Association between acute respiratory disease events and the *MUC5B* promoter polymorphism in smokers

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ABSTRACT

A single-nucleotide polymorphism (rs35705950) in the mucin 5B (*MUC5B*) gene promoter is associated with pulmonary fibrosis and interstitial features on chest CT but may also have beneficial effects. In non-Hispanic whites in the COPDGene cohort with interstitial features (n=454), the *MUC5B* promoter polymorphism was associated with a 61% lower odds of a prospectively reported acute respiratory disease event (P=0.001), a longer time-to-first event (HR=0.57; P=0.006) and 40% fewer events (P=0.016). The *MUC5B* promoter polymorphism may have a beneficial effect on the risk of acute respiratory disease events in smokers with interstitial CT features.

INTRODUCTION

A specific single-nucleotide polymorphism (rs35705950) in the *MUC5B* promoter increases the expression of *MUC5B* and is associated with idiopathic pulmonary fibrosis as well as with more subtle visual and objective parenchymal abnormalities on the chest CT scans of smokers.[1–3] Although the fibrotic risk associated with this polymorphism is clear, its prevalence in whites and the importance of MUC5B in airway defence raise the question of whether the polymorphism may have shorter-term beneficial effects on airway clearance and acute respiratory illnesses at the expense of a longer-term risk of fibrosis.[4–6] We therefore hypothesised that the rs35705950 polymorphism might decrease the risk of acute respiratory disease (ARD) events in smokers and that this effect would be most evident in those with interstitial features on chest CT as these may represent phenotypic evidence of increased *MUC5B* expression in some cases.

METHODS

Detailed methods are provided in the online Supplementary file 1. Briefly, using data from the COPDGene study, we measured the percentage of lung occupied by interstitial and emphysematous features using a previously described automated method.[3] We defined participants as having interstitial and/or emphysematous features if those features occupied >10% of their lung volume and performed sensitivity analyses using a 5% threshold.[3] ARD events occur in smokers with and without COPD and were defined as intermittent episodes of increased shortness of breath, cough and/or change in sputum quality requiring a change in treatment, including antibiotics and/or steroids.[7] ARD events were assessed prospectively and were categorised as severe if they required an emergency room visit or hospitalisation.[7] Due to the small number of participants who were homozygous for the minor allele (TT, n=53 (0.8%)), participants were considered to have the *MUC5B* promoter polymorphism if they had at least one copy of the minor allele. Secondary analyses of the primary outcomes assuming an additive effect of the number of copies of the minor allele were also performed. In addition, due to concern that the effect of each feature type may confound the relationship between the other and ARD events, secondary analyses were also performed in which the effect of each parenchymal feature type was evaluated in those with otherwise 'normal' parenchyma, that is, the effect of interstitial features was evaluated in those without emphysema, and the effect of emphysema was evaluated in those without interstitial features. Finally, due to racial differences in the prevalence of the *MUC5B* promoter polymorphism, all analyses were stratified by race and the primary analyses were performed in non-Hispanic whites (NHW) (minor allele frequency in NHW=19.0%, in blacks=4.4%).

RESULTS

As shown in the online Supplementary table E1, 6863 participants had completed clinical, imaging and genetic data, of which 72.8% were NHW (n=4999). As shown in the online Supplementary table E2), 43.4% of NHW (n=2167) reported an ARD event over a mean of 6.0 years of follow-up, with a mean total number of events of 2.3. In NHW with interstitial features (n=454), those who had the *MUC5B* promoter polymorphism (n=97) had a 61% lower odds of an ARD event than those without the polymorphism (OR 0.39; 95% CI 0.22 to 0.69, P=0.001). Those with the polymorphism also had a longer time-to-first ARD event (HR 0.57; 95% CI 0.38 to 0.85, P=0.006) and reported 40% fewer ARD events (incidence rate ratio=0.60; 95% CI 0.40 to 0.91, P=0.016) (table 1). Similar results were found using an additive genetic model (online Supplementary table E3). NHW individuals with interstitial features who had the *MUC5B* promoter polymorphism also exhibited a lower odds of severe ARD event than those without the polymorphism (OR 0.48; 95% CI 0.23 to 0.97, P=0.042) (online

Table 1 Effect of *MUC5B* genotype on acute respiratory disease events: results stratified by parenchymal feature type

	Odds of reporting ARD event		Time-to-first ARD event		Number of ARD events	
	OR (95% CI)	P	HR (95% CI)	P	Incident rate ratio (95% CI)	P
Entire cohort						
All races (n=6663)	0.94 (0.81 to 1.10)	0.436	0.96 (0.87 to 1.06)	0.433	0.97 (0.86 to 1.09)	0.600
White (n=4999)	0.91 (0.77 to 1.07)	0.240	0.93 (0.84 to 1.04)	0.217	0.97 (0.86 to 1.10)	0.643
Black (n=1864)	1.09 (0.65 to 1.81)	0.754	1.18 (0.82 to 1.72)	0.375	0.89 (0.54 to 1.44)	0.626
Those with interstitial features						
All Races (n=856)	0.54 (0.34 to 0.87)	0.010	0.67 (0.47 to 0.94)	0.020	0.65 (0.44 to 0.96)	0.030
White (n=454)	0.39 (0.22 to 0.69)	0.001	0.57 (0.38 to 0.85)	0.006	0.60 (0.40 to 0.91)	0.016
Black (n=402)	1.25 (0.48 to 3.21)	0.650	1.11 (0.55 to 2.34)	0.772	0.69 (0.28 to 1.74)	0.437
Those with emphysematous features						
All races (n=1907)	1.10 (0.83 to 1.46)	0.522	1.05 (0.90 to 1.23)	0.546	1.03 (0.86 to 1.22)	0.766
White (n=1551)	1.06 (0.78 to 1.53)	0.710	1.01 (0.86 to 1.19)	0.875	1.03 (0.86 to 1.23)	0.747
Black (n=356)	0.73 (0.22 to 2.41)	0.608	0.82 (0.37 to 1.82)	0.631	0.53 (0.19 to 1.47)	0.225

All effects expressed as those with *MUC5B* promoter polymorphism (GT and TT) compared with those without the polymorphism (GG).
Odds of reporting an ARD event assessed using logistic regression, time-to-first ARD event assessed using Cox regression and number of ARD events assessed using negative binomial regression.
All analyses presented are adjusted for age, sex, body mass index, current smoking status, pack years of smoking, per cent predicted FEV_1, a reported history of gastro-oesophageal reflux disease, the total Saint George's Respiratory Questionnaire Score, the clinical centre and a reported history of an ARD in the year prior to study enrolment.
ARD, acute respiratory disease.

Supplementary table E4). These findings were not present in those with emphysematous features (table 1 and online Supplementary table E4).

In NHW with the *MUC5B* promoter polymorphism (n=948), interstitial features were associated with a lower odds of any ARD event (OR 0.57; 95% CI 0.34 to 0.97, P=0.037) (online Supplementary table E5A). The presence of interstitial features had a similar effect when compared with the 'normal' subgroup, that is, those without emphysema (OR 0.44; 95% CI 0.23 to 0.85, P=0.014) (online Supplementary table E6A). In NHW participants, the presence of the *MUC5B* promoter polymorphism significantly interacted with interstitial features to reduce the odds of ARD events (adjusted P interaction=0.016) (online Supplementary table E7). This finding was present even when those with emphysema were excluded (online Supplementary table E8). By contrast, emphysematous features were associated with increased risk of most measures of ARD and this risk was not modified by the *MUC5B* polymorphism (online Supplementary tables E5B, E6B, E7–8).

Finally, in NHW participants who had >5% of their lung occupied by interstitial features, those who had the *MUC5B* promoter polymorphism had a longer time-to-first ARD event than those without the polymorphism (HR 0.84; 95% CI 0.72 to 0.99, P=0.040), but did not have a statistically significant difference in their overall odds or number of ARD events (online Supplementary table E9).

DISCUSSION

In NHW ever-smokers in the COPDGene cohort with objective interstitial lung features, the presence of a *MUC5B* promoter polymorphism (rs35705950) was associated with a decreased odds of ARD events (both total and severe), a longer time-to-first event and fewer cumulative events. Additionally, the effect of interstitial CT features on the odds of reporting an ARD event was significantly modified by the presence of the polymorphism. These effects were specific to interstitial features and did not appear to be due to the concomitant presence of emphysema.

The prevalence of this polymorphism in certain racial groups raises the question of whether it may have a beneficial effect.[1] Given the importance of MUC5B in mucociliary clearance and airway defence, one potential benefit the polymorphism may confer is a decreased risk of respiratory episodes such as ARD events.[4] In the healthy human respiratory tract, MUC5B is the predominant component of secreted mucus, but the relative concentrations of it and the next most abundant mucin, MUC5AC, vary in disease.[8] For instance, in COPD, although MUC5B still predominates and both MUC5B and MUC5AC increase with disease severity, the proportional increase in MUC5AC is far greater than that of MUC5B.[6] Additionally, in asthma, an increase in MUC5AC and a relative decrease in MUC5B produces a MUC5AC-dominated mucus, which is associated with impaired mucus transport and may contribute to the viscous plugs.[9] Therefore, one plausible possible mechanism for our findings is that the polymorphism, which results in increased expression of *MUC5B*, helps to normalise the MUC5AC to MUC5B ratio and therefore improves airway clearance (figure 1).

It should be stressed that our findings are preliminary and should be interpreted with caution. Further work using bronchoscopic, sputum, nasopharyngeal and/or peripheral blood samples is needed to verify that CT interstitial features represent phenotypic evidence of increased *MUC5B* expression and to determine whether increased expression is causally related to fibrosis. Additional work will also be needed to confirm our findings, especially given our use of a significance threshold of 0.05 in the setting of multiple analyses and to evaluate for inadvertent confounding, that is, that the polymorphism is not beneficial, but rather that alternative genetic and clinical factors that also induce interstitial features are simply more harmful. Similarly, our primary results may be biased due to conditioning on a common effect in our multivariable models.[10] However, as there was little change in the results in the secondary analyses only adjusted for covariates unrelated to genetic status, this is unlikely to be the case (online Supplementary table E10). Future replication of our primary findings, combining independent cohorts and/or utilising cohorts with higher event

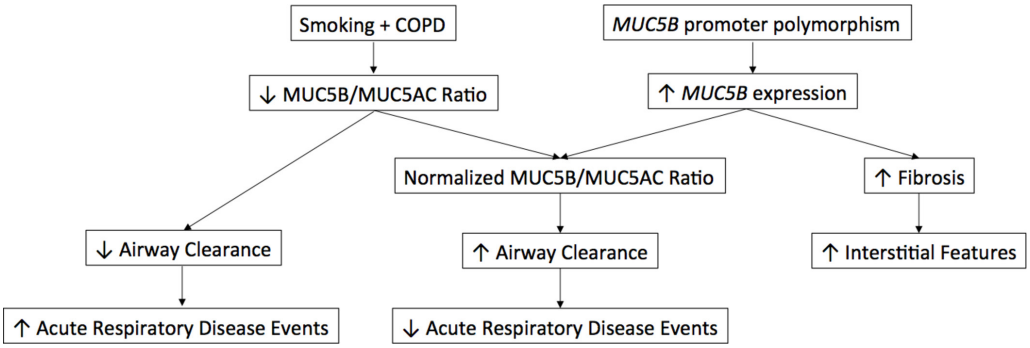

Figure 1 Proposed schema for the relationship between the *MUC5B* promoter polymorphism, interstitial features and ARD events. Note that we hypothesise that those individuals with interstitial features are those with the greatest evidence of increased *MUC5B* expression and therefore those most likely to have the normalised MUC5B/MUC5AC ratio. ARD, acute respiratory disease.

rates, is also needed to overcome additional limitations such as the lack of associations seen between the polymorphism and outcomes in African-American participants. Additional replication in cohorts of never-smokers and those with more advanced fibrotic disease is needed as well. Finally, longer term studies investigating which individuals with the polymorphism go on to develop advanced pulmonary fibrosis are also needed.

In summary, we found that in ever-smokers with objective interstitial features on chest CT, a specific single-nucleotide polymorphism (rs35705950) in the promoter of the gene that encodes mucin 5B was associated with reductions in multiple measures of ARD events, a major driver of COPD death and disability. Future studies are needed to replicate these findings in other population and to continue to investigate their pathophysiological mechanisms.

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Contributors The authors meet criteria for authorship as recommended by the International Committee of Medical Journal Editors. SYA, RH, RSJE and GRW designed the study and wrote the initial manuscript. SYA performed the statistical analyses. RH, JCR and RSJE wrote the feature detection algorithm. All authors contributed to the production of the final of the manuscript.

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