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Current smoking with or without chronic bronchitis is independently associated with goblet cell hyperplasia in healthy smokers and COPD subjects

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COPD, chronic bronchitis (CB) and active smoking have all been associated with goblet cell hyperplasia (GCH) in small studies. Active smoking is strongly associated with CB, but there is a disconnect between CB clinical symptoms and pathology. Chronic cough and sputum production poorly correlate with the presence of GCH or COPD. We hypothesized that the primary determinant of GCH in ever smokers with or without airflow obstruction is active smoking. Goblet Cell Density (GCD) was measured in 71 current or former smokers [32 subjects without COPD and 39 COPD subjects]. Endobronchial mucosal biopsies were stained with Periodic Acid Schiff-Alcian Blue, and GCD was measured as number of goblet cells/mm basement membrane. GCD was divided into tertiles based on \log_{10} transformed values. \log_{10} GCD was greater in current smokers compared to former smokers. Those with classically defined CB or SGRQ defined CB had a greater \log_{10} GCD compared to those without CB. Current smoking was independently associated with tertile 3 (high \log_{10} GCD) whereas CB was not in multivariable regression when adjusting for lung function and demographics. These results suggest that GCH is induced by active smoke exposure and does not necessarily correlate with the clinical symptoms of CB.

Abbreviations

ANOVA	Analysis of variance
ATS	American thoracic society
BMI	Body mass index
CB	Chronic bronchitis
COPD	Chronic obstructive pulmonary disease
CT	Computed tomography
FEV ₁	Forced expiratory volume in 1 s

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FVC	Forced vital capacity
GCD	Goblet cell density
GCH	Goblet cell hyperplasia
GOLD	Global initiative for chronic obstructive lung disease
mMRC	Modified medical research council
SGRQ	Saint George's respiratory questionnaire
SPIROMICS	Subpopulations and intermediate outcome measures in COPD study

Chronic Obstructive Pulmonary Disease (COPD) is a major cause of morbidity and is the fourth leading cause of death in the United States¹. Chronic bronchitis (CB) is a common phenomenon in smokers with and without COPD and is characterized by chronic cough and phlegm. CB increases risk of respiratory exacerbations, is associated with higher mortality, and hastens lung function decline². The most well-established risk factor for developing CB is active smoking^{3,4}.

The pathologic correlate of CB is thought to be goblet cell hyperplasia (GCH). Small airway mucus plugging is more commonly seen in COPD and increases as the degree of airflow obstruction worsens⁵. GCH has been shown to involve the peripheral airways in surgical lung specimens from those with CB⁶ and in large airway endobronchial mucosal biopsies in smokers with airflow obstruction^{7,8}. We have previously shown that GCH was greater in the large airways in those with CB compared to those without CB⁸. Additionally, mucus burden has prognostic significance; one study in lung volume reduction surgery patients found that small airway mucous metaplasia inversely correlated with changes in lung function after surgery⁹, whereas another study found that the degree of small airway mucus luminal occlusion correlated with mortality¹⁰.

However, there is a disconnect between respiratory symptoms and the magnitude of GCH. Although active smoking is the primary risk factor for CB, not all smokers develop CB, and CB can affect former smokers as well³. One study in advanced emphysema patients found no relationship between cough and sputum symptoms and degree of small airway mucus impaction¹¹, while an established pathologic measure of mucous gland hyperplasia has little to no correlation with clinical symptoms¹². We sought to analyze GCH as it related to smoking status and chronic bronchitis in smokers with and without airflow obstruction. Given the disconnect between the clinical syndrome of CB and airway pathology, we hypothesized that GCH would be more commonly seen in current smokers compared to ex-smokers and would not necessarily associate with CB.

Materials and methods

The Subpopulations Intermediate Outcome Measures in COPD Study (SPIROMICS) is a prospective cohort study that has enrolled 2981 subjects across four strata (1) Never smokers (NS), (2) Current or former smokers without COPD (HS), (3) Mild/Moderate COPD, and (4) Severe COPD). Goblet Cell Density (GCD) was measured in 71 subjects in strata 2–4 (current or former smokers with and without COPD) in which endobronchial mucosal biopsies were obtained during the SPIROMICS bronchoscopy substudy¹³. Methods of measuring GCD were performed as previously described⁸. Briefly, biopsies were stained with Periodic Acid Schiff-Alcian Blue. Goblet cells from 4–6 endobronchial specimens were counted and related to the length of basement membrane using Image J¹⁴. If there were fewer than 4 acceptable samples, the subject was excluded ($n = 28$ out of 99 total subjects). GCD was expressed as the number of goblet cells per millimeter of basement membrane. Two observers performed the measurements in a double-blinded fashion. GCD was then \log_{10} transformed to make the distribution of values assume a more Gaussian distribution and were divided into tertiles (1 = low, 2 = medium, 3 = high GCD). Ethical approval for this study was obtained for this study from the SPIROMICS Observational Safety Monitoring Board and this study was carried out in accordance with the Declaration of Helsinki. All participants were enrolled with informed consent in accordance with and under the approval of the Institutional Review Board (IRB) of participating sites (Columbia University Medical Center- Division of General Medicine, New York, NY; Wake Forest Baptist Health- Center for Genomics and Personalized Medicine Research, Winston Salem, NC; University of Utah- Pulmonary Lung Health Research Center, Salt Lake City, UT; University Of Michigan- Pulmonary and Critical Care Medicine, Ann Arbor, MI; University of California at Los Angeles- Pulmonary and Critical Care Medicine, Los Angeles, CA; University of California at San Francisco- Airway Clinical Research Center; San Francisco, CA; University of North Carolina at Chapel Hill—Genomics and Informatics Center—Collaborative Studies Coordinating Center, Chapel Hill, NC; National Jewish Health- Division of Pulmonary, Critical Care, and Sleep Medicine, Denver, CO; Johns Hopkins University- Pulmonary and Critical Care Medicine, Baltimore, MD; University of Illinois- Breathe Chicago Center, Chicago, IL; University of Alabama at Birmingham- Lung Health Center, Birmingham, AL; Temple University- Dept. of Thoracic Medicine and Surgery, Philadelphia, PA; University of Iowa- Internal Medicine, Iowa City, IA).

Airway total mucin concentration was measured in induced sputum samples in a subset of subjects ($n = 7$ in tertile 1, $n = 15$ in tertile 2, $n = 6$ in tertile 3) using previously described methods¹⁵. Sputum was induced by inhalation of hypertonic saline by subjects who had a forced expiratory volume in 1 s of more than 35% of the predicted value, according to protocol¹⁶ and American Thoracic Society and European Respiratory Society standards¹⁷.

Statistics. Statistics were performed using SPSS v25 (IBM Corp., Armonk, NY). Intraclass correlation coefficients between the two observers for goblet cells and GCD were calculated. Differences between groups were assessed using either unpaired *t* tests or one way ANOVA for continuous variables and chi squared tests for categorical variables. Multivariable logistic regression was performed with Tertile 3 (High GCD) as the dependent variable of interest with current smoking, CB classic definition and CB SGRQ definition in separate models with demographics and FEV₁% predicted as covariates. Additionally, multivariable linear regression was performed with the same covariates for \log_{10} GCD. A *p* value of less than 0.05 was considered statistically significant.

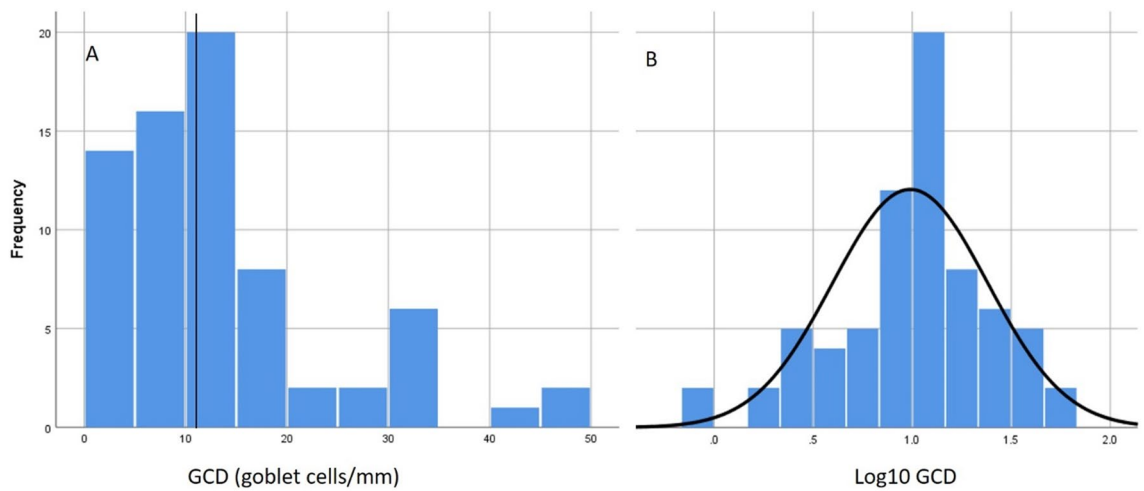


Figure 1. (A) Distribution of goblet cell density measurements. Vertical line represents median value. (B) Distribution of \log_{10} goblet cell density measurements.

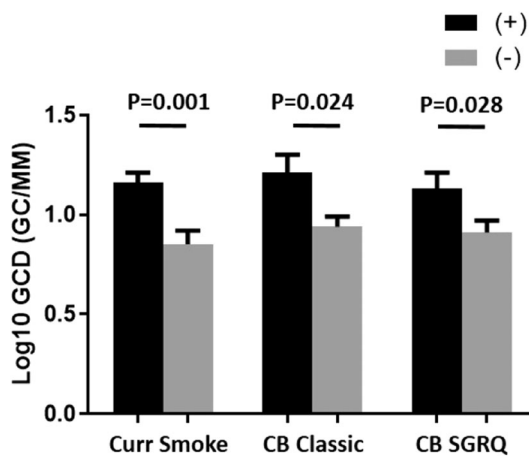


Figure 2. \log_{10} goblet cell density by chronic bronchitis or smoking groups. Data expressed as mean \pm SE. GCD goblet cell density, CB chronic bronchitis, SGRQ Saint George's respiratory questionnaire.

Ethics approval and consent to participate. Each site had IRB approval and all subjects provided informed consent to participate.

Consent for publication. All authors provide their consent to participate. There are no individual subject data that are within this manuscript.

Results

Intraclass correlation coefficients between the two observers for goblet cells and GCD were 0.932 and 0.968, respectively ($p < 0.0001$ for both). See Fig. 1a for a histogram of the distribution of GCD. Figure 1b shows the distribution of \log_{10} GCD. The median value for GCD was 11.034 GC/mm. \log_{10} GCD was greater in those that were currently smoking compared to former smokers (1.16 ± 0.28 [$n = 31$] vs. 0.85 ± 0.42 [$n = 40$], $p = 0.001$). Those with CB defined using the classic definition (cough and phlegm for > 3 months/year for at least 2 consecutive years) had a greater \log_{10} GCD compared to those without CB (1.21 ± 0.31 [$n = 13$] vs. 0.94 ± 0.39 [$n = 58$], $p = 0.024$). Similarly, those with CB by the SGRQ definition (cough and phlegm almost every day or several days a week for the past 4 weeks) had a greater \log_{10} GCD compared to those without CB (1.13 ± 0.39 [$n = 25$] vs. 0.91 ± 0.39 [$n = 43$], $p = 0.028$). See Fig. 2. Sputum mucin concentration was neither different between those with classic CB compared to those without classic CB (2980 ± 1850 vs. 1724 ± 1279 $\mu\text{g/mL}$, $p = 0.061$) nor different between those with SGRQ CB compared to those without SGRQ CB (2090 ± 1688 vs. 1919 ± 1370 $\mu\text{g/mL}$, $p = 0.767$).

The characteristics of the three tertiles are shown in Table 1. 5.0% of ex-smokers and 33.3% of current smokers had classically defined CB. Tertiles 2 and 3 tended to be younger than tertile 1 (64.9 ± 7.5 , 59.8 ± 9.3 , 59.8 ± 8.2 years in tertiles 1, 2 and 3, respectively, $p = 0.06$). The groups were similar in gender, race distribution and body mass index. 16.7% of those in tertile 1 were current smokers, whereas in tertiles 2 and 3 the percentages

	Tertile 1 (n = 24)		Tertile 2 (n = 24)		Tertile 3 (n = 23)	
Age (years)	64.9	± 7.5	59.8	± 9.3	59.8	± 8.2
Gender (% male)	13 (54.2)		13 (54.2)		16 (69.6)	
Race (% Caucasian)	20 (83.3)		16 (66.7)		16 (69.6)	
BMI (kg/m ²)	29.1	± 4.6	28.4	± 4.5	26.9	± 5.7
Current smoker [n (%)]*	4 (16.7)		12 (50.0)		14 (63.6)	
Pack year history	45.2	± 19.8	48.3	± 25.7	43.5	± 20.6
CB classic def [n (%)]	2 (8.3)		4 (16.7)		7 (30.4)	
CB SGRQ def [n (%)]	5 (22.7)		8 (34.8)		12 (52.2)	
FEV ₁ %Pred	95.9	± 20.8	84.1	± 16.4	85.4	± 19.6
FEV ₁ /FVC (%pred)	92.2	± 15.5	83.8	± 14.7	84.0	± 16.7
No COPD [n(%)]	15 (62.5)		7 (29.2)		10 (43.5)	
COPD [n(%)]	9 (37.5)		17 (70.8)		13 (56.5)	
6MWD (m)	477	± 92	439	± 88	466	± 66
SGRQ score	16.1	± 17.3	26.1	± 17.6	23.7	± 19.1
mMRC dyspnea score	0.30	± 0.64	0.65	± 0.89	0.61	± 0.72
CAT score	7.45	± 7.58	12.52	± 8.46	11.83	± 7.95
Exac hx (exac.pt/year)	0.08	± 0.28	0.54	± 1.18	0.41	± 0.80
Sev Exac Hx (exac.pt/year)	0.04	± 0.20	0.17	± 0.38	0.09	± 0.43
GCD (cells/mm) [†]	4.30	± 2.18	11.20	± 2.00	26.06	± 10.59
Mucin conc (µg/mL)	1185	± 967	2211	± 1482	2312	± 1915

Table 1. Baseline characteristics. *BMI* body mass index, *CB* chronic bronchitis, *SGRQ* Saint George's Respiratory Questionnaire, *FEV₁* forced expiratory volume in 1 s, *FVC* forced vital capacity, *6MWD* 6-min walk distance, *mMRC* modified medical research council, *CAT* COPD assessment test, *Exac Hx* exacerbation history, *Sev Exac Hx* severe exacerbation history, *GCD* goblet cell density. * $p = 0.004$, [†] $p < 0.0001$.

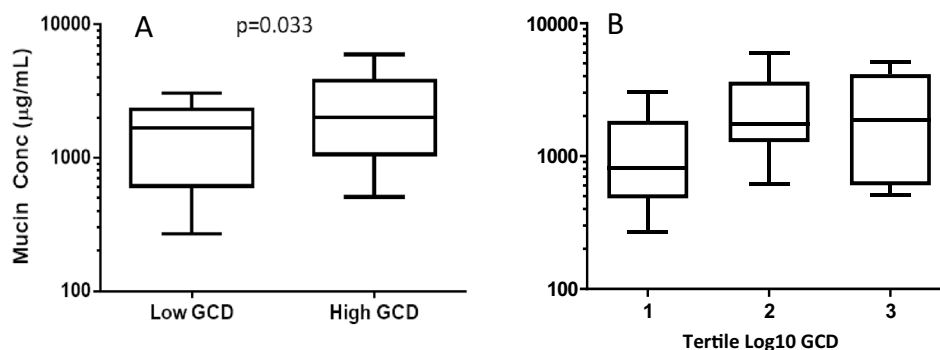


Figure 3. Induced sputum mucin concentrations in the (A) low and high GCD groups and the (B) three tertiles of \log_{10} GCD.

of those that were current smokers were 50.0% and 63.6%, respectively ($p = 0.004$). The percentages of subjects in each tertile with Classic CB or SGRQ CB were not significantly different. Lung function tended to be lower in tertiles 2 and 3 compared to tertile 1 (FEV_1 95.9 ± 20.8 , 84.1 ± 16.4 , 85.4 ± 19.6 predicted for tertiles 1, 2 and 3, respectively, $p = 0.069$). 6-min walk distance, SGRQ scores, mMRC dyspnea scores and exacerbation histories were similar between tertiles. CAT scores tended to be worse in tertiles 2 and 3 compared to tertile 1 (7.45 ± 7.58 , 12.52 ± 8.46 , 11.83 ± 7.95 for tertiles 1, 2, and 3, respectively, $p = 0.08$). Mucin concentrations were greater in tertiles 2 and 3 but the differences were not statistically significant (1185 ± 967 , 2211 ± 1482 , 2312 ± 1915 µg/mL in tertiles 1, 2 and 3, respectively, $p = 0.279$). When GCD was divided into two groups by the median value into Low GCD and High GCD, mucin concentrations were higher in the High GCD group (2492 ± 1691 vs. 1289 ± 838 µg/mL, $p = 0.033$). See Fig. 3.

Multivariable logistic regressions for tertile 3 (high \log_{10} GCD) using current smoking, Classic CB and SGRQ CB in separate models are shown in Table 2. Current smoking was independently associated with High GCD after adjusting for other covariates (OR 4.15, 95% CI 1.17, 14.71). Neither Classic CB nor SGRQ CB were associated with High GCD (OR 3.14, 95% CI 0.86, 11.51 and OR 2.42, 95% CI 0.77, 7.56, respectively). Multivariable linear regressions for \log_{10} GCD using current smoking, Classic CB and SGRQ CB in separate models are shown in Table 3. Model one uses current smoking, Classic CB or SGRQ CB as independent variables of interest with age,

	OR	95% CI	
Current smoking	4.15	1.17	14.71
CB classic def	3.14	0.86	11.51
CB SGRQ def	2.42	0.77	7.56

Table 2. Multivariable logistic regression for Tertile 3 (high GCD). Covariates include age, gender, race, and FEV₁%predicted. Current smoking, CB classic definition and CB SGRQ definition were in separate models.

	Model 1			Model 2		
	Estimate	S.E	p	Estimate	S.E	p
Current smoking	0.265	0.103	0.012	0.226	0.100	0.028
CB classic def	0.257	0.115	0.029	0.218	0.111	0.054
CB SGRQ def	0.190	0.098	0.058	0.118	0.100	0.240

Table 3. Multivariable linear regression for log₁₀ GCD. Model one is adjusted for age, gender, and race; model two is adjusted for age, gender, race, and FEV₁%predicted. Current smoking, CB classic definition and CB SGRQ definition were each tested in separate models with the covariates. The bolded p values are considered statistically significant.

gender and race as covariates. Model two adjusts for age, gender, race, and FEV₁%predicted. Current smoking and Classic CB were independently associated with log₁₀ GCD in model one (estimate 0.265, SE 0.103, p = 0.012 and estimate 0.257, SE 0.115, p = 0.029, respectively). However, in model two, only current smoking was independently associated with log₁₀ GCD (estimate 0.226, SE 0.100, p = 0.028).

Discussion

We showed that GCD was greater in those that were currently smoking and in those with CB. Similarly, those with high log₁₀ GCD were more likely to be current smokers but not CB using either definition. Using multivariable logistic regression, we showed that current smoking, but not CB, was independently associated with high log₁₀ GCD. Similar results were seen with multivariable linear regression after adjusting for lung function and demographics. These results suggest that GCH is induced by active smoke exposure and does not necessarily translate into the clinical symptoms of CB.

This is the first study that demonstrates the complex interrelationship between CB, smoking, and GCH in a large cohort of extensively characterized smokers with and without airflow obstruction. A prior study of epithelial mucin stores compared goblet cell measures in 24 active smokers and 19 nonsmoking controls; mucin stores were greater in the smokers, especially those with airflow limitation⁷. However, it was not clear whether the smokers had CB. In a prior report, we contrasted the GCD in 15 subjects with moderate to severe COPD, 12 smokers without COPD, and 11 healthy nonsmokers⁸. Interestingly, we found that the smokers without airflow obstruction had the greatest degree of GCH while those with CB had a greater GCD than those without CB⁸. Another study revealed that GCH, assessed using qualitative measures, was more commonly seen in habitual tobacco smokers compared to nonsmoking controls¹⁸. Our data expand upon these findings in a much larger cohort with the addition of the relationship of current smoking with CB.

We also showed that mucin concentrations in induced sputum were greater in those with high GCD. This is the first study that has shown a relationship between large airway GCH and sputum mucins. It has been speculated that mucus is expectorated from large airways to produce sputum, suggesting sputum mucin concentration and large airway GCH should be related, but few studies have addressed the correspondence between these two metrics of mucus production. This association may have clinical relevance as well, as sputum mucins have been related to CB, respiratory exacerbations and peripheral airway disease in 917 subjects in the SPIROMICS cohort^{15,19}. Unlike this larger study, our analysis did not show a statistically greater degree of sputum mucins in chronic bronchitics. This may be due to the small sample size or that some mucins arise from submucosal glands that we were not able to examine.

The prevalence of CB ranges anywhere from 12.2% in smokers without airflow obstruction to 74% in some COPD cohorts^{20–22}. CB has been related to an accelerated rate of lung function decline, worse health related quality of life, increased mortality, and an increased risk of respiratory exacerbations in individuals with and without COPD^{20,23–25}. In the Copenhagen City Heart Study, chronic mucus hypersecretion was associated with an increased rate of FEV₁ decline over time²⁴. Analyses of the COPD Gene study and SPIROMICS showed that CB in those without airflow obstruction was associated with respiratory exacerbations^{20,26}, and in those with airflow obstruction CB was associated with a two-fold increased rate of exacerbations in longitudinal follow-up²⁷. In the Tucson Epidemiologic Survey of Airway Obstructive Disease, younger patients with CB had a greater mortality compared to those without CB²⁸. In a study of nearly 48,000 men and women, CB was associated with an increased duration of hospitalization and a greater all-cause mortality²⁵.

The most well described risk factor for CB is active smoking. A large study of more than 1,700 Finnish men showed that over 30 years the cumulative incidence of CB in continued smokers was 42%²⁹. A meta-analysis revealed that the relative risk of CB from current smoking was 3.41³⁰. There is also evidence that smoking cessation decreases CB. Over five years, an analysis of the COPDGene study showed that ex-smokers who resumed smoking were more likely to develop CB and that current smokers who quit were more likely to have their CB resolve³¹. A larger study of over 4,000 subjects followed from ages 20 to 64 showed that those who smoked were more likely to develop chronic mucus hypersecretion and quitting smoking resulted in decreased chronic mucus hypersecretion⁴.

GCH is one of the pathologic foundations for CB. The primary mechanisms responsible for excessive mucus in CB are overproduction and hypersecretion by goblet cells and/or decreased clearance of mucus. Mucus hypersecretion develops as a consequence of cigarette smoke exposure^{32,33}, acute and chronic viral infection³⁴ and inflammatory cells may activate mucin gene transcription³⁵. The increased epithelial gene expression is associated with metaplastic responses in club cells to assume a goblet cell morphology characterized by overproduction of mucus and hypersecretion associated with increased degranulation. This hypersecretion is compounded by difficulty in clearing secretions because of poor ciliary function, defective mucus hydration, distal airway occlusion, and ineffective cough secondary to respiratory muscle weakness and reduced peak expiratory flow in COPD^{5,35–37}.

Unlike the tight correlation between emphysema and lung function, the relationship between airway pathology, physiology and symptom severity is only moderate at best. Large airway GCH correlates poorly with the degree of airflow obstruction³⁸ or chronic phlegm³⁹. Small airway disease has been found in surgical lung specimens from those with advanced emphysema, with no clinical or radiographic evidence to suggest its presence preoperatively^{5,9,40,41}. More importantly, the degree of small airway GCH is difficult to detect clinically by cough or sputum burden¹¹. Our findings shed light on the subject and improve our current understanding. Although both active smokers and those with CB had increased GCD, only current smoking was independently associated with it in multivariable analysis.

There are several limitations that are worthy of mention. Firstly, although the size of the cohort analyzed is large for a bronchoscopy study, its size in comparison to the entire SPIROMICS cohort is small. Secondly, mucin concentrations on the induced sputum samples were only available for 28 subjects, making the distinction of mucin concentrations between groups suboptimal. There was also a lack of never smokers to serve as a control group. Lastly, there may have been within subject differences in GCD based on the areas sampled which could not be assessed.

Nonetheless, we have shown that goblet cell hyperplasia is related more so to current smoking as opposed to the presence or absence of chronic bronchitis, no matter how it is defined. These findings suggest that goblet cell hyperplasia may exist in the absence of chronic bronchitis, again emphasizing the disconnect between clinical symptoms and airway pathology. However, our findings also suggest that current smoking may cause goblet cell hyperplasia before chronic bronchitis develops or independently from chronic bronchitis. These findings need to be validated in other studies and their clinical relevance need to be better defined.

Data availability

More information about the study and how to access SPIROMICS data is at www.spiromics.org.

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Author contributions

V.K. conceived and designed the analysis plan, performed data analysis and contributed significantly to the writing of the manuscript; S.J. generated the data and helped with the data analysis; H.Z. performed the data

analysis; M.K., R.C.B., J.M.W., S.A.C., M.D., M.K.H., R.P., C.B.C., I.B., R.B., J.L.C., R.J.K., S.L.O., W.K.O., S.I.R., F.J.M., and P.G.W. contributed to the data analysis and writing of the manuscript.

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Competing interests

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Additional information

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