value was calculated by the use of two-sample Student’s t test with unequal variances using the natural log-transformed CC10 levels.

Cc10 measurements were available in 101 subjects and was not equal to total population.
higher than in current smokers after adjustment for age, sex, and pack-years of smoking. Therefore, former smokers had 1.70 (95% CI, 1.23-2.36) times higher plasma CC10 levels than current smokers, a difference that was statistically significant ($P < 0.01$; Table 3).

In the multivariate-adjusted model, age showed a strong and significant positive association with plasma lnCC10; for each 10-year increase in age, plasma lnCC10 values increased by 0.33 ng/mL [95% CI, 0.14-0.52; $P < 0.01$; corresponding to ~40% (95% CI, 15-68%) increase in CC10 values; data not shown]. In addition, sex seemed to be an important determinant as men had 1.38 (95% CI, 1.01-1.86) times higher plasma CC10 levels than women ($P < 0.05$; data not shown). Pack-years of cigarette consumption was not associated with plasma CC10 levels. However, analysis of plasma CC10 levels and time since smoking cessation in the 20 former smokers with available specimens showed that plasma lnCC10 levels seemed to increase with increased time since smoking cessation, although this association did not reach statistical significance ($r = 0.22, P = 0.35$).

Analysis of BAL CC10 levels after adjustment for potential confounders showed similar trends. Former smokers tended to have higher BAL CC10 levels than current smokers [adjusted mean ratio, 1.60 (95% CI, 0.92-2.80) for absolute BAL (ng/mL) CC10 levels and 1.35 (95% CI, 0.86-2.10) for normalized BAL (ng/µg protein) CC10 levels, respectively], but the associations did not achieve the same level of significance as plasma CC10 ($P = 0.094$ and 0.193, respectively; Table 3). Age, pack-years of smoking, and time since smoking cessation (in former smokers) were not associated with absolute or normalized BAL CC10. Sex was, however, significantly associated with the normalized BAL CC10. Men had 1.63 (95% CI, 1.07-2.48) times higher normalized BAL CC10 levels than women ($P < 0.05$, data not shown).

The Associations between CC10 Levels and Smoking Status Stratified by Sex. Although there were limited numbers of participant specimens available for subgroup analyses, we did an exploratory analysis of the associations between smoking status, sex, and CC10 levels by including interaction terms in the multivariate regression models. A significant interaction was observed between sex and smoking status on plasma lnCC10 and on normalized BAL lnCC10 (ng/µg protein), although not on the absolute BAL lnCC10 levels ($P$ interaction $= 0.012$, 0.035, and 0.351, respectively; data not shown). Therefore, plasma and normalized BAL CC10 levels were included in the secondary analysis stratified by both sex and smoking status. No significant interactions were present between smoking status and age or pack-years of smoking, on CC10 levels in plasma, or BAL (data not shown).

The associations between CC10 levels and smoking status stratified by sex are shown in Table 4. After adjustment for age and pack-years of smoking, female current smokers exhibited significantly lower plasma or normalized BAL CC10 levels than male current smokers ($P < 0.01$), suggesting that tobacco-induced decrease in CC10 was more profound in women than in men. The reason for this sex difference cannot be explained

The Associations between CC10 Levels and Smoking Status. The associations between plasma or BAL CC10 levels and smoking status as well as various demographic factors were first determined by univariate regression analysis. Age showed a statistically significant positive association with plasma lnCC10, whereas male sex showed a statistically significant positive association with normalized BAL lnCC10 levels ($P < 0.01$ and $P < 0.05$, respectively; data not shown). In the univariate analysis, former smoking status showed a statistically significant positive association with plasma lnCC10 and a trend toward an association with absolute BAL lnCC10 levels ($P < 0.01$ and $P = 0.059$, respectively; Table 3).

We then used multivariate regression models to evaluate the associations between plasma CC10 levels and smoking status after taking into account the potential confounders. Plasma lnCC10 in former smokers was 0.53 ng/mL (95% CI, 0.21-0.86) in men. Thereason for this sex difference cannot be explained.

### Table 3. Unadjusted and multivariate-adjusted associations between CC10 levels and smoking status (former versus current smokers)

<table>
<thead>
<tr>
<th>CC10</th>
<th>Unadjusted</th>
<th>Multivariate-adjusted*</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>lnCC10 coefficient (95% CI)</td>
<td>CC10 mean ratio (95% CI)</td>
</tr>
<tr>
<td>Plasma (ng/mL)</td>
<td>0.60 (0.25, 0.95)</td>
<td>1.82 (1.28, 2.59)</td>
</tr>
<tr>
<td>BAL (ng/mL)</td>
<td>0.52 (–0.02, 1.07)</td>
<td>1.68 (0.98, 2.92)</td>
</tr>
<tr>
<td>BAL (ng/µg protein)</td>
<td>0.32 (–0.13, 0.76)</td>
<td>1.38 (0.88, 2.14)</td>
</tr>
</tbody>
</table>

*Adjusted for age, pack-years of smoking, and sex.
by differences in cumulative tobacco exposure as assessed by pack-years of smoking ($P > 0.05$; data not shown). Interestingly, whereas female former smokers had significantly higher plasma and BAL CC10 levels than female current smokers [mean ratio 3.32 (95% CI, 1.82-6.05) and 2.92 (95% CI, 1.26-6.75) for plasma and BAL CC10 levels, respectively], male current and former smokers tended to have more similar values (Table 4). Thus, we conclude that the magnitude of association between CC10 levels and smoking status may be different between women and men.

To explore the potential reasons for the observed sex difference in the effect of smoking cessation on CC10 levels, we asked whether pack-years of cigarette consumption and time since smoking cessation to enrollment were different among female and male former smokers using regression and the Student’s $t$ test. No statistically significant differences were found between these two groups (data not shown).

**Discussion**

The goal of the present study was to determine the effect of long-term smoking cessation on CC10, an anti-inflammatory protein secreted by bronchiolar Clara cells that is abundantly present in BAL and measurable in blood. Several previous studies have shown alterations in CC10 resulting from a variety of pulmonary insults. Decreased CC10 levels have been documented in serum and BAL from current smokers compared with healthy nonsmokers by several investigators (10, 22-27). However, whether the decrease in CC10 associated with tobacco exposure is permanent or reversible has not been addressed previously. We now show that a significant relationship exists between CC10 levels and current versus former smoking status. On average, former smokers had ~1.7 times higher plasma CC10 levels than current smokers, a difference that was statistically significant. In addition, former smokers also had higher BAL CC10 levels than current smokers, although not with the same level of significance. Although we did not have access to specimens from nonsmokers for direct comparison with former smokers, the magnitude of the difference between former and current smokers is in keeping with previous reports by Bernard et al. (10) and Shijubo et al. (24), who found the mean ratio of CC10 levels in nonsmokers to current smokers to be 1.29 or 1.48 in serum and 2.13 or 2.18 in BAL, respectively. This suggests that at least some of the damage associated with tobacco smoke, as reflected by CC10 levels, may be repaired by long-term smoking cessation.

Previous data from several studies suggest that decreased CC10 expression occurs early during pulmonary carcinogenesis, and that down-regulation of CC10 is involved in carcinogenic progression rather than having an “innocent bystander” effect (10, 17-20, 32, 33). For example, enforced expression of CC10 in cancer cell lines results in a less malignant phenotype with loss of invasiveness, decreased metalloproteinase expression, altered adhesive properties, and decreased anchorage-independent growth (19, 34). It is not known, however, whether restoration of CC10 early after carcinogenic pulmonary injury would prevent the progression to cancer and whether CC10 correlates with lung cancer risk. Although formal testing with CC10 replacement therapy is needed to address the former question, our finding that former smokers have higher CC10 levels supports the hypothesis that increased CC10 correlates with decreased lung cancer risk. Although lung cancer risk remains elevated for years after smoking cessation when compared with lifetime nonsmoking, the lung cancer risk of former smokers is decreased when compared with the risk of persistent smokers (5). Additional studies will be needed to evaluate the relationship between CC10 and lung cancer risk more precisely.

Our plasma CC10 measurements are consistent with the serum values reported for current smokers in the literature that ranged from 7.9 to 66 ng/mL (10, 22-25), although they are approximately five times higher than another report that also used an ELISA to measure serum CC10 (24). The discrepancy is likely due to methodologic differences due to our use of a very sensitive competitive ELISA and different antibodies, differences in study populations, and the measurement of CC10 in plasma rather than serum. CC10 is known to be highly protein bound, and thus, its levels are likely lower in serum, after depletion of coagulations factors and other associated proteins, than in plasma. Specifically, CC10 binds fibrinectin with high affinity, and fibrinectin levels in serum are only one-ninth of plasma values (35, 36). It is therefore likely that some CC10 is lost with fibrinectin in serum.

In this study, higher plasma CC10 levels are found to be significantly associated with older age in both the unadjusted and multivariate-adjusted models, a finding that is consistent with previous publications (22, 23, 27). This is probably due to reduced renal function with increasing age because serum CC10 levels are significantly associated with renal function (25). In contrast, BAL CC10 levels were not affected by age. Furthermore, sex seemed to be associated with plasma and normalized BAL CC10 levels after multivariate adjustment ($P < 0.05$; data not shown), a finding for which there is disagreement in the literature (25, 37). The disagreement may result from different CC10 assays and/or different study populations used in the published studies. More studies using a single assay and similar populations (e.g., people with similar histologic changes in the bronchial epithelium or lung function) are needed to clarify this question.
The finding that long-term smoking cessation is associated with higher CC10 levels in women than men although female current smokers have lower CC10 levels than male current smokers is particularly intriguing. If CC10 is a sensitive measure of damage to the lung, as is suggested by multiple previous studies, then one explanation of these data would be that women may sustain a greater insult to the lung from tobacco exposure, but may have greater ability to repair some of the damage after smoking cessation. Sex-related differences in response to tobacco have, indeed, been shown previously (38). Several studies suggest that women are at a higher risk of developing lung cancer than men from smoking, possibly due to the sex differences in tobacco carcinogen metabolic activation and detoxification, although not all the data are consistent (38-41). Furthermore, estrogen may bind to estrogen receptors present in normal and malignant lung cells and thereby stimulate growth factor signaling pathways (40). Conversely, evidence exists that repair of oxidative damage by the enzyme 8-oxoguanine DNA N-glycosylase, whose decreased activity is associated with increased lung cancer risk, is impaired in older men compared with women (42, 43). These data suggest that gender-related differences in DNA repair capacity do indeed exist, and older women may have more preserved capacity to repair damage than men. It needs to be emphasized that our results are from a subgroup analysis in our study, consisting of a relatively small number of cases, and thus, these results should only be considered to be as hypothesis generating. However, the fact that measurements obtained from different sample types (plasma and BAL) showed similar results suggests that these results may indeed be real.

Our study has several important strengths. It is the first report documenting the effect of long-term smoking cessation on CC10 measurements. A previous small study of eight subjects showed that BAL CC10 levels increase at 3, 6, and 9 months after smoking cessation, although CC10 levels returned to baseline by 15 months (44). However, no prior studies have examined the effect of long-term smoking cessation on CC10 levels. In addition, this study is the largest CC10 study with BAL samples, demonstrating a wide range of values in people at risk for lung cancer. These measurements will help to inform future studies by defining the range of values that can be anticipated.

Certain limitations to the current study deserve consideration. First, although we found a significant correlation between plasma and BAL CC10 levels, consistent with previous studies, this correlation was not very strong (10, 24). This may be due to technical issues such as dilutional variability in obtaining BAL samples, which we tried to account for by performing analyses on both absolute and protein-normalized BAL measurements. There are multiple technical issues, such as the amount of fluid used for lavage, filtration, and concentration, which differentially affect concentrations of proteins of large versus small molecular size (45, 46). The BAL technique was done in a consistent manner in this study, but it is therefore difficult to compare BAL measurements across studies, and it remains unclear whether the absolute or normalized measurements are more appropriate to use for analysis. The weak correlation could also be due to differences in intravascular leakage through the damaged lung epithelial barrier caused by a variety of factors that could not be assessed or controlled for in this study (24). Second, there were limited numbers of participants in the study, especially former smokers, and as such, the study had limited power and precision in determining the associations. Third, due to the design of the chemoprevention trial, there was not a healthy nonsmoking comparison group. We therefore could not precisely determine whether the CC10 levels in former smokers returned to the levels expected in never-smokers or remained reduced. It should be noted, however, that the magnitude of the change observed in our study is consistent with changes noted by other investigators studying current and never-smokers, as discussed above (10, 24). Furthermore, this study included only subjects with bronchial dysplasia, who are generally thought to be at higher risk for subsequent lung cancer than smokers without dysplasia. Subsequent studies will need to determine if the results can be extrapolated to the general population. Finally, we were not able to control for other potential confounders that may alter the results. For example, some studies have reported an association between body mass index and CC10 levels, but the literature is contradictory, and we were not able to control for this poorly characterized potential confounder (25, 47, 48).

In summary, the results from this study suggest that at least some of the damage associated with tobacco smoking may be repaired by long-term smoking cessation and further support the concept that CC10 may be a sensitive marker of tobacco-induced lung injury. When taken together with data showing the decrease in CC10 expression during lung carcinogenesis and the partial reversion of the neoplastic phenotype upon overexpression of CC10, the results raise the intriguing possibility that CC10 could be a marker of lung cancer risk or a blood-based early detection marker. Large studies are needed to confirm our observations and to further explore the clinical utility of CC10.

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References


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