

**ACE2, TMPRSS2 AND FURIN GENE EXPRESSION IN THE AIRWAYS OF PEOPLE WITH ASTHMA –
IMPLICATIONS FOR COVID-19**

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31 **CONFLICTS OF INTEREST**

32 The authors do not have conflicts of interest relevant to the subject matter of this article.

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34 **KEY WORDS**

35 COVID-19; ACE2; TMPRSS2; furin; asthma; bronchial brush; bronchial biopsy; IL-
36 17; Th2

ABSTRACT

To-date, there has not been a clear signal suggesting that asthma or treatment with inhaled steroids are a risk factor for severe COVID-19 disease. We have therefore explored ACE2 receptor mRNA expression, and co-factors for Sars-CoV-2 infectivity (TMPRSS2 and furin) in bronchial brushes and biopsies from people with asthma and healthy controls, and looked for relationships between asthma severity, Th2- and IL-17 dependent gene signatures, and clinical demographics (age, sex). We have looked at a cohort of 356 research participants from previously described studies. The only significant association was a positive correlation between ACE2 and IL-17-dependent gene expression, and an inverse correlation between ACE2 and Th2-cytokine-dependent gene expression. These data 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.

50 **CAPSULE SUMMARY**

51 Expression of mRNA for ACE2, the Sars-CoV-2 receptor, is similar in the lower airways of healthy
52 controls and people with mild-severe asthma. Altered ACE2 expression is unlikely to confer enhanced
53 COVID-19 pneumonia risk in asthma.

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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 (WHO). Several risk factors have been identified for severe COVID-19-associated pneumonia including increased age and the presence of co-morbidities, 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. Co-factors 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 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 2nd-5th generation airways were placed into RNAlater. Bronchial brush ACE2 expression data were available from 356 patients (88 healthy volunteers and 268 asthmatics [mild to moderate asthma 125; severe asthma: 143]), across five asthma/healthy volunteer cohorts, Leicester UK (n=34) ⁴, the multicenter Bronchoscopic Exploratory Research Study of Biomarkers in Corticosteroid-refractory Asthma (BOBCAT) study (n=54) ⁵, the Severe Asthma Research Program (SARP) 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 asthmatics from the Leicester and BOBCAT cohorts. For bronchial brush data, the five data sets were combined into a single data set after first adjusting for batch effects by separately mean centring each of the five 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.

The clinical characteristics of the participants (median [Q1:3]) for healthy volunteers vs people with asthma were respectively as follows: age - 25(22-34) vs 40(27-49) years, FEV₁% predicted - 101(93-109) vs 71.5(58-88) %, and FEV₁/FVC - 82(78-85) vs 72(62-79). Overall, there was no difference for ACE2, TMPRSS2 or furin mRNA expression between people with asthma compared to healthy controls (p=0.96), 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-moderate and severe asthma (**Fig.1A-C**). ACE2, TMPRSS2 or furin gene expression were not correlated.

There were weak but highly significant inverse and positive correlations between ACE2 expression and the expression of Th2-dependent and IL-17(Th17)-dependent epithelial gene signatures respectively, defined as previously ⁴ (**Fig,1D-E**). Similar observations were noted in bronchial biopsies with no differences in ACE2 gene expression between healthy volunteers, mild to moderate and severe asthmatics (p=0.43)(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 asthmatics 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 asthmatics that 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 STAT3 pathway⁹. 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 Th2 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 Th17 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 corticosteroids¹⁰. In the same paper, 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 it will be important to understand further the effects of Th2 and IL-17-driven inflammation, and inhaled corticosteroids on airway epithelial cell ACE2 expression, and the susceptibility of these cells to infection and replication by SARS-CoV2.

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FIGURE LEGEND

A-C) ACE2, TMPRSS2 and Furin gene expression in bronchial brush samples are not increased in mild-moderate or severe asthma compared to health. **D)** There is a weak positive correlation between ACE2 gene expression and an IL-17-dependent gene expression signature in bronchial brushes samples (health and asthma combined). **E)** There is a weak inverse correlation between ACE2 gene expression and a Th2-dependent gene expression signature in bronchial brushes samples (health and asthma combined).

Figure 1

