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ACE2, TMPRSS2, and furin gene expression in the airways of people with asthma—implications for COVID-19

To the Editor:

Coronavirus disease 2019 (COVID-19) is caused by a novel zoonotic coronavirus known as severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) and has been identified as a pandemic by the World Health Organization. Several risk factors have been identified for severe COVID-19–associated pneumonia including increased age and the presence of comorbidities, in particular diabetes, cardiovascular disease, and tobacco smoking.[1] However, a number of reports have failed to identify excess risk in patients with respiratory airway diseases such as asthma.[2]

SARS-CoV-2 infects people by binding to the angiotensin-converting enzyme 2 (ACE2) receptor, a transmembrane endopeptidase that cleaves both angiotensin 1 and 2, and which is expressed by epithelial cells in several organs including the airways. Cofactors facilitating SARS-CoV-2 infectivity are the transmembrane peptidase serine 2 (TMPRSS2), which cleaves the SARS-CoV-2 spike protein, and possibly the protease furin.[3] Understanding the expression of ACE2, TMPRSS2, and furin in the airways of people with asthma may help determine whether asthma itself or treatment with inhaled or oral corticosteroids may alter susceptibility to SARS-CoV-2 infection and potentially related disease severity. We have therefore explored the RNA expression of ACE2, TMPRSS2, and furin in human bronchial brushes and biopsies from previously described cohorts of people with asthma of varying corticosteroid treatment intensity (as an index of severity) and healthy controls.

Airway brushes and biopsies were collected at bronchoscopy with written informed consent and ethical approvals. Airway brushes were placed into RNAprotect and airway biopsies from the second- to fifth-generation airways were placed into RNAlater. Bronchial brush ACE2 expression data were available from 356 patients (88 healthy volunteers and 268 patients with asthma [mild to moderate asthma, 125; severe asthma, 143]), across 5 asthma/healthy volunteer cohorts, Leicester, UK (n = 34),[4] the multicenter Bronchoscopic Exploratory Research Study of Biomarkers in Corticosteroid-refractory Asthma (n = 54),[5] the

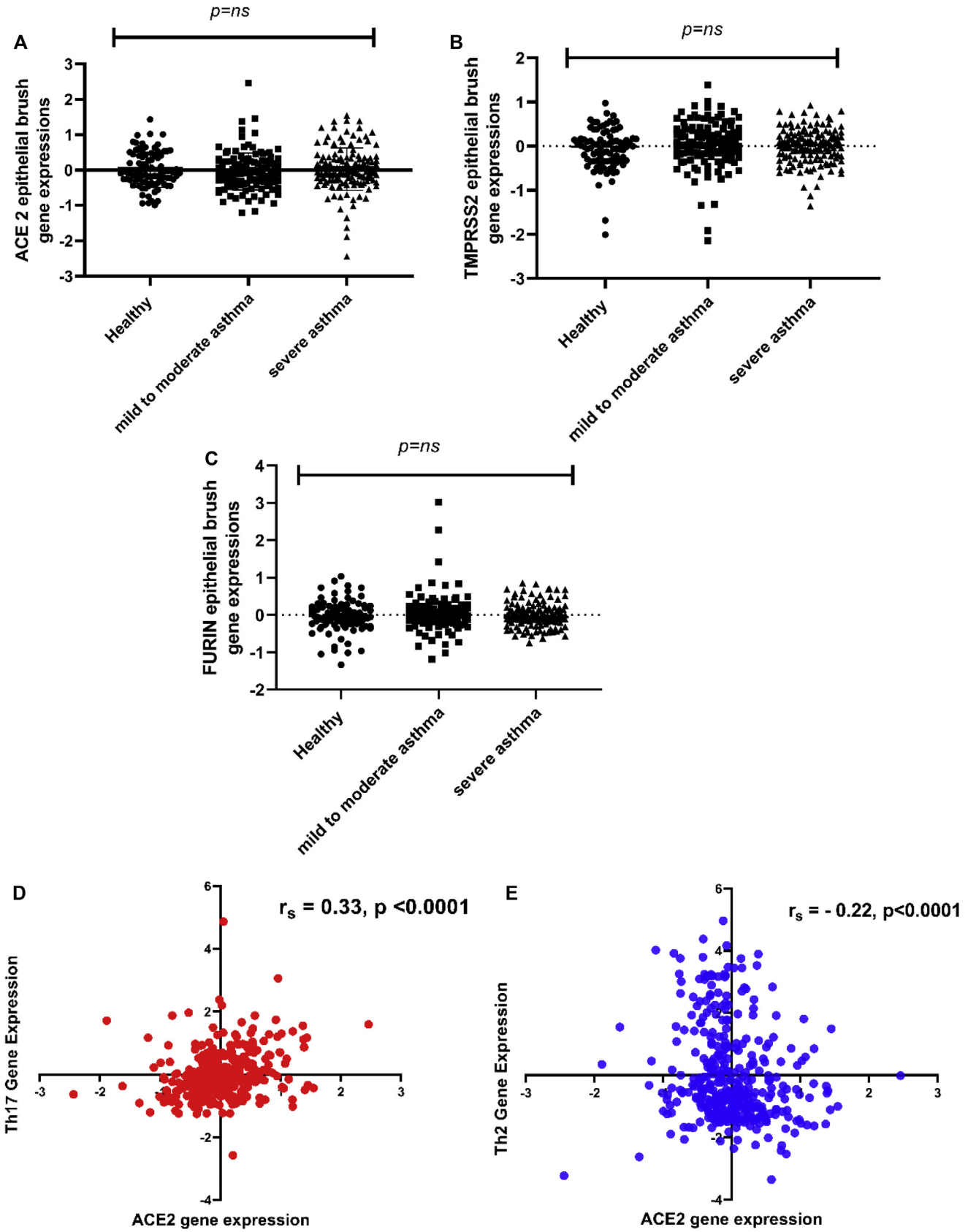

FIG 1. A-C, ACE2, TMPRSS2, and furin gene expression in bronchial brush samples is not increased in patients with mild to moderate or severe asthma compared with healthy controls. **D**, There is a weak positive correlation between ACE2 gene expression and a T_H17–dependent gene expression signature in bronchial brushes samples (healthy control and patients with asthma combined). **E**, There is a weak inverse correlation between ACE2 gene expression and a T_H2-dependent gene expression signature in bronchial brushes samples (healthy control and patients with asthma combined).

Severe Asthma Research Program cohort (n = 154),[6] and Southampton, UK (n = 114).[7,8] Bronchial biopsy ACE2 expression data (n = 94) were available in 17 healthy volunteers and 77 patients with asthma from the Leicester and Bronchoscopic Exploratory Research Study of Biomarkers in Corticosteroid-refractory Asthma cohorts. For bronchial brush data, the 5 data sets were combined into a single data set after first adjusting for batch effects by separately mean centering each of the 5 data sets. The same method was applied to the biopsy data. The Wilcoxon rank-sum test and the Kruskal-Wallis test were used to test for between-group differences in ACE2 expression. The Spearman rank correlation was used when reporting correlations. All analyses were performed using R statistics, version 3.6.0, and figures were generated in GraphPad Prism 8.1.2 (GraphPad Software, San Diego, Calif).

The clinical characteristics of the participants (median [Q1:3]) for healthy volunteers versus people with asthma were respectively as follows: age—25 (22-34) versus 40 (27-49) years, FEV_1% predicted—101 (93-109) versus 71.5 (58-88), and FEV_1/forced vital capacity—82 (78-85) versus 72 (62-79). Overall, there was no difference for ACE2, TMPRSS2, or furin mRNA expression between people with asthma compared with healthy controls (P = .96), no significant differences in ACE2 expression between males and females, and no correlation between ACE2 gene expression and age (data not shown). There were no differences in ACE2, TMPRSS2, or furin gene expression between healthy volunteers and people with mild to moderate and severe asthma (Fig 1, *A-C*). ACE2, TMPRSS2, or furin gene expressions were not correlated.

There were weak but highly significant inverse and positive correlations between ACE2 expression and the expression of T_H2-dependent and IL-17 (T_H17)-dependent epithelial gene signatures, respectively, defined as previously[4] (Fig 1, *D* and *E*). Similar observations were noted in bronchial biopsies, with no differences in ACE2 gene expression between healthy volunteers and patients with mild to moderate asthma and patients with severe asthma (P = .43) (not shown).

These data would suggest that differences in ACE2, TMPRSS2, and furin epithelial and airway gene expression are unlikely to confer enhanced COVID-19 pneumonia risk in patients with asthma across all treatment intensities and severity. It is therefore possible that the risk of severe COVID-19 pneumonia is no greater than the background population risk in patients with asthma in the absence of other known risk factors such as diabetes and cardiovascular disease. This would support current guidance on the use of inhaled steroids and rescue prednisolone in patients with asthma who experience exacerbations during the COVID-19 pandemic.

A previous mouse model of infection demonstrated that ACE2 inhibits neutrophil infiltration and lung inflammation by limiting IL-17 signaling by reducing the activity of the signal transducer and activator of transcription 3 pathway.[9] However, our observations in bronchial brush airway epithelial cells identified a positive correlation between ACE2 gene expression and a previously described IL-17–dependent gene expression signature, with an inverse association with T_H2 gene expression. It is possible that ACE2 protein expression in the airways might not mirror the RNA expression, which is a limitation of our study. Furthermore, the precise relationship between other host immunoregulatory factors that may modify the risk of severe COVID-19 pneumonia and asthma, as well as corticosteroid exposure, which may induce T_H17 immunity in asthma, have not been examined here directly. However, our data are in keeping with a recent report demonstrating that in nasal brushings from children, ACE2 expression was inversely correlated with markers of type 2 immunity, with no influence of sex or use of nasal corticosteroids.[10] In the same article, it was shown that segmental bronchial allergen challenge in adults with mild asthma led to decreases in ACE2 expression, and that IL-13 reduces ACE2 expression on cultured bronchial epithelial cells.

In summary, these data suggest that it will be important to understand further the effects of T_H2 and IL-17–driven inflammation, and of inhaled corticosteroids on airway epithelial cell ACE2 expression, and the susceptibility of these cells to infection and replication by SARS-CoV-2.

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A possible role for B cells in COVID-19? Lesson from patients with agammaglobulinemia

To the Editor:

An epidemic of coronavirus SARS-CoV-2 has become the focus of scientific attention.[1] The high infectivity of SARS-CoV-2 and rapid rise in the number of patients affected reflects the lack of preexisting immunity as reported by the World Health Organization (https://www.who.int/emergencies/diseases/novel-coronavirus-2019). The clinical presentation of coronavirus disease 2019 (COVID-19) is variable, ranging from lack of symptoms to severe respiratory distress and multiorgan failure requiring intensive care unit admission and mechanical ventilation. Treatment of COVID-19 requires in-depth knowledge of the immune-mediated mechanisms of the disease. To date, we have identified 7 patients with primary antibody deficiencies (PADs) and COVID-19 infection: 5 had common variable immune deficiencies (CVIDs) and 2 had agammaglobulinemia (1 with X-linked agammaglobulinemia and 1 with autosomal recessive agammaglobulinemia).[2] All of the patients with PADs had defective antibody production. Patients with agammaglobulinemia lack B lymphocytes, whereas patients with CVID have dysfunctional B lymphocytes. In patients with agammaglobulinemia, the COVID-19 course was characterized by mild symptoms, short duration, and no need for treatment with an immune-modulating drug blocking IL-6, and it had a favorable outcome. In contrast, patients with CVIDs presented with a severe form of the disease requiring treatment with multiple drugs, including antiretroviral agents and IL-6–blocking drugs, as well as mechanical ventilation (Table I). The strikingly different clinical course of COVID-19 in patients with agammaglobulinemia compared with that in patients with CVIDs cannot be explained by the levels of serum immunoglobulins, which were similarly low in all patients with PADs at diagnosis and were maintained at adequate and comparable levels in all patients by immunoglobulin substitutive therapy (see Table E1 in this article's Online Repository at www.jacionline.org). A detailed COVID-19 clinical history, laboratory data, type and dosage of administered treatment, and disease timing are provided for each patient in Case Reports in this article's Online Repository (at www.jacionline.org). The lung high-resolution computed tomography (HRCT) of a patient with CVID at hospital admission for COVID-19 showed extensive ground glass opacities associated with areas of alveolar consolidation in the upper and lower lobes, with the alveolar component predominating over the interstitial component. (Fig 1, *A*). On treatment, the lung HRCT showed a reduction in the extent of ground glass opacities and areas of alveolar consolidation. (Fig 1, *B*). In contrast, the lung HRCT of a patient with agammaglobulinemia performed at the time of COVID-19 was unchanged from lung HRCT performed 1 year earlier and showed bronchiectasis and sequelae of a right lung pneumonectomy done when the patient was 18 years old (Fig 1, *C* and *D*). All patients with PADs are equally vulnerable to most bacterial infections because antibodies are important in blocking infectivity and preventing diseases. In addition, antibodies have a role in the immune response to viral infections.[3] Patients with agammaglobulinemia are susceptible to a limited number of viral infections only—mainly norovirus and enteroviruses such as polioviruses,[4] with an increased incidence of postvaccination poliomyelitis due to the oral attenuated Sabin vaccine.[5] CVIDs patients are susceptible to rhinoviruses, noroviruses, and herpesviruses that in turn play a role in driving an underlying inflammatory condition. Because only patients with agammaglobulinemia had a mild course of COVID-19, we speculate on a possible role of B lymphocytes in the SARS-CoV-2–induced inflammation. We have already shown that children appear to better contain SARS-CoV-2 in the early phase of infection, possibly because their B cells are able to generate natural antibodies in a timely manner on encounter with novel pathogens when compared with B cells from adults.[6] The role of inflammation in aggravating the clinical picture of subjects with COVID-19 has already been described. Treatment with drugs such as IL-6 inhibitors aimed at reducing the cytokine storm syndrome and lung inflammation associated with a profound increase in level of cytokines such as IL-6 and increased level of ferritin[7] have already been carried out, initially on an individual basis and currently within clinical trials. Of note, our patients with CVID who required IL-6–blocking treatment (3 of 5) presented with increased serum ferritin levels (see the Online Repository). It has been demonstrated that B cells produce IL-6 to drive germinal center formation. In patients unable to carry on the physiologic immune response, IL-6 produced by B cells may increase the level of inflammation. Lack of B-cell–derived IL-6 abrogates spontaneous autoimmune germinal center formation in a mouse model, resulting in protection from systemic autoimmunity.[8] Thus, it appears that cytokine storm syndrome may play a significant role in the respiratory failure in COVID-19 infection. The role of B cells in determining lung inflammatory disorders is also demonstrated by the observation that granulomatous-lymphocytic interstitial lung disease, which occurs in 10% of patients with CVID, can be treated with B-cell–depleting drugs.[9] COVID-19 treatments might contemplate the possibility of dampening the inflammatory functions of B cells and blocking cytokine production by monocytes and dendritic cells. Our data represent the first description of COVID-19 in patients affected with primary antibody defects, offer useful insights to the putative mechanisms underlying the immunologic response to the infection, and suggest possible clues to novel therapeutic targets.

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