Current Smoking Status Is Associated With Lower Quantitative CT Measures of Emphysema and Gas Trapping

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Purpose: The purposes of this study were to evaluate the effect of smoking status on quantitative computed tomography CT measures of low-attenuation areas (LAAs) on inspiratory and expiratory CT and to provide a method of adjusting for this effect.

Materials and Methods: A total of 6762 current and former smokers underwent spirometry and volumetric inspiratory and expiratory CT. Quantitative CT analysis was completed using open-source 3D Slicer software. LAAs were defined as lung voxels with attenuation values ≤ -950 Hounsfield units (HU) on inspiratory CT and ≤ -856 HU on expiratory CT and were expressed as percentage of CT lung volume (%$LAA_{I\text{-}950}$ and %$LAA_{E\text{-}856}$). Multiple linear regression was used to determine the effect of smoking status on %$LAA_{I\text{-}950}$ and %$LAA_{E\text{-}856}$ while controlling for demographic variables, spirometric lung function, and smoking history, as well as total lung capacity (%$LAA_{I\text{-}950}$) or functional residual capacity (%$LAA_{E\text{-}856}$). Quantile normalization was used to align the %$LAA_{I\text{-}950}$ distributions for current and former smokers.

Results: Mean %$LAA_{I\text{-}950}$ was 4.2 ± 7.1 in current smokers and 7.7 ± 9.7 in former smokers ($P < 0.001$). After adjusting for confounders, %$LAA_{I\text{-}950}$ was 3.5 percentage points lower and %$LAA_{E\text{-}856}$ was 6.0 percentage points lower in current smokers than in former smokers ($P < 0.001$). After quantile normalization, smoking status was an insignificant variable in the inspiratory regression model, with %$LAA_{I\text{-}950}$ being 0.27 percentage points higher in current smokers ($P = 0.13$).

Conclusions: After adjusting for patient demographics and lung function, current smokers display significantly lower %$LAA_{I\text{-}950}$ and %$LAA_{E\text{-}856}$ than do former smokers. Potential methods for adjusting for this effect would include adding a fixed value (eg, 3.5%) to the calculated percentage of emphysema in current smokers, or quantile normalization.

Key Words: quantitative computed tomography, emphysema, air trapping, chronic obstructive pulmonary disease, smoking

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Quantitative computed tomography (QCT) is useful for evaluating disease severity in patients with chronic obstructive pulmonary disease (COPD).[1–11] In particular, lung densitometry measures help quantify the extent of emphysema on inspiratory CT,[2–4] as well as expiratory gas trapping.[5–7] Confounders known to affect CT densitometry include sex, age, and smoking history.[8–11] More recently, several studies showed that current smokers display significantly less "emphysema" by QCT measures than do former smokers.[12–15] Furthermore, smoking cessation leads to distinct increases in the QCT extent of emphysema.[14,15] A current hypothesis to explain this surprising phenomenon asserts that inflammation due to cigarette smoke exposure may effectively mask emphysema by increasing local lung tissue density[14,15] (Fig. 1). It is also suggested that the increase in apparent percentage of emphysema after smoking cessation may be due to declines in sputum production[14] or clearing of accumulated soot and tar.[15] Still others propose that these findings may be driven by a survivor effect, if those with more rapidly progressing disease are more likely to reduce or quit smoking.[12] These studies, though, have been too small to control for possible confounding factors such as age and severity of COPD. Likewise, the cross-sectional study design is insufficient to address any possible survivor effect. Furthermore, the effect of current smoking on QCT measures of expiratory gas trapping has not been evaluated.

The purposes of the current study were to evaluate the effect of current smoking status on QCT measures of emphysema while controlling for known and possible confounders, as well as to develop a means of adjustment to account for this effect. In addition, we sought to determine whether the current smoker phenomenon also affects QCT measures of expiratory gas trapping.

MATERIALS AND METHODS

Subjects

Between November 2007 and April 2011, 10,192 current and former smokers were recruited from 21 clinical sites in the United States to participate in the COPDGene

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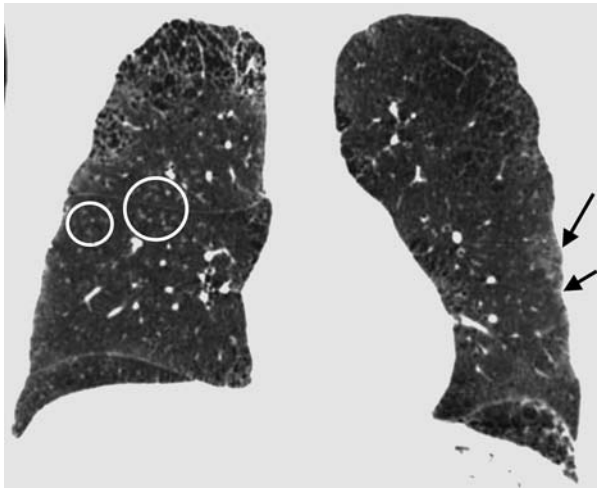

FIGURE 1. Examples of CN on CT. Coronal CT image of a GOLD 1 subject with moderately extensive visual extent of emphysema. However, the quantitative emphysema score was only 5.1%. The emphysema may have been masked on quantitative assessment by the presence of centrilobular nodules (circled) and patchy ground-glass abnormality (arrows).

study. The subjects were all non-Hispanic whites or African-Americans and ranged in age from 45 to 80 years with smoking histories of at least 10 pack-years. The data collected included inspiratory and expiratory CT scans, demographic and medical history questionnaires, and spirometry. Subjects with lung fibrosis and bronchiectasis were excluded from the analysis. Of the full cohort, 6762 subjects who had undergone full QCT analysis were randomly paired on the basis of smoking status to achieve a cohort with equal numbers of current and former smokers within each GOLD stage of disease severity[16] to carry out quantile normalization as described below. Detailed demographic information, including smoking history and average spirometric findings for the cohort, can be found in Table 1. All subjects met the criteria to be classified under the GOLD system for COPD based on postbronchodilator spirometric findings of forced expiratory volume in 1 second (FEV_1) and forced vital capacity (FVC).[16] Spirometric reference values[17]were used to calculate % predicted values of FEV_1 and FVC to classify disease severity. A total of 3438 (50.8%) subjects were classified as COPD cases (GOLD stages 1 to 4), and the remaining 3324 were smoking controls without evidence of obstruction. Distribution of subjects based on the GOLD staging criteria is provided in Table 2. A subset of 500 CT scans from an equal number of current and former smokers was visually scored by an expert radiologist blinded to smoking status for the presence and extent of centrilobular nodularity (CN)[18] within each lobe. In addition, 386 subjects indicated inhaled corticosteroid use on the questionnaire. IRB-approved written informed consent was obtained for every subject before enrollment. All data were collected and protected in accordance with HIPAA regulations.

CT Image Acquisition

Study subjects underwent 2 volumetric CT scans according to a standardized technique.[19] One scan was performed at full inspiration (total lung capacity, TLC), with the second at the end of a normal expiration (functional residual capacity, FRC). Tube potential was set at 120 kVp for all scans, and images were acquired with an effective mAs of 200 for inspiration and 50 for expiration.[19] Scans were performed using 11 different scanner models from 1 of 3 manufacturers: General Electric Medical Systems (n = 2511), Siemens (n = 3879), and Philips (n = 386). High-resolution scanning and reconstruction techniques were implemented to obtain thin-section, contiguous slices. To achieve near-isotropic voxels, scans were reconstructed with differing slice thicknesses of 0.625, 0.75, and 0.9 mm, depending on the parameters permitted by each scanner model, while the corresponding slice intervals were 0.625, 0.5, and 0.45 mm. The images were reconstructed using manufacturer-specific "smooth" convolution kernels: Standard, B31f, and B. Protocol adherence was routinely evaluated in all cases, and individual scan quality was visually assessed for the presence of motion artifacts, inclusion of all lung parts, as well as subjective measures of adequacy of inspiration/expiration. Scanners were calibrated on a weekly basis to maintain internal consistency, while monthly scans were collected on a standard phantom to track and verify the consistency of CT attenuation measurements.

Image Analysis

CT images were analyzed using open-source 3D Slicer software,[20] which provided automated lung segmentations

TABLE 1. Demographics of the Study Subjects

	Total Cohort	Former Smokers	Current Smokers	*P*
Males, N (%)	3646 (54)	1729 (51)	1917 (57)	< 0.001
Non-Hispanic white, N (%)	4772 (71)	2962 (88)	1810 (54)	< 0.001
Smoking duration (y)	36.2 ± 10.5	33.0 ± 11.2	39.4 ± 8.6	< 0.001
Years since quitting	NA	14.1 ± 11.1	NA	NA
Pack-years	44.0 ± 24.8	43.9 ± 26.0	44.2 ± 23.6	0.55
Age	60.1 ± 9.0	64.1 ± 8.6	56.2 ± 7.5	< 0.001
BMI	28.5 ± 6.0	29.3 ± 5.9	27.8 ± 5.9	< 0.001
Height (cm)	170.1 ± 9.5	169.6 ± 9.5	170.6 ± 9.5	< 0.001
FEV_1 (% predicted)	79.3 ± 24.7	78.9 ± 25.1	79.7 ± 24.4	0.18
FVC (% predicted)	90.5 ± 17.0	89.9 ± 16.6	97.1 ± 17.4	0.005
FEV_1/FVC	0.67 ± 0.15	0.66 ± 0.16	0.67 ± 0.15	< 0.001
Inhaled corticosteroids, N (%)	386 (6)	227 (7)	159 (5)	< 0.001

Years since quitting refers to the average reported length of time since smoking cessation in former smokers. One pack-year is equivalent to an average of 1 pack of cigarettes smoked a day for 1 year. Except for Sex, Race, and Inhaled corticosteroid use variables, all values represent mean ± SD.

NA indicates not applicable.

TABLE 2. Mean $\%LAA_{I\text{-}950}$ and $\%LAA_{E\text{-}856}$ by GOLD Stage and Smoking Status

		$\%LAA_{I\text{-}950}$		Normalized $\%LAA_{I\text{-}950}$			$\%LAA_{E\text{-}856}$	
	N	Former	Current	Former	Current	N	Former	Current
Controls	3324	2.8 ± 3.1	1.4 ± 1.8	2.1 ± 2.5	2.1 ± 2.5	2998	12.5 ± 9.7	9.6 ± 9.1
GOLD 1	672	7.0 ± 6.4	3.9 ± 4.7	5.4 ± 5.5	5.5 ± 5.5	600	23.2 ± 11.3	18.0 ± 11.9
GOLD 2	1750	9.3 ± 8.8	4.8 ± 5.8	7.1 ± 7.2	7.1 ± 7.2	1603	30.9 ± 15.1	23.6 ± 15.2
GOLD 3	766	18.5 ± 12.4	10.7 ± 11.2	14.6 ± 10.0	14.7 ± 10.0	701	49.8 ± 16.4	39.6 ± 18.1
GOLD 4	250	29.2 ± 12.8	19.1 ± 13.5	24.2 ± 13.1	24.2 ± 13.1	234	65.9 ± 10.8	57.0 ± 16.3
Cases (1-4)	3438	12.4 ± 11.5	7.0 ± 9.0	9.7 ± 9.7	9.7 ± 9.7	3138	36.4 ± 19.1	28.5 ± 18.9
Total	6762	7.7 ± 9.7	4.2 ± 7.1	6.0 ± 8.1	6.0 ± 8.1	6136	24.7 ± 19.4	19.3 ± 17.7

All values except N represent mean ± SD.

All relationships between former and current smokers for $\%LAA_{I\text{-}950}$ and $\%LAA_{E\text{-}856}$ are significant at the level $P < 0.001$.

GOLD: controls—$FEV_1/FVC \geq 0.7$, $FEV_1 \geq 80\%$ predicted; GOLD 1: $FEV_1/FVC < 0.7$, $FEV_1 \geq 80\%$ predicted; GOLD 2: $FEV_1/FVC < 0.7$, FEV_1 50%-79% predicted; GOLD 3: $FEV_1/FVC < 0.7$, FEV_1 30%-49% predicted; GOLD 4: $FEV_1/FVC < 0.7$, $FEV_1 < 30\%$ predicted.

and densitometric measures. The segmented lung volume on inspiratory scans provided a measure of CT-derived TLC (TLC_{CT}), whereas the same measure on expiratory scans defined CT-derived FRC (FRC_{CT}). Previously established prediction equations[21] provided methods to determine predicted values for plethysmographic TLC and FRC for each subject. We compared the predicted plethysmographic values to TLC_{CT} and FRC_{CT} as an objective measure of adequacy of inspiration/expiration. The density mask technique was used with thresholds for lung attenuation set at −950, −910, and −856 HU. On inspiratory CT scans, low-attenuation areas (LAAs) were defined as voxels ≤ -950 HU and expressed as percentage of TLC_{CT} ($\%LAA_{I\text{-}950}$).[22] Likewise, on expiratory scans, LAAs were defined as voxels ≤ -856 HU, expressed as a percentage of FRC_{CT} ($\%LAA_{E\text{-}856}$).[22] Estimated tissue volume was also assessed on the basis of voxel attenuation values[23] (Table 3). Data relating to the 15th percentile of lung attenuation (Perc15) and mean lung attenuation for inspiratory scans are provided in Supplemental Digital Content 1 (http://links.lww.com/IGC/A318).

Statistical Analysis

Multiple linear regression was utilized to determine the effect of current smoking status on QCT-derived $\%LAA_{I\text{-}950}$ and $\%LAA_{E\text{-}856}$. The models controlled for age, sex, race, height, weight, FEV_1/FVC, smoking history in years, and average daily cigarette consumption over the course of the subjects' smoking history (cigarettes per day) by self-report, as well as TLC_{CT} ($\%LAA_{I\text{-}950}$) or FRC_{CT} ($\%LAA_{E\text{-}856}$). Additional models included corticosteroid use and time since quitting for former smokers. Further modeling was performed separately for the COPD cases and smoking controls. Scanner type associations and interaction terms for current smoking status with average cigarettes smoked per day and FEV_1/FVC were also evaluated. A 2-sided Wald test was used to evaluate statistical significance, and a variable was considered to be statistically significant if its corresponding P value was < 0.05. Diagnostics, including residual plots, were performed to verify the appropriateness of all fitted models. Statistical analyses were performed using JMP 10 (copyright © 2012 SAS Institute Inc.), the SAS/STAT software package, Version 9.2, of the SAS System for Windows XP (copyright © 2002–2008 SAS Institute Inc.), and the R software package (R Core Team, 2012).

TABLE 3. Mean %Tissue Volume by GOLD Stage and Smoking Status

	%Tissue Volume		
	Former	Current	*P*
Controls	14.8 ± 2.4	16.5 ± 3.1	< 0.001
GOLD 1	13.3 ± 2.1	14.8 ± 2.6	< 0.001
GOLD 2	13.7 ± 2.5	14.9 ± 2.6	< 0.001
GOLD 3	12.4 ± 2.7	13.8 ± 2.9	< 0.001
GOLD 4	10.9 ± 1.9	12.0 ± 2.6	0.002
Cases (1-4)	13.1 ± 2.6	14.4 ± 2.8	< 0.001
Total	13.9 ± 2.6	15.4 ± 3.1	< 0.001

All values represent mean ± SD.

Tissue volume of each voxel, $V_{(x)}$, is estimated by voxel HU attenuation value based on the equation:

$$V_{(x)} = v_{(x)} \frac{HU_{(x)} - HU_{air}}{HU_{tissue} - HU_{air}}$$

where $v_{(x)}$ is the voxel volume, $HU_{(x)}$ is the attenuation value of the voxel, $HU_{air} = -1000$, and $HU_{tissue} = 55$.[23] Total tissue volume of all lung voxels is presented as % of TLC_{CT}.

Adjustment Procedures

Quantile normalization was used to align the $\%LAA_{I\text{-}950}$ distributions for current and former smokers so that the statistical properties were similar for both groups[24]; the normalizations were performed on subjects within each GOLD stage separately. Quantile normalization has its origins in high-dimensional genomic data analysis. The main goal of this procedure is to conform 2 or more technical replicates believed to have the same distribution. The procedure essentially works by making the quantiles equal, thus aligning the 2 distributions so that they are the same. The original purpose of this technique was to remove variation due to technical issues from 2 samples, thus making them similar. In this paper the goal was to align the distributions of $\%LAA_{I\text{-}950}$ for former and current smokers so that they have a similar center, spread, and shape: this removes the effect of smoking status on QCT measures.

A simpler adjustment method was implemented using the difference in mean $\%LAA_{I\text{-}950}$ between former and current smokers. Within each GOLD stage the mean difference was added to the scores for current smokers, whereas the former smokers' scores remained unchanged. This method aligned the mean $\%LAA_{I\text{-}950}$ scores, but underlying differences in the distributions still persisted. This method simply shifted or scaled the distributions so that they would have the same center or spread but had no impact on conforming the shapes of the distributions to be similar.

RESULTS

Table 2 displays average values of $\%LAA_{I\text{-}950}$ and $\%LAA_{E\text{-}856}$ stratified by GOLD stage. Within each stage of COPD severity, as well as for the control smokers, $\%LAA_{I\text{-}950}$ and $\%LAA_{E\text{-}856}$ were both significantly lower for current smokers than for former smokers (all $P < 0.001$). In the subset of 500 subjects who underwent visual scoring, current smokers were significantly more likely than former smokers to be scored for the presence of CN ($P < 0.001$) (Table 4). Tables 5 and 6 display the results of multiple linear regression for $\%LAA_{I\text{-}950}$ and $\%LAA_{E\text{-}856}$, respectively. After adjusting for possible confounders, current smoking status was a statistically significant component in both models. In current smokers, $\%LAA_{I\text{-}950}$ was 3.5 percentage points lower than in former smokers ($P < 0.001$), whereas $\%LAA_{E\text{-}856}$ was 6.0 percentage points lower ($P < 0.001$). Inhaled corticosteroid use was significantly associated with $\%LAA_{I\text{-}950}$ but not with $\%LAA_{E\text{-}856}$, whereas time since quitting showed converse associations. Inhaled corticosteroid use and time since quitting did not contribute much or alter the other associations and thus were excluded from subsequent models. The apparent negative association between height and %LAA in the multivariate analyses is probably because CT lung volume, which correlates with height, was also included in the model. Adjustment for scanner model had minimal effect on the models shown here or the associations found with the other variables and hence were not included. Models for the visually scored subset adjusting for CN presence and extent are provided in Supplemental Digital Content 1, http://links.lww.com/IGC/A318.

The effect size of current smoking status was larger in the COPD cases than in the smoking controls for both outcomes, yet remained statistically significant ($P < 0.001$) in all comparisons. For the cases, $\%LAA_{I\text{-}950}$ was 4.7 percentage points lower in current smokers, whereas it was just 1.1 percentage points lower in the control smokers (Table 7). Similarly, in the stratified models, $\%LAA_{E\text{-}856}$ was 6.6 percentage points lower in current smokers with COPD and 4.0 percentage points lower in control smokers (Table 7). Models for Perc15 and mean lung attenuation showed similar trends to those seen for $\%LAA_{I\text{-}950}$ and $\%LAA_{E\text{-}856}$, and are provided in Supplemental Digital Content 1 (http://links.lww.com/IGC/A318).

Figure 2 displays the distributions of $\%LAA_{I\text{-}950}$ from minimum to maximum values in each group. Original and normalized distributions are displayed in black for current smokers and in gray for former smokers. After quantile normalization, the distributions for former and current smokers are nearly identical, and the corresponding normalized mean $\%LAA_{I\text{-}950}$ values can be found in Table 2. Table 8 provides the percentile distributions of $\%LAA_{I\text{-}950}$ for current and former smokers before and after normalization, as well as after the mean difference adjustment. Quantile normalization successfully aligned the distributions, whereas the more basic mean difference adjustment resulted in added divergence. The normalized values of $\%LAA_{I\text{-}950}$ were used as the outcome variable for the models in Table 9. Upon application of this corrective factor, smoking status was not related to the normalized outcome in the combined multivariate model. Although it remained a significant component in models separated by COPD diagnosis, its direction of effect was reversed and the effect size diminished so that current smokers were associated with slightly higher values of $\%LAA_{I\text{-}950}$ than were former smokers.

DISCUSSION

In this study, we confirmed previous findings that density-based CT measures of emphysema and air trapping were consistently lower in current smokers than in former smokers, including those without COPD and those with varying degrees of COPD severity. We found that this current smoker effect persisted after adjustment for disease severity and other potential confounders but was not present in current smokers who did not have visible centrilobular nodules. After adjustment, the difference in $\%LAA_{I\text{-}950}$ between current and former smokers was 3.5%, and the difference for $\%LAA_{E\text{-}856}$ was 6.0%. When quantile normalization was performed to align the distributions of $\%LAA_{I\text{-}950}$ in current and former smokers, the estimate of the effect of current smoking was eliminated or reversed.

Prior research has consistently noted that current smokers display significantly lower QCT scores of "emphysema" than do former smokers.[12–15] Although smaller, with 894 subjects, the cohort examined by Grydeland et al[12] was similar in many ways (54% former smokers, 59% men, mean age 59.8 y) to the COPDGene cohort and yielded results with respect to the current smoker effect that were in line with those presented here (-4.86% $LAA_{I\text{-}950}$ for current vs. former smokers). However, the authors suggested that the effect was most likely a result of survivor effect. In a study by Camiciottoli et al,[13] using a threshold of 6.8% for $LAA_{I\text{-}950}$ as a cutoff for the presence of emphysema, former smokers were nearly twice as likely to have emphysema than were current smokers (20.8% and 37.6%, respectively). This study was relatively small (n = 266) and did not present specific values of $LAA_{I\text{-}950}$ for their subjects. Furthermore, the study subjects were recruited from a lung cancer screening trial, and the authors attributed their findings to the nature of the study recruitment. The study presented here offers the opportunity to explore the current smoking effect in a large cohort that permits adequate

TABLE 4. Prevalence of CN and Associated $\%LAA_{I\text{-}950}$ and $\%LAA_{E\text{-}856}$ Scores in 500 Current and Former Smokers

	CN Present			CN Absent		
	N (%)	$\%LAA_{I\text{-}950}$	$\%LAA_{E\text{-}856}$	N (%)	$\%LAA_{I\text{-}950}$	$\%LAA_{E\text{-}856}$
Current smokers	140 (56)	3.9 ± 5.3	22.7 ± 16.3	110 (44)	14.7 ± 13.8	40.9 ± 23.8
Former smokers	83 (33)	7.7 ± 8.6	26.1 ± 16.7	167 (67)	17.3 ± 14.3	42.6 ± 22.2
P	< 0.001	< 0.001	0.13	< 0.001	0.13	0.54

All values except N represent mean ± SD.

TABLE 5. Multiple Linear Regression Analysis for $\%LAA_{I\text{-}950}$

	Model 1A		Model 1B		Model 1C		Model 1D	
	Estimate	*P*	Estimate	*P*	Estimate	*P*	Estimate	*P*
Current smoker	−3.52	< 0.001	−3.55	< 0.001	−3.60	< 0.001	−3.65	< 0.001
Age	−0.0001	0.99	−0.0002	0.99	0.020	0.24	0.024	0.18
Sex (F)	0.598	0.005	0.597	0.005	0.588	0.005	0.586	0.006
Race (black)	2.91	< 0.001	2.94	< 0.001	2.89	< 0.001	2.92	< 0.001
Cigarettes per day	0.015	0.02	0.013	0.042	0.017	0.012	0.015	0.025
Smoking duration (y)	−0.015	0.09	−0.013	0.15	−0.034	0.03	−0.036	0.025
Weight (kg)	−0.061	< 0.001	−0.061	< 0.001	−0.061	< 0.001	−0.061	< 0.001
Height (cm)	−0.065	< 0.001	−0.066	< 0.001	−0.065	< 0.001	−0.066	< 0.001
FEV_1/FVC	−33.52	< 0.001	−33.10	< 0.001	−33.47	< 0.001	−33.04	< 0.001
TLC_{CT}	1.86	< 0.001	1.89	< 0.001	1.86	< 0.001	1.89	< 0.001
Inhaled corticosteroid	—	—	0.740	0.018	—	—	0.747	0.016
Years since quitting	—	—	—	—	−0.027	0.14	−0.031	0.08
	$R^2 = 0.55$		$R^2 = 0.56$		$R^2 = 0.55$		$R^2 = 0.56$	

Model 1A is the standard multiple linear regression model constructed for all subsequent comparisons. Model 1B adjusts the standard model for inhaled corticosteroid use. Model 1C adjusts the standard model for years since quitting in former smokers. Model 1D adjusts the standard model for both inhaled corticosteroid use and years since quitting in former smokers.

TABLE 6. Multiple Linear Regression Analysis for $\%LAA_{E\text{-}856}$

	Model 2A		Model 2B		Model 2C		Model 2D	
	Estimate	*P*	Estimate	*P*	Estimate	*P*	Estimate	*P*
Current smoker	−5.95	< 0.001	−5.93	< 0.001	−6.12	< 0.001	−6.11	< 0.001
Age	0.171	< 0.001	0.168	< 0.001	0.213	< 0.001	0.214	< 0.001
Sex (F)	1.83	< 0.001	1.79	< 0.001	1.81	< 0.001	1.77	< 0.001
Race (black)	5.63	< 0.001	5.42	< 0.001	5.61	< 0.001	5.39	< 0.001
Cigarettes per day	0.009	0.38	0.010	0.33	0.011	0.26	0.013	0.21
Smoking duration (y)	−0.018	0.16	−0.017	0.18	−0.059	0.015	−0.062	0.01
Weight (kg)	−0.096	< 0.001	−0.096	< 0.001	−0.096	< 0.001	−0.096	< 0.001
Height (cm)	−0.268	< 0.001	−0.266	< 0.001	−0.268	< 0.001	−0.267	< 0.001
FEV_1/FVC	−47.71	< 0.001	−47.64	< 0.001	−47.63	< 0.001	−47.55	< 0.001
FRC_{CT}	10.22	< 0.001	10.19	< 0.001	10.21	< 0.001	10.18	< 0.001
Inhaled corticosteroid	—	—	0.354	0.45	—	—	0.368	0.43
Years since quitting	—	—	—	—	−0.055	0.044	−0.060	0.027
	$R^2 = 0.80$		$R^2 = 0.80$		$R^2 = 0.80$		$R^2 = 0.80$	

Model 2A is the standard multiple linear regression model constructed for all subsequent comparisons. Model 2B adjusts the standard model for inhaled corticosteroid use. Model 2C adjusts the standard model for years since quitting in former smokers. Model 2D adjusts the standard model for both inhaled corticosteroid use and years since quitting in former smokers.

TABLE 7. Multiple Linear Regression Analysis for $\%LAA_{I\text{-}950}$ and $\%LAA_{E\text{-}856}$: Cases and Controls

	$\%LAA_{I\text{-}950}$				$\%LAA_{E\text{-}856}$			
	Cases		Controls		Cases		Controls	
	Estimate	*P*	Estimate	*P*	Estimate	*P*	Estimate	*P*
Current smoker	−4.73	< 0.001	−1.12	< 0.001	−6.62	< 0.001	−4.00	< 0.001
Age	0.036	0.053	0.021	< 0.001	0.255	< 0.001	0.138	< 0.001
Sex (F)	1.17	0.001	−0.192	0.11	2.47	< 0.001	1.08	0.001
Race (black)	3.23	< 0.001	0.561	< 0.001	6.05	< 0.001	3.59	< 0.001
Cigarettes per day	0.025	0.019	0.008	0.055	0.038	0.011	−0.019	0.11
Smoking duration (y)	−0.025	0.10	−0.004	0.39	−0.034	0.10	−0.020	0.17
Weight (kg)	−0.108	< 0.001	−0.005	0.036	−0.175	< 0.001	−0.015	0.044
Height (cm)	−0.036	0.10	−0.045	< 0.001	−0.231	< 0.001	−0.270	< 0.001
FEV_1/FVC	−46.19	< 0.001	−6.27	< 0.001	−58.02	< 0.001	−12.18	< 0.001
CT lung volume (L)	2.29	< 0.001	0.892	< 0.001	9.77	< 0.001	10.48	< 0.001
	$R^2 = 0.56$		$R^2 = 0.23$		$R^2 = 0.78$		$R^2 = 0.55$	

CT lung volume refers to TLC_{CT} for $\%LAA_{I\text{-}950}$ and FRC_{CT} for $\%LAA_{E\text{-}856}$.

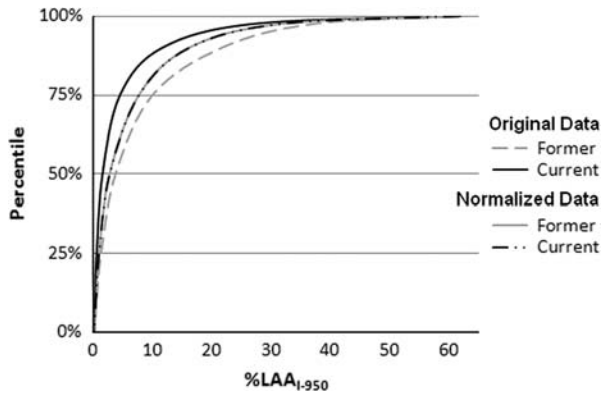

FIGURE 2. Distribution of $\%LAA_{I\text{-}950}$ by smoking status: original versus normalized data. Distributions of $\%LAA_{I\text{-}950}$ are plotted from minimum to maximum value within each smoking status group where the values at the 50th percentile represent the medians of each distribution.

adjustment for differences in disease severity and other potential confounders.

The finding that severity of "emphysema" increases after smoking cessation suggests that the smoking effect is a real phenomenon. Shaker et al[14] followed up a group of 36 subjects who quit smoking after baseline CT scans. They defined emphysema as voxels ≤ -910 HU ($\%LAA_{I\text{-}910}$) and collected 15th percentile of lung attenuation (Perc15) as well. Half of the group took inhaled corticosteroids during the study period, whereas the others received placebo. The authors found a statistically significant increase in $\%LAA_{I\text{-}910}$ of 2.6 percentage points, and Perc15 fell by 4.9 HU. Interestingly, the effect was driven by the treatment group. When evaluated alone, the placebo group did not display significant changes in lung density measures. However, the study size was too small to achieve a statistically significant difference between the groups and thus limits strong conclusions. A study by Ashraf et al[15] displayed similar results in a group of 77 subjects who quit smoking for at least 2 years after baseline CT scans. The group had an average decrease in Perc15 of 6.2 HU after 1 year and a further decrease of 3.6 HU after the second year. Beyond 2 years, no further change was detected. In addition, 18 subjects relapsed during the study period and exhibited an increased Perc15 of 3.7 HU.

TABLE 8. Distributions of $\%LAA_{I\text{-}950}$ Before and After Quantile Normalization and Mean Difference Adjustment by Smoking Status

	Original $\%LAA_{I\text{-}950}$		Normalized $\%LAA_{I\text{-}950}$		Adjusted $\%LAA_{I\text{-}950}$	
Percentile	**Former**	**Current**	**Former**	**Current**	**Former**	**Current**
Maximum	61.20	61.91	61.56	61.56	61.20	71.98
97.5%	37.03	26.91	31.55	31.54	37.03	34.45
90.0%	21.65	11.59	16.14	16.18	21.65	18.11
75.0%	9.92	4.43	7.64	7.65	9.92	9.01
Median	3.68	1.46	2.68	2.69	3.68	4.84
25.0%	1.23	0.49	0.89	0.89	1.23	2.14
10.0%	0.48	0.21	0.36	0.36	0.48	1.66
2.5%	0.17	0.08	0.13	0.13	0.17	1.50
Minimum	0.01	0.00	0.02	0.02	0.01	1.40

Cigarette smoking causes accumulation of inflammatory cells in the lung, particularly around respiratory bronchioles (respiratory bronchiolitis). Accumulation of this soft tissue attenuation material may result in an increase in CT attenuation within individual voxels, resulting in a relative decrease in the percentage of LAAs. The fact that current smoking was associated with a higher proportion of subjects with CN supports this possibility. This hypothesis is further supported by the findings by Shaker et al[14] cited above, showing that former smokers who took inhaled corticosteroids had a larger decrease in lung density measures than did the untreated group. This suggests that the anti-inflammatory effect of the medication may have played a part in emphasizing the decreased QCT lung density. Our study supports the inflammatory hypothesis by showing that subjects taking inhaled corticosteroids had a relative increase in LAAs, but we found no significant difference in this effect between former and current smokers. The effect size of smoking status did not change significantly when corticosteroid use was added to the model, suggesting that the smoking effect was not affected by corticosteroid use. Although Langerhans cell histiocytosis may also cause CN, this is a relatively rare complication of smoking, and therefore we believe that most of the centrilobular nodules seen in our subjects were due to respiratory bronchiolitis.

The sample size of the current data set provided an opportunity to use the quantile normalization technique to eliminate the confounding role of smoking status in QCT measures. This technique has not previously been utilized in a data set of this type but offers a unique approach to this sort of correction. The normalization was performed separately in each GOLD stage for 2 reasons: to control for pulmonary function and because univariate assessments of $\%LAA_{I\text{-}950}$ (Table 2) showed differing effect sizes and variances between the current and former smokers within each GOLD stage. Upon normalization, each subject was assigned a new value for $\%LAA_{I\text{-}950}$. The new distributions of the current and former smoking groups became almost perfectly aligned.

To test the impact of the normalization, the multivariate models were developed again with the normalized $\%LAA_{I\text{-}950}$ value as the outcome of interest. In the combined model of all subjects, smoking status became a statistically insignificant component. Although the overall R^2 of the model was not changed, the effect size was greatly reduced and the direction reversed. Meanwhile, the associations of the other variables remained relatively stable. In the separate case and control models, smoking status remained a significant component, but like the combined model the effect size was substantially reduced, and the direction of the effect was reversed. Quantile normalization may prove to be a relevant technique to adjust for smoking status in the evaluation of QCT lung densitometry. Alternative strategies would include adding a fixed number (eg, 3.5%) to the measured % of LAAs in current smokers, or incorporating the smoking status into a multivariate model.

The current study is not without limitations. Inclusion of subjects from multiple clinical centers introduces variations due to scanner differences. However, the scanner model did not affect the models shown here. Because of the cross-sectional nature of this study, we were unable to determine how long it takes for this smoking effect to manifest or to reverse its course upon smoking cessation. We did attempt to control for subjects' time since quitting,

TABLE 9. Multiple Linear Regression Analysis for Quantile Normalized $\%LAA_{I\text{-}950}$: Cases and Controls

	Cases		Controls		All Subjects	
	Estimate	***P***	**Estimate**	***P***	**Estimate**	***P***
Current smoker	0.976	0.001	0.251	0.023	0.267	0.13
Age	0.055	0.002	0.020	< 0.001	0.011	0.28
Sex (F)	1.12	0.001	−0.161	0.16	0.611	0.002
Race (black)	2.84	< 0.001	0.545	< 0.001	2.25	< 0.001
Cigarettes per day	0.031	0.002	0.006	0.14	0.016	0.009
Smoking duration (y)	−0.028	0.045	−0.001	0.75	−0.022	0.005
Weight (kg)	−0.097	< 0.001	−0.007	0.003	−0.056	< 0.001
Height (cm)	−0.028	0.17	−0.042	< 0.001	−0.055	< 0.001
FEV_1/FVC	−44.83	< 0.001	−6.14	< 0.001	−32.82	< 0.001
TLC_{CT} (L)	2.10	< 0.001	0.883	< 0.001	1.74	< 0.001
	$R^2 = 0.54$		$R^2 = 0.18$		$R^2 = 0.56$	

but the association was statistically unimportant and made no meaningful impact on other model variables. Although we sought to control for severity of disease in the model, we cannot exclude the possibility that some of the apparent differences between current and former smokers might be due to a survivor effect. This paper did not analyze any zonal or lobar difference in lung attenuation, but this might be a topic for future research.

The quantile normalization technique used here controlled for COPD severity based on the GOLD stage but did not account for other variables that may be interrelated. Also, as the procedure requires paired data, we were obliged to exclude data for >1400 subjects who could not be paired. Those excluded subjects were disproportionately represented in the GOLD 3 and 4 categories, as there were fewer current smokers in those groups to be paired with former smokers. However, this exclusion actually helped provide a cohort that was seemingly more representative of the general COPD population. The COPDGene cohort was recruited with an intentional overabundance of GOLD 3 and 4 subjects compared with the general population. Most of the study subjects evaluated here will soon be undergoing 5-year follow-up scans. This should allow us to address the effect of smoking cessation in subjects who quit smoking before follow-up.

In summary, smoking status has a substantial effect on lung densitometry measures, and thus it is important to consider this variable in subjects being evaluated by QCT. In population studies, quantile normalization may be a helpful method to adjust lung density measures to reduce or eliminate the confounding effect of smoking status.

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