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Tobacco Smoking Increases the Lung Gene Expression of ACE2, the Receptor of SARS-CoV-2

To the Editor:

On March 11, 2020, the World Health Organization declared the coronavirus disease (COVID-19) outbreak a pandemic. As of May 28, 2020, laboratories had confirmed 5,701,337 COVID-19 cases, and 357,668 deaths had been reported in 216 countries, areas, or territories (1). COVID-19 is caused by a new type of pathogenic coronavirus, severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), which is phylogenetically similar to SARS-CoV, with approximately 80% identity between the genomes (2). SARS viruses affect the respiratory tract and cause an acute respiratory response through the same cell-entry receptor, ACE2 (angiotensin-converting enzyme 2), which is the only experimentally confirmed SARS-CoV-2 receptor. SARS-CoV-2 infection also uses activation of the spike proteins found on the surface of the virus for cellular entry. The best candidates for priming spike proteins are two host cell enzymes called Furin and TMPRSS2 (2). In the current severe global emergency, to enable effective prevention and care, it is imperative to identify potential risk factors, such as cigarette smoking, which is a substantial risk factor for various important bacterial and viral infections.

Some of the results of this study were previously published in preprint form (medRxiv, https://www.medrxiv.org/content/10.1101/2020.02.05.20020107v3).

Methods

We evaluated a comprehensive set of transcriptomic data sets to investigate the associations of smoking with *ACE2*, *FURIN*, and *TMPRSS2* gene expression in lung tissues. Two data sets were generated using normal lung tissues from patients with lung adenocarcinoma: a Caucasian RNA-sequencing (RNA-seq) data set from The Cancer Genome Atlas ($n = 48$) (3) and an Asian RNA-seq data set from the Gene Expression Omnibus (GSE40419, $n = 74$) (4). We included three polyethnic microarray data sets of gene expression in healthy small airway epithelium samples (GSE63127, $n = 230$ [5]; GSE19667, $n = 116$ [6]; and GSE5058, $n = 24$ [7]) and large airway epithelium samples (GSE7895, $n = 104$ [8]). In addition, we analyzed three microarray data sets of samples derived from healthy subjects and patients with chronic obstructive pulmonary disease (COPD), including small airway epithelium samples from current smokers (from GSE5058, $n = 26$ [7]),

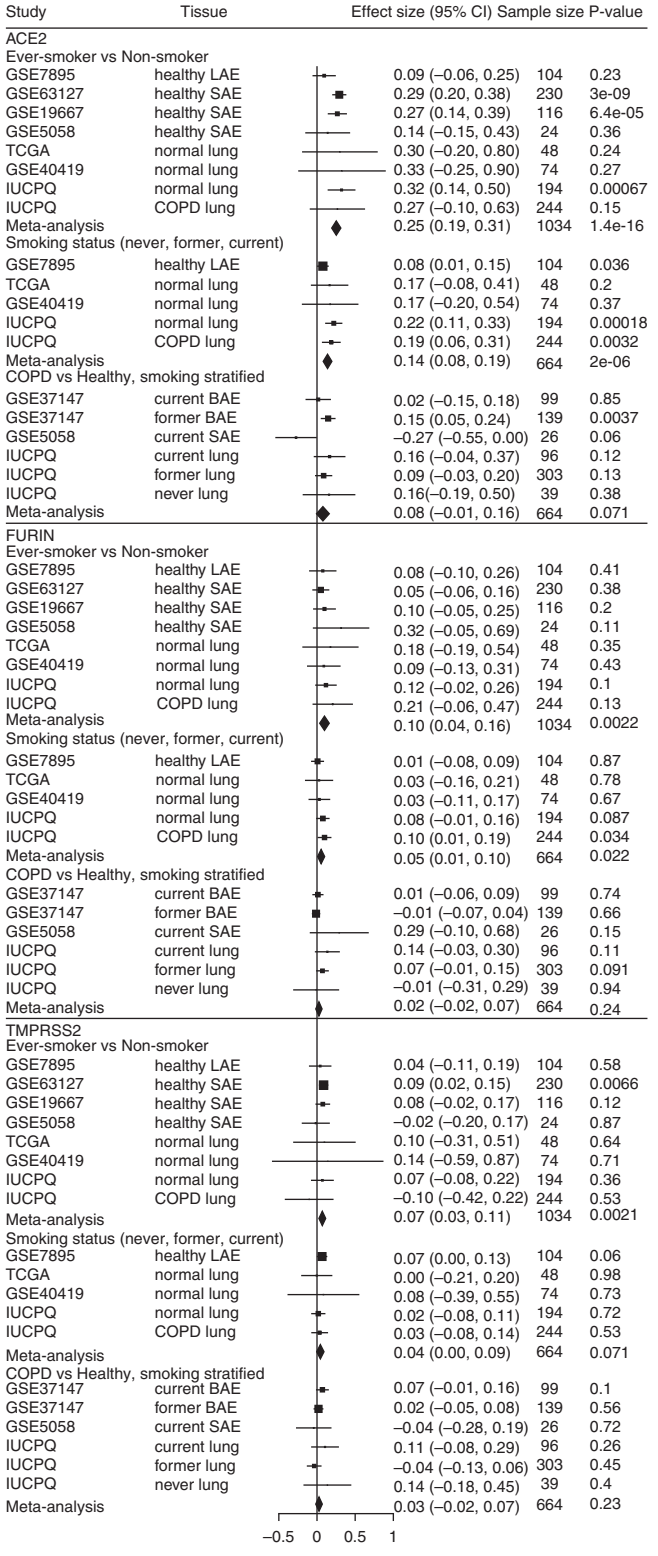

Figure 1. Forest plots for the effects of smoking on *ACE2*, *FURIN*, and *TMPRSS2* pulmonary gene expression. Nonsmokers/never-smokers and ever-smokers (including current and former smokers) were identified in each original study based on self-reported smoking history. For each gene, the top panel shows a comparison of ever-smoker and nonsmoker groups, the middle panel shows the association of *ACE2* gene expression with

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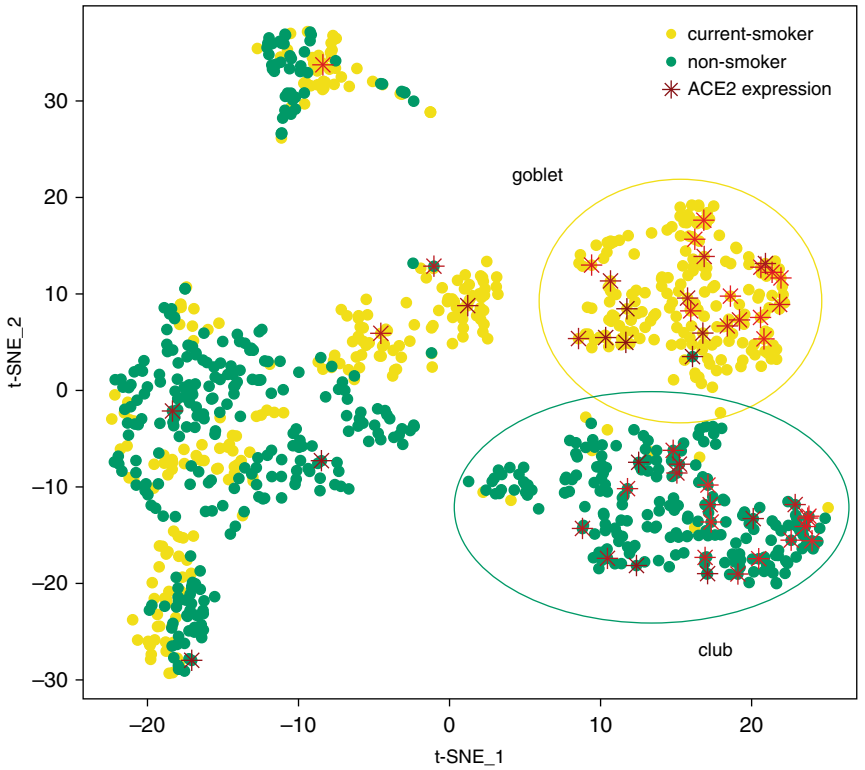

Figure 2. *ACE2* expression in single-cell transcriptomics of bronchial epithelium cells from never-smokers and current smokers. A *t*-distributed stochastic neighbor embedding (t-SNE) plot of single-cell transcriptome profiles from never-smokers and current smokers is shown. *ACE2* expression is shown by red stars. Cell types were identified based on gene expression of markers (11). Confirming the original study, we observed that smokers exhibited a remodeled cell composition in bronchial epithelium with a loss of club cells and extensive hyperplasia of goblet cells.

bronchial airway epithelium samples from current and former smokers (GSE37147, $n = 238$ [9]), and lung samples from white patients ($n = 438$) who underwent lung cancer surgery at the Institut Universitaire de Cardiologie et de Pneumologie de Québec (10). RNA-seq data sets were generated with the Illumina HiSeq platform and microarray data sets were generated with Affymetrix arrays. All of the data were deidentified, and the study approvals were obtained in the original studies (2–10). A total of 1,286 assay results were evaluated. We considered the fragments per kilobase per million mapped reads for RNA-seq data and robust multiarray average values for microarray data to represent normalized gene expression. All data were $\log_2$ transformed to improve normality.

Smoking status (never, former, or current smoker) was identified based on self-reported smoking history. Association tests were performed using a linear model with $\log_2$ *ACE2*, *FURIN*, or *TMPRSS2* gene expression as the dependent variable and smoking status or COPD status as an independent variable. A meta-analysis was performed by pooling the effect sizes and SEs estimated from each study using a random-effects model. Age and sex were included as covariates. Although we did not observe significant associations of age and sex with the expression of *ACE2* and *FURIN*, we found a negative correlation between *TMPRSS2* expression and age in some of the data sets. Data management, statistical analyses, and visualizations were performed using R 3.6.1.

A single-cell RNA-seq data set (GSE131391) (11) was also analyzed. In this analysis, bronchial epithelial cells, single ALCAM$^+$ epithelial cells, and CD45$^+$ white blood cells were profiled from six never-smokers and six current smokers. Sequencing read counts in single cells were downloaded, and subsequent data analyses, including data normalization, high variable feature selection, data scaling, dimension reduction, and cluster identification, were performed using the Seurat 3.0 package. We used SCANNER for data visualization and cell type identification.

Results

We identified upregulation of pulmonary *ACE2* gene expression in ever-smokers compared with nonsmokers in all data sets, irrespective of tissue subset or COPD status (Figure 1). A

Figure 1. (*Continued*). smoking status (never, former, or current smoker), and the bottom panel shows the comparison of chronic obstructive pulmonary disease (COPD) and healthy groups, stratified by smoking status. For each study, the estimated effect size and 95% confidence intervals (CIs) are plotted. The size of the squares is proportional to the weights, which were estimated by the standard "inverse-variance" method for random-effects models in meta-analysis. BAE = bronchial airway epithelium; IUCPQ = Institut Universitaire de Cardiologie et de Pneumologie de Québec; LAE = large airway epithelium; SAE = small airway epithelium; TCGA = The Cancer Genome Atlas.

meta-analysis showed that ever-smoking significantly and substantially increased pulmonary *ACE2* expression by 25% (P value = 1.4×10^{-16}; Figure 1). Similarly, smoking status (never, former, or current smoker) was also significantly associated with *ACE2* pulmonary expression in the meta-analysis ($\beta = 0.14$, $P = 2.0 \times 10^{-6}$; Figure 1). The significant effect of smoking on *ACE2* pulmonary expression identified in this study may suggest an increased risk for viral binding and entry of SARS-CoV and SARS-CoV-2 in lungs of smokers. *FURIN* was also upregulated by smoking, but to a lower extent compared with *ACE2*. *TMRPSS2* gene expression in lung was not associated with smoking (Figure 1).

We also evaluated the effect of COPD on gene expression. When we stratified the data by smoking status, we observed a trend ($\beta = 0.08$, $P = 0.07$) for higher *ACE2* levels in patients with COPD, but the results were not consistent across data sets (Figure 1). In the Institut Universitaire de Cardiologie et de Pneumologie de Québec data, *ACE2* expression was upregulated in patients with COPD ($P = 0.0006$), but the effect was attenuated after adjustment for smoking status ($P = 0.03$).

We further evaluated the effect of smoking on *ACE2* pulmonary expression in single bronchial epithelial cells from six never-smokers and six current smokers. We found that smoking remodeled cells in the bronchial epithelium, with a loss of club cells and extensive hyperplasia of goblet cells. The *ACE2* gene was mainly expressed in goblet cells in smokers, and in club cells in never-smokers (Figure 2). This result is consistent with a very recent study that found the highest ACE2 expression in alveolar type II cells (which derive from club cells) and in a transient secretory cell type in subsegmental bronchial branches (12). This may indicate that smokers have a risk of COVID-19 infection complications based on their *ACE2* expression profiles, which could contribute to variations in infection susceptibility, disease severity, and treatment outcome.

Despite a significant increase in the prevalence of electronic cigarettes (E-cigs), to date no studies have compared single (E-cig only) and dual (E-cig and tobacco) users. The mechanisms underlying tobacco-related upregulation of *ACE2* pulmonary expression, as well as the degree to which smoking affects infection susceptibility and clinical manifestations, are unknown. Further mechanistic studies are needed to address these issues. Although our knowledge is currently limited, this study indicates that smoking could be a risk factor for COVID-19 by affecting *ACE2* expression, and provides valuable information for identifying and stratifying more susceptible populations. ■

Author disclosures are available with the text of this letter at www.atsjournals.org.

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