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Association of *MUC5B* promoter polymorphism with interstitial lung changes after COVID-19: A preliminary observation

Sir,

The minor (*T*) allele of rs35705950 a single-nucleotide polymorphism (SNP) in the mucin 5B (*MUC5B*) gene promoter is thought to increase the risk of sporadic idiopathic pulmonary fibrosis (IPF) as well as interstitial lung abnormalities (ILAs) in the general population[1,2]. Older age, male gender, tobacco smoke exposure and air pollution are other risk factors for ILA[3]. It has been recently reported that this SNP was associated with fewer COVID-19-related hospitalizations and post-COVID-19 pneumonia events, but similar severe outcomes and mortality[4]. In another study, COVID-19 survivors having one or two copies of the rs35705950 risk allele had higher plasma levels of mucin 5B[5]. In the former study, the authors captured only short-term outcomes and invited future studies with extended follow up to analyze the effect of this SNP on post-COVID-19 lung abnormalities. Furthermore, prior studies on the association between COVID-19 infection and *MUC5B* have been performed in Caucasian populations; however, there is no study from India. Hence, this study aimed to determine the association of this SNP with interstitial changes in the lung, six months after recovery from severe COVID-19 pneumonia. The term post-COVID-19 interstitial changes (PCICs) has been used for these lung abnormalities.

Cases were selected from a prospective database[6] of consecutive subjects followed up after recovery from severe, hospitalization-requiring COVID-19 pneumonia between January 2021 and February 2022 after clearance from the Institutional Ethics Committee. The study participants provided blood samples at enrolment and underwent thin-section computed tomography (CT) of the chest at discharge and at six months follow up. We included individuals for the current study if a blood sample and a follow up CT chest were available. Those with pre-existing interstitial lung disease (ILD) based on history, prior chest imaging or chest imaging at admission were excluded from this study. Two radiologists adjudicated the presence of PCICs on chest CT.

We defined cases as individuals with PCICs on the six-month chest CT as per the Fleischner Society criteria for ILAs[3]. Accordingly, PCICs included non-dependent lung abnormalities (reticulation, architectural distortion, traction bronchiectasis, honeycombing and cysts) involving five per cent or more of a lung zone (upper, middle and lower zones of the lungs delineated by the inferior aortic arch and right inferior pulmonary vein) in individuals in whom an established ILD was not suspected. The participants in the comparator group were those without PCICs. We recorded the demographic details, smoking status, comorbid illness and other clinical details, including the neutrophil–lymphocyte ratio (NLR) at admission, the requirement of mechanical ventilation (MV), length of hospital stay (LOS) and the chest CT findings (including PCICs) at six months. The cases were further categorized as having subpleural or non-subpleural PCICs, and fibrotic (traction bronchiectasis, honeycombing or architectural distortion) or non-fibrotic PCICs. Genotyping of the *MUC5B* gene SNP rs35705950 was done using PCR, followed by Sanger sequencing, as described previously[7]. The minor allele frequency of this SNP were compared between the cases and participants in the comparator group. Furthermore, the association of this SNP with PCICs was evaluated in multivariable-adjusted regression analyses with age, sex and smoking as confounders. A secondary regression analysis was performed, including clinically important variables (NLR at admission, MV and LOS) associated with post-COVID-19 lung abnormalities[6]. For subpleural

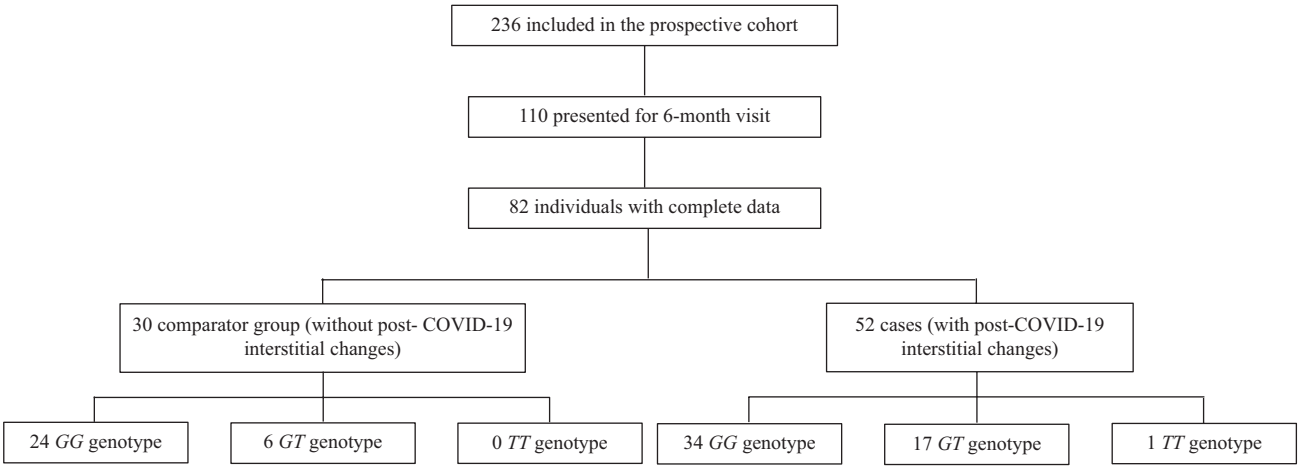

Figure. Study flow and major findings. COVID-19, coronavirus disease 2019; *GG*, *GT* and *TT* genotypes represent wild-type, heterozygous and homozygous subjects for the risk allele (*T*) of the rs35705950 polymorphism in the *MUC5B* gene.

PCICs and fibrotic PCICs only univariate analyses were performed.

Of the 236 individuals in the prospective database, 110 presented for follow up at six months (Figure). Detailed analyses of the radiologic and physiologic parameters of some of these individuals was recently published[6]. We included 82 participants (52 cases with PCICs and 30 individuals in the comparator group without PCICs) with available data in the current study (Table). About 57.7 per cent (30/52) of the cases had Grade 1 or higher breathlessness on the modified Medical Research Council (mMRC) scale compared to 30 per cent (9/30) in the comparator group (P=0.02). Fourteen and 21 participants had subpleural and fibrotic PCICs, respectively. There was substantial agreement (Cohen's κ=0.71) between the two radiologists in the adjudication of the presence of PCICs.

GG, *GT* and *TT* genotypes of the *MUC5B* rs35705950 SNP were found in 34 (65.4%), 17 (32.7%) and 1 (1.9%) cases, and among 24 (80%), 6 (20%), and 0 individuals in the comparator group (Figure). The allele frequencies conformed to the Hardy–Weinberg equilibrium in both the cases (P=0.49) as well as in the comparator group (P=0.54). The minor *MUC5B* *T*-allele appeared at a higher frequency in the cases than in the comparator group [18.3 *vs.* 10%; odds ratio (OR), 2.01; 95% confidence intervals (CI), 0.76-5.35], but was not significant (P=0.16). Conversely, 75 per cent of the individuals with a variant allele (*GT* or *TT* genotype) had PCICs at six months as compared to 58.6 per cent of those without the variant allele (wild-type, *GG* genotype); P=0.16. The OR for PCICs among those with a variant genotype [heterozygous (*GT*) or homozygous (*TT*)] versus those with wild type (*GG*) was 2.12 (95% CI, 0.73-6.12; P=0.17). Only four covariates were considered in the multivariable model (genotype, age, sex and smoking), as these are usual factors associated with interstitial lung diseases in previous studies. We could not correct for factors in the primary analysis that reflected COVID-19 severity such as the NLR, respiratory support and length of hospital stay as the sample size was low (52 cases and 30 subjects in the comparator group) that could allow considering only 3-5 covariates. Multivariate analysis revealed a similar OR of 2.11 (Table). A secondary multivariate regression analysis revealed an OR of 2.48 (95% CI, 0.73-8.37) after correcting for age, sex, smoking, NLR at admission, MV and LOS (P=0.15). A variant genotype was significantly associated with subpleural PCICs (OR, 6.36; 95% CI, 1.85-21.85; P=0.003) but not with fibrotic PCICs (OR, 1.73; 95% CI, 0.61-4.94; P=0.31).

The minor allele frequency among the comparator group without PCICs was similar to that found in the general Indian population (11.8%), while that among the cases with PCICs was higher, but lower than in Indians with IPF (32.1%)[7]. The *MUC5B* rs35705950 polymorphism is known to be associated with mucin overproduction, which predisposes to the risk of IPF and ILAs[1,2]. The same SNP may be associated with longer survival in IPF, the reasons for which remain unknown[7,8]. It is proposed that higher mucin production might help in the clearance of pathogens, thus protecting the carriers of this SNP from frequent respiratory infections[4]. Similar mechanisms might be at play in the context of COVID-19. While the

Table. Characteristics of cases and comparator group and logistic regression analyses for factors associated with post-COVID-2019 interstitial lung changes

Parameter	Comparator group (n=30)	Cases (n=52)	P
Age (yr), mean±SD	54.4±12.2	54.6±11.8	0.92
Male sex, n(%)	21 (70)	35 (67.3)	0.8
Smokers, n(%)	7 (23.3)	14 (26.9)	0.72
Comorbid illnesses, n (%)			
Any	27 (90)	42 (80.8)	0.36
Major	26 (86.7)	41 (78.8)	0.38
Others	4 (13.3)	6 (11.5)	1
Parameters during acute illness			
NLR at admission, meadian (IQR)	9.9 (6.3-18.9)	12.9 (7.4-24.7)	0.22
Respiratory support during acute illness			0.05
Oxygen supplementation only, n(%)	29 (96.7)	40 (76.9)	
Mechanical ventilation, n(%)	1 (3.3)	11 (21.2)	
Length of hospital stay (days), mean±SD	9.9±5.5	19±10.8	<0.001
Parameters at six months			
Dyspnoea severity, mMRC scale [median (IQR)]	0 (0-1)	1 (0-1)	0.01
Grade 0	21 (70)	22 (42.3)	
Grade 1	8 (26.7)	20 (38.5)	
Grade 2	1 (3.3)	9 (17.3)	
Grade 3	0	1 (1.9)	
Resting oxygen saturation, mean±SD	98.3±1.1	98.6±1.1	0.38
Forced vital capacity, l, mean±SD	2.72±0.81	2.46±0.61	0.17
Forced vital capacity, % predicted, mean±SD	80.6±11.1	74.7±13.2	0.07
Six-minute walk distance, m, mean±SD	417±109	419±73	0.94
% predicted, mean±SD	88.4±21	89.3±14.4	0.85
Abnormalities on chest CT, n(%)			
Normal	4 (13.3)	0	0.02
Ground-glass opacities	16 (53.3)	42 (80.8)	0.009
Consolidation	1 (3.3)	3 (5.8)	1
Parenchymal bands	7 (23.3)	45 (86.5)	<0.001
Reticulation	1 (3.3)	22 (42.3)	<0.001
Traction bronchiectasis	1 (3.3)	22 (42.3)	<0.001

Univariate and multivariate logistic regression analyses for PCICs at six months*

Parameter	OR (95% CI)	*P*	aOR (95% CI)	*P*
Variant genotype at *MUC5B* SNP rs35705950	2.12 (0.73-6.12)	0.17	2.11 (0.72-6.17)	0.17
Age	1 (0.96-1.04)	0.92	1 (0.96-1.04)	0.98
Male sex	1.13 (0.43-2.99)	0.8	1.04 (0.38-2.84)	0.98
Smoking	1.21 (0.43-3.44)	0.72	1.23 (0.42-3.56)	0.7

*The values represent either mean±SD, median (IQR), or n (%), unless specified otherwise. Major comorbidities included diabetes mellitus, hypertension, coronary artery disease, chronic kidney disease, obesity and obstructive airway disease. Other comorbidities included hypothyroidism, rheumatoid arthritis, Cushing's syndrome, ulcerative colitis and cardiac arrhythmias. OR, odds ratio; aOR, adjusted OR; CI, confidence interval; IQR, interquartile range; COVID-19, coronavirus disease 2019; PCICs, post-COVID-19 interstitial changes; mMRC, modified Medical Research Council; NLR, neutrophil–lymphocyte ratio; SD, standard deviation; CT, computed tomography

SNP protects against hospitalization-requiring severe disease by helping in the clearance of the virus, the mucus overproduction might predispose to PCICs after recovery from acute COVID-19 pneumonia. A recent study[5] found that COVID-19 survivors who had one or two copies of the rs35705950 risk allele had higher plasma levels of mucin 5B. Further investigation is required into the downstream effects of this polymorphism in the lung tissue of individuals with COVID-19 both in the acute as well as post-acute phases. Longer follow up is required to reveal whether such PCICs progress to ILD over months to years as happens with ILAs in a proportion of the affected individuals[9]. Furthermore, it can be hypothesized that ILAs in the general population that are detected incidentally might result from undiagnosed viral illnesses. We have specifically avoided using the term ILAs for the interstitial changes in this study as ILAs are encountered incidentally in asymptomatic individuals, while several participants of this study had symptoms. Nevertheless, if our study results are confirmed, it suggests that those who have recovered from severe COVID-19 and have the *MUC5B* promoter polymorphism may be followed up more closely with imaging and physiological testing. In fact, up to 45 per cent of individuals with severe COVID-19 may have mMRC Grade 1 breathlessness at one year[10]. Electrical impedance tomography may identify regional inhomogeneity in the lungs of several such individuals despite them having normal pulmonary function tests[10].

Our study had limited power due to the small sample size. The ideal method would have been to include a larger sample and perform whole genome sequencing (WGS) to discover novel variants associated with PCICs. However, resource-intensive techniques like WGS were not feasible and only about half of the participants presented for the six-month visit, and only a third had the availability of chest CT and blood samples. Therefore, the study sample is are not representative of the entire population. Telephonic follow up revealed that most individuals who were lost to follow up did not present to the hospital as they were asymptomatic (data not shown). Thus, the included sample is likely enriched for those who were more likely to have symptoms and possibly PCICs, introducing a selection bias. Due to the small sample size, we adjusted for only a few confounders (age, sex and smoking) while not for measures of COVID-19 severity and treatment. However, the exploratory analysis, including NLR, MV and LOS, as covariates in the multivariable-adjusted analysis did not significantly alter the results. We also did not perform multivariate analysis for fibrotic or subpleural PCICs due to the small numbers. Only the CT abnormalities included in the definition of ILAs were considered and hence, we did not study other defects. As the adjudication of PCICs was based on visual assessment of abnormalities at a threshold of five per cent involvement of the lung zones, there is a potential for the misclassification of cases and individuals in the comparator group. This is especially so in the setting of post-COVID-19 recovery, wherein several individuals had minor interstitial abnormalities not meeting the ILA criteria (Table). In the absence of pre-COVID-19 chest CT, we cannot be certain that the interstitial abnormalities were indeed due to COVID-19 infection or were pre-existing. Furthermore, our follow up duration of six months was relatively short. Thus, the findings of this study should be interpreted with caution due to potential biases that might have originated from the selection process and other clinical confounders and underline the need for examining the proposed hypothesis in a larger sample. Despite these limitations, our study has the strength of presenting novel findings in this hitherto less-researched field.

Overall, the results presented here are only hypothesis generating and suggest the need for larger studies in diverse populations to explore the association between *MUC5B* promoter polymorphism and PCICs.

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