

Are patients with chronic rhinosinusitis with nasal polyps at a decreased risk of COVID-19 infection?

To the Editor:

Severe acute respiratory syndrome-coronavirus-2 (SARS-CoV-2) uses the SARS-CoV receptor angiotensin-converting enzyme 2 (ACE2) for entry to the cell and the transmembrane serine protease 2 (TMPRSS2) for S protein priming.^{1,2} Higher ACE2 expression was recently reported in nasal compared to throat tissue.³ In fact, higher SARS-CoV-2 viral load was detected in nasal compared to throat swabs obtained from COVID-19-infected patients. This was attributed to the difference in ACE2 expression between both tissues.⁴ Recently, we have also shown that the upper airway expresses more SARS-CoV-2 entry genes, ACE2 and TMPRSS2, compared to the lower airway.⁵ Moreover, Hou et al.⁶ have recently established that multiciliated cells are the main cell types expressing ACE2 in nasal tissue and infected with SARS-CoV-2. Moreover, Sungnak et al.,⁷ by analyzing data of single-cell RNA-sequencing from healthy human nasal epithelial cells, showed that ACE2 and TMPRSS2 are co-expressed in nasal epithelium with genes involved in host innate immunity, referring to the potential role of these cells in initiating SARS-CoV-2 infection. Therefore, the level of SARS-CoV-2 receptors in nasal tissue may determine the level of viral infectivity because these receptors are not upregulated following infection.¹ With that in mind, we decided to investigate potential factors that may affect the expression of SARS-CoV-2 receptors and hence the risk of infectivity with COVID-19 in various phenotypes of sinonasal inflammation.

To achieve this, gene-expression data sets were used to determine the expression pattern of SARS-CoV-2 cell entry genes, ACE2 and TMPRSS2, in the inflamed uncinuate process from patients with chronic rhinosinusitis without nasal polyps (CRSsNPs) and nasal polyps from patients with CRS with nasal polyps (CRSwNP) (see Supplementary

Methods). A reduction in ACE2 expression was observed in the inflamed uncinuate tissue of CRSsNP patients but not to a significant level (Fig. 1A). Interestingly, a significant reduction in the expression of ACE2 and TMPRSS2 was observed in the nasal polyps of CRSwNP patients compared to healthy controls (Fig. 1A). This data implies that patients with CRSwNP might have a lower risk of SARS-CoV-2 infection due to lower expression levels of SARS-CoV-2 cell entry genes.

To our knowledge, the impact of allergic inflammation on the level of expression of ACE2 and TMPRSS2 remains poorly understood. In an in vitro study, interleukin 4 (IL4) has been shown to reduce ACE2 gene expression levels in SARS-CoV-infected cells.⁸ Moreover, ACE2 was shown to be less activated in eosinophilic and allergic airway inflammation conditions,⁹ which may indicate a potential regulatory effect of such conditions on these receptors. In our study, we investigated the expression levels of interferon γ (IFN γ), IL17, and type 2 cytokines in the tissues of these CRS patients. Significantly higher levels of IL4, IL5, and IL13 cytokines were observed in nasal polyp tissue from CRSwNP patients, whereas IFN γ and IL17 cytokines were more elevated in the uncinuate tissue (Fig. 1A). This suggests that type 2 inflammation may downregulate ACE2 and TMPRSS2 expression.

To further investigate, we used another data set of nasal polyp tissue from eosinophilic and non-eosinophilic CRSwNP patients. Interestingly, a reduction in expression of ACE2 and TMPRSS2 receptors was observed in the eosinophilic nasal polyp tissue compared to healthy controls. In contrast, an increase was observed in both receptors in non-eosinophilic nasal polyp tissue compared to controls (Fig. 1B). In addition, ACE2 and TMPRSS2 expression in non-eosinophilic polyp tissue was significantly higher than in eosinophilic tissue ($p = 0.0378$ and $p = 0.0039$, respectively).

We then assessed the level of expression of IFN γ , IL17, and type 2 cytokines in eosinophilic vs non-eosinophilic tissue from CRSwNP patients. As expected, eosinophilic polyp tissue had higher levels of expression of IL4, IL5, and IL13 cytokines, whereas higher levels of IFN γ and IL-17 were observed in non-eosinophilic polyp tissue (Fig. 1B). It was also observed that ACE2 has positive correlation with IFN γ ($r = 0.86$, $p = 0.003$), but not with IL17. ACE2 levels negatively correlated with IL5 ($r = 0.709$, $p = 0.033$) and IL13 ($r = 0.682$, $p = 0.043$) (Supplementary Fig. 1A-C). No significant correlation was found between ACE2 and IL4 levels. Correlations of TMPRSS2 with the abovementioned cytokines IFN γ , IL17, IL4, IL5, and IL13 were not significant.

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Additional supporting information may be found online in the Supporting Information section at the end of the article.

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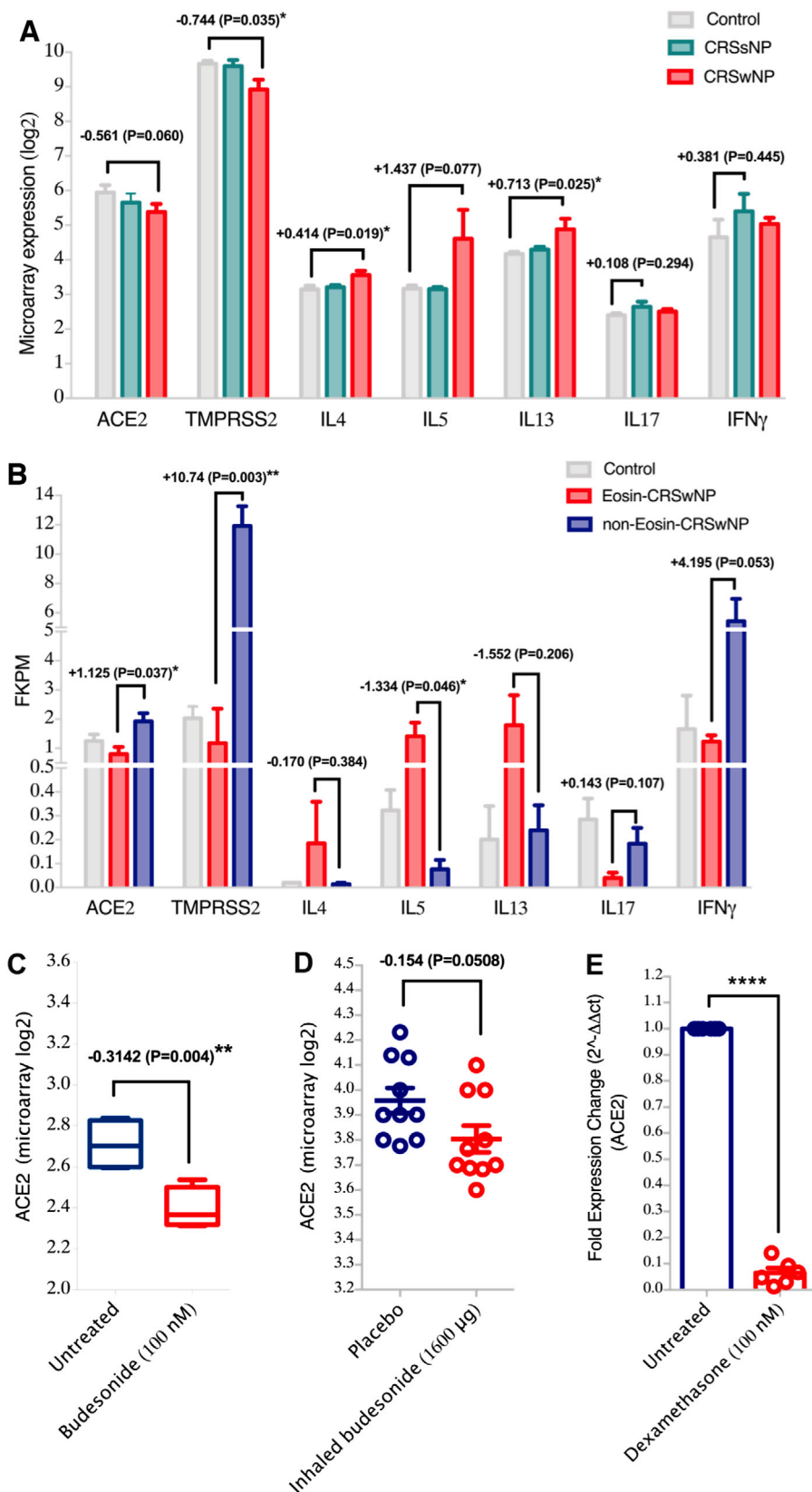


FIGURE 1. Reduced expression of SARS-CoV-2 host entry genes, TMPRSS2 and ACE2, in the nasal polyps of CRSwNP patients. (A) Gene expression of ACE2 and TMPRSS2 as well as proinflammatory cytokines in the inflamed unciniate tissues of CRSsNP patients (n = 3), nasal polyps of CRSwNP patients (n = 3), and unciniate tissues of healthy controls (n = 3). The data presented shows lower expression of ACE2 and TMPRSS2 in the nasal polyps of CRSwNP patients compared to normal unciniate tissue. Accordingly, gene expression levels of type 2 cytokines, IL4, IL-5, and IL13 were higher in the nasal polyps of CRSwNP patients; whereas IL17 and IFN γ cytokines were more elevated in the unciniate tissue of CRSsNP patients. (B) Gene expression of ACE2 and TMPRSS2 as well

Next, the differential expression of ACE2 and TMPRSS2 was assessed in datasets of human ethmoid sinus cells treated independently with IL4, IFN γ , or IL17.⁶ IFN γ and IL17 increased the expression of both ACE2 and TMPRSS2 in these cells (Supplementary Fig. 2A,B), whereas IL4 suppressed their expression levels (Supplementary Fig. 2C). We further evaluated the expression of ACE2 and TMPRSS2 in a data set of IL13-treated human nasal epithelial cells isolated from nasal turbinates. IL13 also suppressed ACE2, but increased the expression of TMPRSS2 in these cells (Supplementary Fig. 2D), consistent with recent reports.^{6,10}

This data suggest that eosinophilic inflammation and the associated type 2 cytokines downregulate the expression of ACE2 in nasal tissue of CRS patients and thus may have a protective role against COVID-19 infection. In contrast, our data indicates that IFN γ increases ACE2 expression, in line with previously published data.¹¹ This may suggest that neutrophilic inflammation and the associated cytokines such as IFN γ and IL17 may upregulate COVID-19 receptors levels and thus facilitate COVID-19 infectivity. This data is also in line with our previous finding⁵ indicating that patients with chronic obstructive pulmonary disease, characterized with neutrophilic inflammation, have significantly elevated ACE2 and TMPRSS2 lung tissue levels.

CRS patients are usually under prolonged exposure to corticosteroids, which may regulate ACE2 expression. A recent report suggested that in asthmatic subjects, sputum ACE2 expression is significantly lower in patients who are on inhaled corticosteroids compared to those who are not on inhaled corticosteroids.¹² Here, we hypothesized that corticosteroids may regulate ACE2 gene expression. To test this hypothesis, we examined the expression of ACE2 in relation to steroid treatment in 2 publicly available data sets of gene expression data from airway epithelial cells as well as bronchial biopsies. Treatment with budesonide downregulated the expression of ACE2 in both bronchial epithelial cells (Fig. 1C) and bronchial biopsies (Fig. 1D). To confirm this observation, we treated healthy primary bronchial fibroblasts with dexamethasone (100nM for 24 hours) and analyzed ACE2 expression using real-time polymerase chain reaction (RT-PCR). Dexamethasone significantly suppressed ACE2 expression in these cells (Fig. 1E). This suggests that prolonged exposure to corticosteroids may suppresses ACE2 gene expression levels in nasal tissue of CRS patients. Because the effect of corticosteroids on COVID-19 patients is still highly debat-

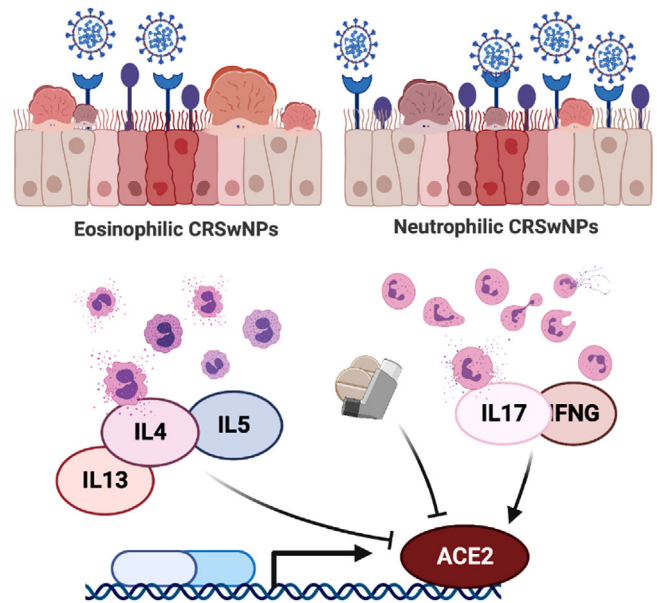


FIGURE 2. The effect of corticosteroids and pro-inflammatory cytokines on the ACE2 gene expression level in the nasal polyps of eosinophilic and non-eosinophilic CRSwNP patients. ACE2 = angiotensin-converting enzyme 2; CRSwNP = chronic rhinosinusitis with nasal polyps.

able, more research is required to confirm our findings and observations.

Moreover, a recent study by Jackson et al.¹³ suggested that atopic asthmatics might be at lower risk of COVID-19-related illness because respiratory allergen exposure and type 2 cytokine IL13 reduces ACE2 gene expression in both nasal and bronchial epithelial cells. The analysis from our study provides further evidence that type 2 or eosinophilic inflammation is biased more toward lowering ACE2 gene expression, in the context of CRS as well, and shows for the first time that steroids may have a protective effect by downregulating ACE2 expression in the nasal tissues of CRS.

The limitation of our study is that although the difference in type 2 cytokines observed between polyp and unciate tissue may contribute to the regulation of these receptors, this does not exclude the possibility that the difference in receptors expression may reflect tissue differences rather than endotype.

In conclusion, as presented in Figure 2, our data suggest that the type of inflammation underlying CRS, as well

as proinflammatory cytokines in nasal polyps of eosinophilic (n = 3), nasal polyps of non-eosinophilic CRSwNP patients (n = 3), and sphenoid sinus mucosa of healthy controls (n = 3). The data presented shows decreased level of ACE2 and TMPRSS2 expression in the nasal polyps of eosinophilic compared to non-eosinophilic CRSwNP patients. Additionally, it shows increased gene expression levels of type 2 cytokines, IL4, IL5, and IL13 in the nasal polyps of eosinophilic compared to non-eosinophilic CRSwNP patients. In other hand, the gene expression levels of IL17 and IFN γ were higher in the nasal polyps of non-eosinophilic compared to eosinophilic CRSwNP patients. (C) Gene expression level of ACE2 in the human bronchial airway epithelial BEAS-2B cells untreated or treated with budesonide (100nM) for 18 hours. The data shows that treatment with budesonide downregulated ACE2 expression levels in the treated compared to untreated BEAS-2B cells. (D) Gene expression level of ACE2 in bronchial biopsies from healthy male, non-smoker, non-allergic volunteers treated with placebo or budesonide (1600 μ g). The data shows that treatment with budesonide downregulated ACE2 expression levels in the budesonide-treated compared to placebo-treated subjects. (E) Gene expression level of ACE2 in the primary bronchial fibroblasts untreated or treated with dexamethasone (100nM) for 24 hours. The data shows that treatment with dexamethasone downregulated ACE2 expression levels in the treated compared to untreated fibroblasts. ACE2 = angiotensin-converting enzyme 2; CRSsNP = chronic rhinosinusitis without nasal polyps; CRSwNP = chronic rhinosinusitis with nasal polyps; IFN = interferon; IL = interleukin; TMPRSS2 = transmembrane serine protease 2.

as corticosteroid treatment, may modulate ACE2 and TM-PRSS2 gene expression levels in the nasal polyps of CR-SwNP patients.

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