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Type 2 inflammation modulates ACE2 and TMPRSS2 in airway epithelial cells

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Background: Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) has dramatically changed our world, country, communities, and families. There is controversy regarding risk factors for severe COVID-19 disease. It has been suggested that asthma and allergy are not highly represented as comorbid conditions associated with COVID-19.

Objective: Our aim was to extend our work in IL-13 biology to determine whether airway epithelial cell expression of 2 key mediators critical for SARS-CoV-2 infection, namely, angiotensin-converting enzyme 2 (*ACE2*) and transmembrane protease, serine 2 (*TMPRSS2*), are modulated by IL-13.

Methods: We determined effects of IL-13 treatment on *ACE2* and *TMPRSS2* expression *ex vivo* in primary airway epithelial cells from participants with and without type 2 asthma obtained by bronchoscopy. We also examined expression of *ACE2* and *TMPRSS2* in 2 data sets containing gene expression data from nasal and airway epithelial cells from children and adults with asthma and allergic rhinitis.

Results: IL-13 significantly reduced *ACE2* and increased *TMPRSS2* expression *ex vivo* in airway epithelial cells. In 2 independent data sets, *ACE2* expression was significantly reduced and *TMPRSS2* expression was significantly increased in the nasal and airway epithelial cells in type 2 asthma and allergic rhinitis. *ACE2* expression was significantly negatively associated with type 2 cytokines, whereas *TMPRSS2* expression was significantly positively associated with type 2 cytokines.

Conclusion: IL-13 modulates *ACE2* and *TMPRSS2* expression in airway epithelial cells in asthma and atopy. This deserves further study with regard to any effects that asthma and atopy may render in the setting of COVID-19 infection. (J Allergy Clin Immunol 2020;146:80-8.)

Key words: *Asthma, IL-13, ACE2, TMPRSS2, nasal epithelial cells, airway epithelial cells, COVID-19, SARS-CoV-2, type 2 inflammation*

Since its recognition in December 2019, the outbreak of COVID-19 caused by severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) infection has generated strong concern among individuals presenting with underlying medical conditions. Individuals with chronic lung diseases or asthma are at higher risk for developing severe complications from COVID-19.[1] Interestingly, among patients with allergy and asthma, there is controversy as to whether allergy and asthma represent comorbid conditions that increase risk for COVID-19. One study that reported 140 cases of COVID-19 in Wuhan, China, indicated no self-reported cases of asthma, allergic rhinitis, atopic dermatitis, or food allergy among infected patients.[2] Moreover, a report by Dong et al suggested a low prevalence of asthma and allergy in pediatric cases and also found a low prevalence of rhinitis and atopic dermatitis.[3] Lupia et al summarized clinical presentations of COVID-19 infection globally and reported a low prevalence of coexisting respiratory disease overall in patients with acute COVID-19 infection.[4] However, a recent report from Seattle, Washington, describing 24 cases of acute lung injury associated with COVID-19 described 3 of 24 patients who had asthma and had been treated with oral corticosteroids within 1 week of presenting because of an asthma exacerbation.[5] A recent publication from the COVID-19–Associated Hospitalization Surveillance Network describing 180 patients admitted to hospitals in the United States from March 1 to 22, 2020, reported that 17% of them carried the diagnosis of asthma.[6] However, information regarding severity, medication requirements, and comorbid conditions of these patients is not currently known. Therefore, the controversy exists, as it is well known that viral infections exacerbate asthma.[7]

For effective host cell entry, SARS-CoV-2 relies on 2 critical proteins, angiotensin-converting enzyme 2 (ACE2)[8] and transmembrane protease, serine 2 (TMPRSS2).[9] Here, we demonstrate that IL-13, a cytokine associated with type 2 asthma, suppresses *ACE2* expression and increases *TMPRSS2* expression in airway epithelial cells from participants with type 2 asthma and atopy. On the basis of these findings, we hypothesized that type 2 cytokines modulate ACE2 and TMPRSS2 expression in the airway epithelial cell in asthma and atopy.

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Abbreviations used
ACE2: Angiotensin-converting enzyme 2
Ang I: Angiotensin I
Ang II: Angiotensin II
CLCA1: Calcium-activated chloride channel regulator 1
FVC: Forced vital capacity
POSTN: Periostin
PPIA: Peptidylprolyl isomerase A
SARS-CoV-2: Severe acute respiratory syndrome coronavirus 2
SERPINB2: Serpin family B member 2
TMPRSS2: Transmembrane protease, serine 2

METHODS

Patient recruitment

Participants were recruited from the population in Tucson, Arizona, and the surrounding areas. Informed consent was obtained from each participant (18-65 years of age). Participants with asthma met the Global Initiative for Asthma criteria for mild and moderate asthma, including the presence of reversibility of airflow obstruction or airway responsiveness with a provocative concentration of methacholine resulting in a 20% decrease in FEV_1 value (according to the National Heart, Lung, and Blood Institute and National Asthma Education and Prevention Program Expert Panel Report 3) not exceeding 8 mg/mL or less than 16 mg/mL if the participants were taking inhaled corticosteroids.[10] The presence of atopy was determined by using skin testing; peripheral eosinophil and fractional exhaled nitric oxide levels were measured. Healthy participants had no evidence of airflow obstruction and no history of pulmonary disease; the presence of atopy was not an exclusion. Exclusion criteria included an exacerbation of asthma within 4 weeks of study that required antibiotics and/or corticosteroids, greater than a 10–pack year history of tobacco use or any cigarette use in the past year, and any other significant medical conditions.

Research bronchoscopy

Participants underwent bronchoscopy with endobronchial-protected brushing as previously described.[11] Brushing of the proximal airways to obtain bronchial epithelial cells was performed under direct visualization by using a separate protected cytologic brush for each pass, for a total of 8 passes. Participants were discharged when their FEV_1 achieved 90% of the prebronchoscopy, postalbuterol value.

Human airway epithelial cell air-liquid interface experiments

Freshly isolated airway bronchial epithelial cells from endobronchial brushings were cultured with PneumaCult-EX Plus (StemCell Technologies, Vancouver, British Columbia, Canada). After reaching confluence, cells were trypsinized and seeded onto 12-mm-diameter collagen-coated polyester Transwell insert membranes (Corning, Lowell, Mass) at a concentration of 5×10^4 per well. The cells were then cultured at the air-liquid interface by using PneumaCult–Air-Liquid Interface (StemCell Technologies) and allowed to differentiate for 2 weeks as previously described.[11] Cells were stimulated with 10 ng/mL of IL-13 (Peprotech, Rocky Hill, N.J) for 48 hours. Epithelial cells were collected for RT-PCR analysis of *ACE2* and *TMPRSS2* (see the section on PCR methods). Conditions were performed in triplicate with appropriate unexposed controls.

Quantitative reverse transcription PCR

Total mRNA was extracted according to instructions provided with the RNeasy Plus Mini Kit (Qiagen), and cDNA was synthesized by using 1 μg of RNA (Applied Biosystems, Foster City, Calif). Real-time PCR was performed on a CFX96 Touch system (Bio-Rad, Hercules, Calif) by using TaqMan probes (Applied Biosystems) specific for *PPIA*, *ACE2*, and *TMPRSS2*. Fold changes for genes of interest were compared with those of the housekeeping gene, *PPIA*, and calculated by using the $2^{-\Delta\Delta CT}$ method data presentation.[12]

Gene expression analysis

Two data sets were downloaded from the Gene Expression Omnibus (https://www.ncbi.nlm.nih.gov/geo/). The data set GSE19187 included gene expression data from a nasal epithelial cell brushing study of 38 children (11 healthy children, 14 children with isolated rhinitis, 7 children with rhinitis and controlled asthma, and 6 children with rhinitis and uncontrolled asthma) on a platform of the Affymetrix Human gene 1.0 ST Array.[13]

The purpose of this study was to establish gene expression signatures of nasal brushings from children with dust mite allergic rhinitis as defined by clinical diagnoses and asthma control staging as determined by the Allergic Rhinitis and Its Impact on Asthma[14] clinical criteria and a positive result of skin prick testing to *Dermatophagoides pteronyssinus* and *Dermatophagoides farina*. Diagnosis of controlled or uncontrolled asthma was based on the National Heart, Lung, and Blood Institute and the National Asthma Education and Prevention Program. Expert Panel Report 3 criteria.[15] Supervised learning and unsupervised clustering were used to predict the different subgroups of patients and define signaling pathways associated with rhinitis and asthma. These profiles were compared with those of primary cultures of human nasal epithelial cells stimulated with IL-4, IL-13, IFN-α, IFN-β, or IFN-γ following *in vitro* differentiation. For our analysis, we used baseline data obtained before any IL-4, IL-13, or IFN exposure.

The second data set, GSE4302, contained gene expression data that stemmed from an airway epithelial brushing study of 86 subjects (28 healthy subjects, 16 smokers, and 42 subjects with mild-to-moderate asthma) in which diagnosis of asthma was based on the National Heart, Lung, and Blood Institute and National Asthma Education and Prevention Program Expert Panel Report 3 criteria.[15] The data are on a platform of the Affymetrix Human Genome U133 Plus 2.0 Array (Thermo Fisher Scientific, Waltham, Mass).[16-19] In this study, participants underwent bronchoscopy with endobronchial brushing and biopsy. However, only epithelial gene expression data were included in the data set. Epithelial cell gene expression was determined by using microarray and PCR analysis. In a subset, epithelial cells were exposed to IL-13 and dexamethasone to further elucidate which genes were modulated by IL-13 and dexamethasone. Participants with a history of oral or inhaled steroid use in the past 4 weeks were excluded. For our analysis we evaluated baseline data obtained before exposure to IL-13 and/or dexamethasone.

Statistical analysis

Analysis of the *ex vivo* data was performed using a mixed effects model with the subject as a random effect. We performed statistical analyses on ΔCt values (Ct_{ACE2}-Ct_{PPIA}). Analysis on this scale (log of concentration) exhibits distributional characteristics that are more consistent with modeling assumptions (normal errors constant within group variance) than the analyses for $2^{-\Delta\Delta Ct}$ that are commonly used.[12] The data were presented per the $2^{-\Delta\Delta Ct}$ once analysis was complete. We used linear mixed effect models to evaluate IL-13 treatment and asthma status on ΔCt values. The mixed model accommodates the nesting structure of participants within the asthma group and treatment replicates for each participant.

For analysis of the public databases, differences among the groups were analyzed by using the Wilcoxon rank sum test or Steel multiple comparison test, as appropriate. The trend among the groups was analyzed by using the Jonckheere-Terpstra test. For categorization of the type 2–low and type 2–high groups in the GSE19187 and GSE4302 data sets, respectively, unsupervised hierarchical clustering including values of 3 type 2 signature genes (calcium-activated chloride channel regulator 1 [*CLCA1*], periostin [*POSTN*], and serpin family B member 2 [*SERPINB2*]) using the Euclidean metric with complete linkage was performed as previously described.[17] Correlations between nonparametric data were undertaken by using the Spearman rank correlation test. Statistical significance was defined as a P value less than .05. All analyses were performed by using R version 3.6.1. software (The R Foundation; http://www.r-project.org/) or GraphPad Prism version 8.4.1 (GraphPad Software, San Diego, Calif).

RESULTS

IL-13 treatment suppresses *ACE2* expression in airway epithelial cells *ex vivo*

Participant demographics for the *ex vivo* study are shown in Table I. The disease of the participants with asthma was mild to moderate in severity based on medication requirements with the patient requiring controller medication. The participants with asthma exhibited good to fair asthma control. All participants with and without asthma had a history of atopy based on their clinical history, presence of allergic rhinitis, and elevated fractional exhaled nitric oxide level. Total and allergen-specific IgE levels were not available for these participants.

IL-13 reduced *ACE2* expression and increased *TMPRSS2* expression in airway epithelial cells from both the asthma and nonasthma atopic groups (Fig 1). As it been suggested that ciclesonide may possess antiviral properties,[20] we excluded the participant with asthma who used inhaled corticosteroids and repeated the analysis. We did not find significant differences in *ACE2* and *TMPRSS2* expression (data not shown).

Gene expression analysis

For the analysis of gene expression data, we used the publicly available results of 2 studies, GSE19187[13] and GSE4302,[16-19] as described in the Methods. For participant characteristics, see Tables E1 and E2 (in the Online Repository available at www.jacionline.org) and the original publications by Giovannini-Chami et al,[13] Woodruff et al,[16] Woodruff et al,[17] Dougherty et al,[18] and Park et al.[19]

ACE2 expression is lower in asthma and allergic rhinitis

In study GSE19187, nasal epithelial cells of participants with asthma and/or allergic rhinitis demonstrated lower *ACE2* expression compared with that of healthy participants (Fig 2, *A*). Assessment of *ACE2* expression by severity of symptoms (healthy, rhinitis without asthma, rhinitis with controlled asthma, and rhinitis with uncontrolled asthma) showed that participants with rhinitis and uncontrolled asthma demonstrated the lowest nasal epithelial cell *ACE2* expression (the Jonckheere-Terpstra trend test, [Fig 2, *B*]). In addition, participants who were categorized as type 2–high on the basis of 3 gene signatures (*CLCA1, POSTN,* and *SERPINB2*),[17] also demonstrated lower nasal epithelial *ACE2* expression than did those categorized as belonging to the type 2–low and healthy control groups (Fig 2, *C*).

We next examined the association of nasal epithelial cell *ACE2* expression with type 2 cytokines. *IL-13*, but not *IL-4* and *IL-5*, was significantly negatively correlated with *ACE2* expression (Fig 3, *A-C*). The type 2–driven genes *CLCA1*, *POSTN*, and *SERPINB2* showed an inverse correlation with *ACE2* expression (see Fig E1 in this article's Online Repository at www.jacionline.org).

Airway epithelial cell *ACE2* expression is lower in type 2–high participants with asthma

In data set GSE4302 containing airway epithelial cell mRNA, *ACE2* expression was lower (albeit not significantly) in the participants with asthma than in the control participants ($P = .097$) (Fig 4, *A*). When participants with asthma were grouped into the categories type 2–high and type 2–low on the basis of 3 gene signature expressions,[17] the type 2–high participants demonstrated lower expression of *ACE2* than did the healthy participants, whereas the type 2–low participants demonstrated expression similar to that in healthy participants (Fig 4, *B*).

ACE2 expression in airway epithelial cells was correlated with type 2 cytokines, *IL-4*, *IL-5*, and *IL-13* in participants with asthma (Fig 5, *A-C*). Furthermore, 2 type 2 cytokine–induced genes (ie, *CLCA1* and *SERPINB2*, but not *POSTN*) were significantly negatively correlated with *ACE2* expression when the group of those with asthma and the group of healthy participants were combined (see Fig E2 in this article's Online Repository at www.jacionline.org).

TMPRSS2 gene expression is increased in allergic rhinitis and asthma

In data set GSE19187, the participants with asthma and/or allergic rhinitis demonstrated increased nasal epithelial cell expression of *TMPRSS2* compared with the healthy participants, whether combined (Fig 6, *A*) or evaluated as separate groups (Fig 6, *B*). Moreover, the type 2–high participants demonstrated significantly higher expression of *TMPRSS2*, whereas the expression by type 2–low participants was similar to that by healthy participants (Fig 6, *C*). There was a significant correlation between nasal epithelial *TMPRSS2* gene expression with *CLCA1*, *POSTN*, and *SERPINB2*, but not with *IL-4*, *IL-5*, or *IL-13* (see Fig E3 in this article's Online Repository at www.jacionline.org).

TMPRSS2 gene expression is positively correlated with type 2 cytokine–induced genes in asthma

Although gene expression of *TMPRSS2* was not different between healthy participants and those with asthma (Fig 7, *A*), *TMPRSS2* expression was higher (albeit not significantly) in type 2–high patients with asthma than in healthy participants ($P = .086$). There was no difference in *TMPRSS2* expression between the healthy participants and the type 2–low participants with asthma ($P = .84$) (Fig 7, *B*).

In addition, a significant correlation between airway epithelial *TMPRSS2* and *IL-4* expression was present when both groups were combined, as well as for the group containing only subjects with asthma (see Fig E4 in this article's Online Repository at www.jacionline.org). This relationship was not demonstrated for *IL-5* and *IL-13* (see Fig E4). Airway epithelial cell *TMPRSS2* expression was significantly positively correlated with expression of *CLCA1* and *SERPINB2*, but not with expression of *POSTN* when the asthma and control groups were combined (see Fig E5 in this article's Online Repository at www.jacionline.org).

DISCUSSION

To our knowledge, this is the first report to show that IL-13 decreases *ACE2* expression and increases *TMPRSS2* expression in airway epithelial cells in asthma and atopy. These *ex vivo* observations are supported by interrogation of 2 public databases, which include children and adults and in which we have shown that *ACE2* expression is reduced and *TMPRSS2* expression is increased in type 2 asthma and rhinitis. In addition, *ACE2* and *TMPRSS2* expression are correlated with type 2 cytokines, although in opposite ways: *ACE2* is inversely correlated with type 2 or type 2–driven genes, whereas *TMPRSS2* expression is

TABLE I. Participant characteristics for the *ex vivo* study

Participant demographic characteristics	Atopic, without asthma	Asthma
Sex (n)		
Females	1	2
Males	3	2
Race (n)		
White	2	2
Black	0	0
Asian	0	0
Hispanic	2	2
Mean age, y, ± SD	31 ± 13	39 ± 14
Mean body mass index, kg/m^2, ± SD	25 ± 3	31 ± 7
Age of onset (n)*		
>12 y	N/A	1
<12 y	N/A	2
Mean duration of asthma, y, ± SD	N/A	33 ± 15
Mean Asthma Control Questionnaire score	N/A	1.22 ± 0.76
Exacerbations per year (n)	N/A	0
FENO concentration (ppb)	41 ± 30	30 ± 8
Atopy (% positive)	100	100
Mean peripheral eosinophil level, %, ± SD	0.75 ± 0.83	4 ± 3
Mean peripheral eosinophil level, abs (× 10^9/mm^3), ± SD	0.05 ± 0.05	0.18 ± 0.11
Mean peripheral neutrophil level,%, ± SD	57 ± 11	53 ± 8
Mean peripheral neutrophil level, abs (× 10^9/mm^3), ± SD	4 ± 2	4 ± 2
Mean FVC, %, ± SD	105 ± 5	95 ± 3
Mean FEV_1, %, ± SD	97 ± 10	90 ± 7
Mean FEV_1/FVC ratio, %, ± SD	76 ± 7	77 ± 3
PC20 level (mg/mL)	N/A	5 ± 1
Medication (n)		
Short acting β-agonist	N/A	4
Leukotriene modifier	N/A	1
ICS	N/A	0
Antihistamine	N/A	2
Combination therapy (ICS/LABA)	N/A	1
Short-acting anticholinergic	N/A	0
Intranasal corticosteroid	0	0

abs, Absolute; *FENO*, fractional exhaled nitric oxide; *ICS*, inhaled corticosteroid; *LABA*, long-acting β-agonist; *N/A,* not available; *PC20*, provocative concentration of methacholine that resulted in a 20% decrease in the FEV_1.
*Age of onset is not available for 1 participant.

positively correlated with type 2 or type 2–driven genes. These analyses are exploratory and provide a direction for future investigation.

Elucidation of the role of ACE2 in asthma is just beginning. The renin-angiotensin system is a critical component in regulating multiple tissue and organ functions, such as those of the cardiovascular system, kidney, lung, and liver, specifically by maintaining homeostasis of blood pressure, electrolyte balance, and inflammatory responses.[21,22] Renin, a protease produced predominantly in the kidneys, cleaves angiotensinogen to generate angiotensin I (Ang I). Subsequently, angiotensin-converting enzyme (ACE) cleaves Ang I to produce angiotensin II (Ang II). Ang II is hydrolyzed by various angiotensinases, such as ACE2. ACE2 is a terminal carboxypeptidase and a type I transmembrane glycoprotein, as well as a potent negative regulator of the renin-angiotensin system. The imbalance in enzymatic activity of ACE/ACE2 has been suggested to be involved in pathogenesis in several diseases, including lung diseases.[23]

In asthma, ACE2 may have a role because of its anti-inflammatory nature and ability to inactivate Ang II and activate Ang1-7, which are the 2 counteractive systems strongly related to asthma. In an ovalbumin-challenged mouse model of type 2 asthma, Ang1-7 modulated ovalbumin-induced increases in total cell counts, eosinophils, lymphocytes, and neutrophils and decreased perivascular and peribronchial inflammation, fibrosis, and goblet cell hyper/metaplasia.[24] These results support an important protective role of Ang1-7 in allergic asthma, as well as the role of ACE2 in reducing inflammation.[24] Type 2 cytokines appear to reduce *ACE2* expression, reducing the anti-inflammatory function of ACE2-generated Ang1-7.

ACE2 also has an additional function in relation to SARS-CoV-2 infection. The spike (S) protein of SARS-CoV-2 binds to ACE2 to mediate virus attachment to host cell membranes. Hoffmann et al[9] demonstrated that host cell entry of SARS-CoV-2 depends on the SARS-CoV receptor ACE2 and can be blocked by a clinically proven inhibitor of the cellular serine protease TMPRSS2, which is used by SARS-CoV-2 for S protein priming. Inhibition of TMPRSS2 could be a therapeutic target in COVID-19. It has been shown that ACE2 is a host factor used in cell entry by 2 additional coronaviruses, SARS-CoV and human coronavirus NL63.[25] TMPRSS2 has been reported to facilitate infection with human coronavirus 229E,[26] Middle East respiratory syndrome–related coronavirus,[27] H1N1,[28,29] H3N2, and H7N9 influenza viruses.[30,31] Taken together, both ACE2 and TMPRSS2 are important host factors for infection with many viruses, with some particularly relevant to asthma. We speculate whether the

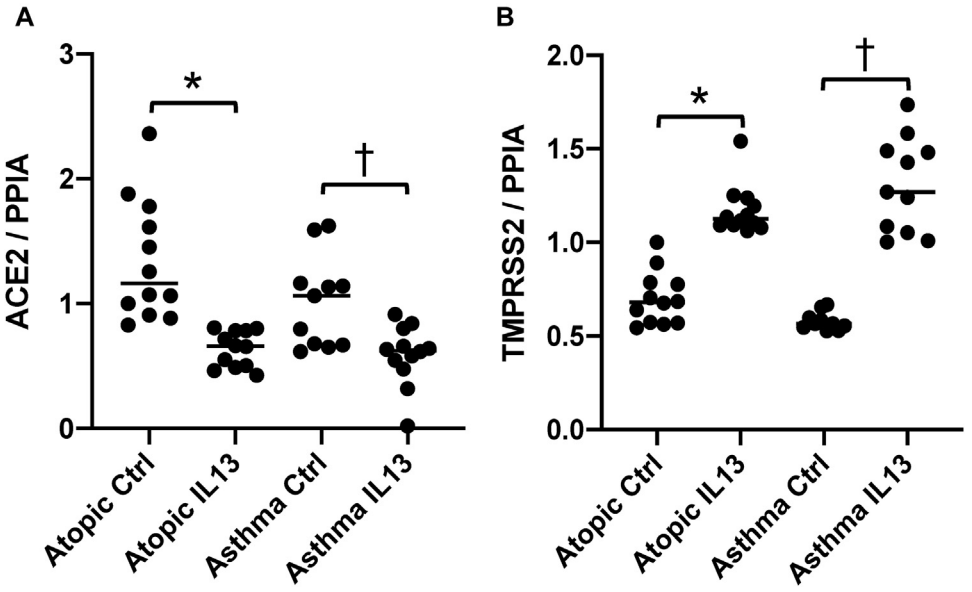

FIG 1. IL-13 exposure reduces *ACE2* and increases *TMPRSS2* expression in airway epithelial cells from participants with asthma and atopy. **A,** IL-13 exposure significantly reduces *ACE2* expression in nonasthma atopic (mean = 1.27 [95% CI = 1.05-1.54] vs mean = 0.62 [95% CI 0.54-0.71]; **P* = .0001) and asthmatic (mean = 0.95 [95% CI = 0.77-1.17] vs mean = 0.47 [95% CI = 0.27-0.82]; †*P* = .038) airway epithelial cells. **B,** IL-13 significantly increases *TMPRSS2* expression in nonasthma atopic (mean = 0.69 [95% CI = 0.62-0.77] vs mean = 1.16 [95% CI = 1.10-1.23]; **P* < .0001) and asthmatic (mean = 0.58 [95% CI 0.55-0.61] vs mean 1.28 [95% CI 1.15-1.44]; †*P* < .0001) airway epithelial cells.

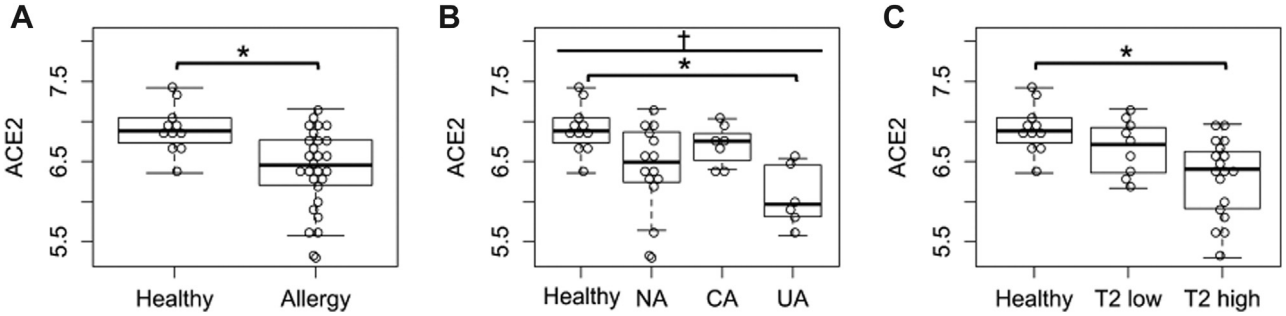

FIG 2. *ACE2* expression is reduced in nasal epithelial cells from type 2 (T2)-high participants with allergy. **A**, In data set GSE19187, participants with asthma and allergic rhinitis demonstrate lower expression of *ACE2*. **P* = .0039. **B,** Participants with uncontrolled asthma (UA) demonstrate lower *ACE2* expression than healthy controls do. **P* = .0053 for the paired comparison; †*P* = .0035. **C,** When subtyped to T2-low and T2-high groups, T2-high participants demonstrate lower *ACE2* expression than healthy controls do. **P* = .0024 for paired comparison. Box plots show medians with interquartile ranges. *CA*, Rhinitis with controlled asthma; *NA*, rhinitis without asthma; *UA*, rhinitis with uncontrolled asthma.

upregulation of *TMPRSS2* gene expression in the upper and lower airways increases susceptibility of patients with asthma to infection by influenza.

The role of type 2 cytokines in *ACE2* and *TMPRSS2* expression is not clear. IL-4 and IL-13, both of which are type 2 cytokines relevant to asthma, reduce *ACE2* expression in Vero E6 cells, a primate kidney epithelial cell line.[32] We have shown similar effects by IL-13 in the upper and lower airways in human asthma and rhinitis. Although we evaluated gene expression, there are conflicting reports regarding the existence of ACE2 protein in the nasal epithelium. Hamming et al reported that ACE2 protein was present in the basal layer of the nonkeratinizing squamous epithelium but not present on the surface of nasal epithelial cells.[33] Bertram et al noted ACE2 and TMPRSS2 immunostaining in sinus epithelium by using antigen retrieval methods.[34] Furthermore, there are 2 forms of ACE2, full-length ACE2 and soluble ACE2, the latter which is shed from the full-length, membrane-bound protein and is considered as a potential therapy.[35] In our study we report overall gene expression of *ACE2* in nasal and airway epithelial cells to include the extracellular domain, but we do not report expression of membrane-bound and soluble forms. Although we speculate that type 2 cytokine stimulation may suppress ACE2 in the central and upper airway to reduce viral entry, evaluation of membrane-bound and soluble forms of ACE2 is the logical next step to determine the significance of this observation.

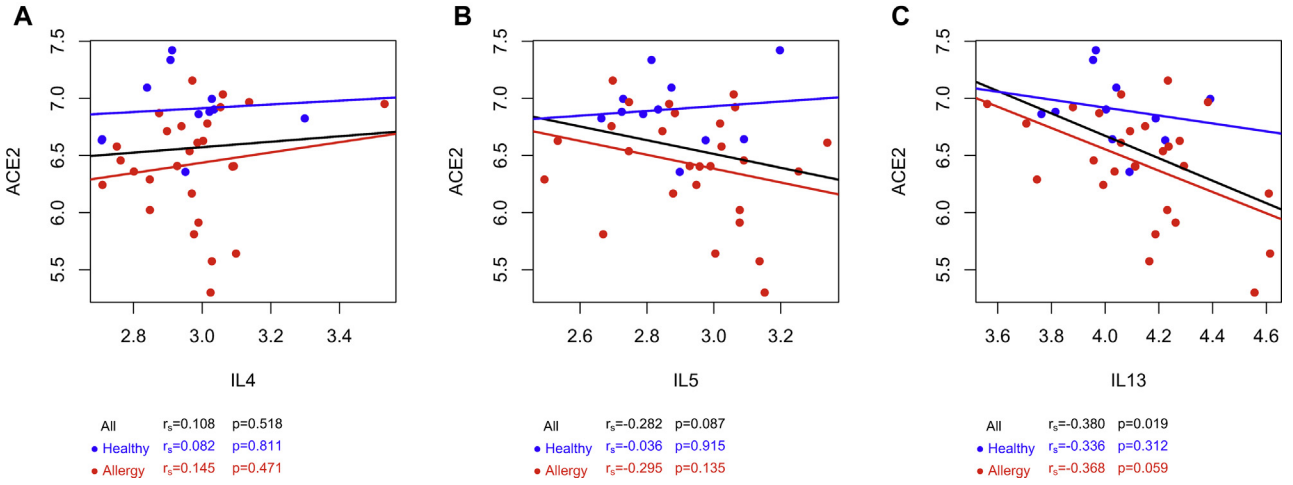

FIG 3. *ACE2* expression is significantly negatively correlated with *IL-13* expression. The data from GSE19187 reveal no significant correlation between *IL-4* and *ACE2* **(A)** and between *IL-5* and *ACE2* **(B)**. **(C)** *IL-13* and *ACE2* are significantly negatively correlated when all groups are combined. r_s, Spearman rank correlation coefficient.

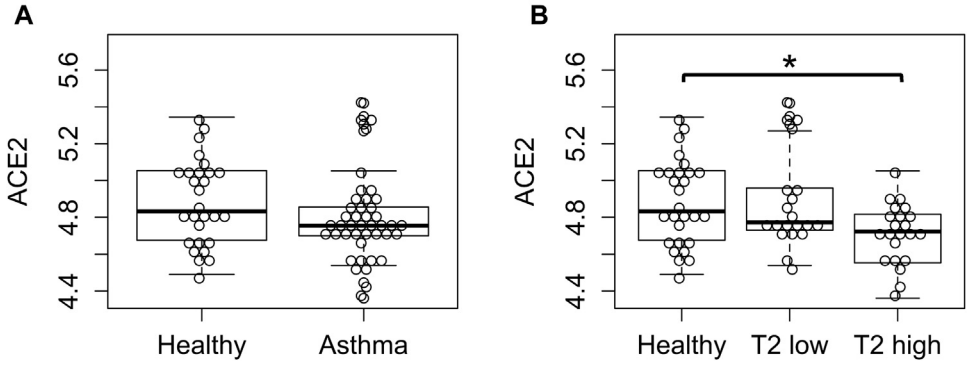

FIG 4. *ACE2* expression is decreased in airway epithelial cells in type 2 (T2)-high asthma. **A,** In the gene expression data set from adult participants in GSE4302, *ACE2* expression is reduced in all asthma participants compared with in healthy participants, with a trend toward significance ($P = .097$). **B,** Patients with T2-high asthma demonstrate lower airway epithelial *ACE2* expression than healthy participants do. *$P = .031$ for the paired comparison. Box plots show medians with interquartile ranges.

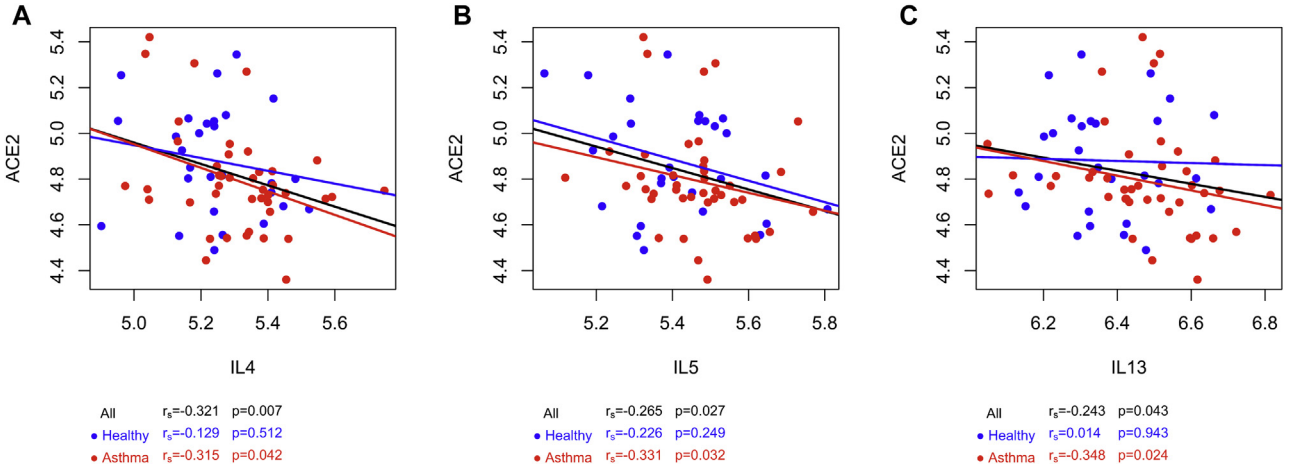

FIG 5. Airway epithelial *ACE2* is significantly negatively correlated with expression of *IL-4*, *IL-5*, and *IL-13*. Analysis of data set GSE4302 reveals a significant negative correlation of *IL-4*, *IL-5*, and *IL-13* with *ACE2* in the airway epithelial cells from participants with asthma and when asthma and healthy groups are combined. r_s, Spearman rank correlation coefficient.

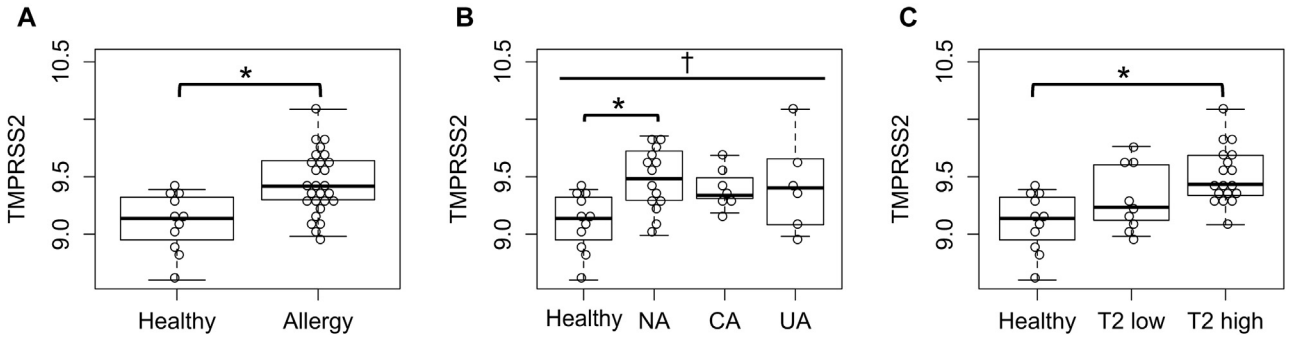

FIG 6. *TMPRSS2* gene expression is higher in nasal epithelial cells from type 2 (T2)-high participants with allergy. In data set GSE19187, participants with asthma and/or allergic rhinitis demonstrate increased nasal epithelial expression of *TMPRSS2* compared with that demonstrated by healthy participants regardless of whether the allergy and asthma groups were combined (*$P = .0022$) **(A)** or evaluated as separate groups (*$P = .018$ for the paired comparison; †$P = .034$) **(B). C,** The T2-high participants demonstrate significantly higher expression of *TMPRSS2*, whereas the expression of *TMPRSS2* by T2-low participants is similar to that demonstrated by healthy participants. *$P = .0012$ for the paired comparison. Box plots show medians with interquartile ranges. *CA*, Rhinitis with controlled asthma; *NA*, rhinitis and no asthma; *UA*, rhinitis with uncontrolled asthma.

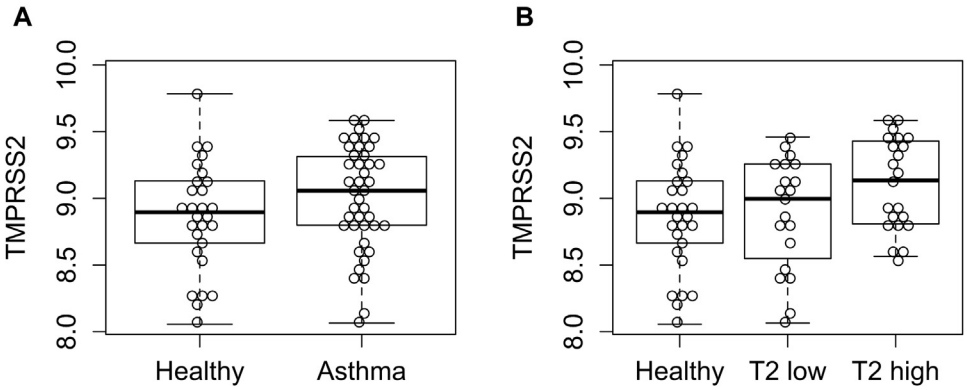

FIG 7. *TMPRSS2* expression is higher in the airway epithelial cells from type 2 (T2)-high asthma, with a trend toward significance. **A,** In the adult asthma study GSE4302, *TMPRSS2* expression is not significantly different between healthy participants and participants with asthma ($P = .13$). **B,** *TMPRSS2* expression tended to be higher in T2-high participants with asthma than in healthy participants ($P = .086$). Box plots show medians with interquartile ranges.

In addition, increased expression of *TMPRSS2* could negate any protective effect of reduced *ACE2* expression. Epidemiologic support for such assertion is suggestive, as studies from China have not identified asthma as a major risk factor for severe COVID-19[2,3] and some reports from the United States have identified asthma as a risk factor for COVID-19[5,6] whereas others have not.[36,37] Additional data from both biologic and epidemiologic studies are warranted.

Whether inhaled or nasal corticosteroids are protective against SARS-CoV-2 infection merits comment, as it has been suggested that ciclesonide may possess antiviral properties owing to its ability to block viral replication.[20] In our *ex vivo* study, 1 of our participants with asthma used inhaled corticosteroids. We excluded this participant and repeated the analysis. We did not find significant differences in *ACE2* and *TMPRSS2* expression (data not shown). Participants whose nasal airway epithelial cell data were included in data set GSE19187 did not receive treatment with nasal corticosteroids for at least 1 month before enrollment. Similarly, participants whose airway epithelial cells are included in data set GSE4302 did not take inhaled or oral corticosteroids for 1 month before enrollment. Thus, the association of *ACE2* and *TMPRSS2* gene expression with type 2 cytokines, atopy, and type 2–high asthma are not dependent on the effect of corticosteroids in this analysis. This issue should be addressed in future investigation.

Our study has several strengths. Using airway epithelial cells from well-phenotyped atopic participants with and without asthma from our own laboratory, we have shown that IL-13 (1) suppresses *ACE2* gene expression and (2) increases *TMPRSS2* expression. We have observed similar results from 2 distinct data sets in children and adults with rhinitis and asthma. This approach adds robustness to the observation that IL-13 modulates the receptor system for COVID-19. Recently, Jackson et al have reported that underlying allergy and allergen exposure reduces the expression of *ACE2*.[38] Our data extend their findings by (1) demonstrating contrasting changes in *TMPRSS2* in response to IL-13 stimulation *ex vivo* and (2) demonstrating that the effect of underlying allergy was type

2–specific when unsupervised hierarchical clustering including type 2–induced genes was used.

The limitations of our study include (1) the fact that our sample size for the *ex vivo* studies was small and (2) the fact that data from publicly available data sets do not include detailed clinical information.

In summary, we have provided evidence that type 2 inflammation suppresses expression of *ACE2* and increases expression of *TMPRSS2* in nasal and airway epithelial cells in asthma and atopy. These observations may provide a foundation to elucidate the relative role of these 2 mediators in cell entry and how type 2 cytokines modulate susceptibility to COVID-19.

We would like to acknowledge Pascal Barbry, PhD, and Prescott Woodruff, MD, for use of their data in databases GSE19187 and GSE4302, respectively. The authors would also like to acknowledge Ronald Schunk, RT, for his assistance with participant recruiting and bronchoscopy and Bianca Sierra for her assistance with *ex vivo* experiments.

Key messages:

- **IL-13 decreases *ACE2* expression and increases *TMPRSS2* expression in bronchial epithelial cells *ex vivo*.**
- ***ACE2* and *TMPRSS2* are modulated by type 2 inflammation in the upper and lower airways.**

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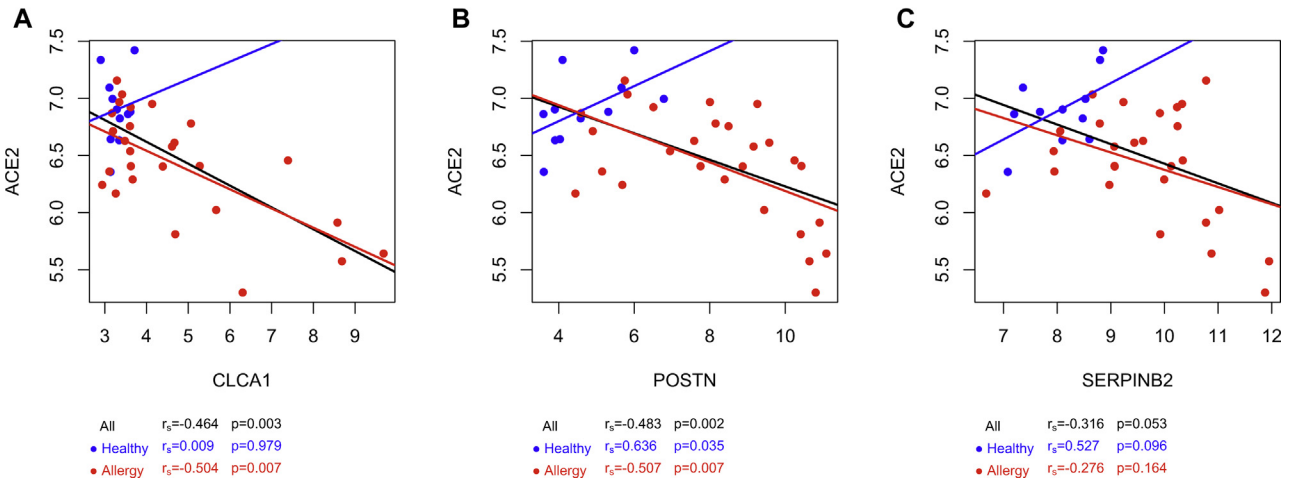

FIG E1. Expression of *ACE2* and the type 2 (T2)-driven genes *CLCA1* and *SERPINB2* is significantly inversely correlated in nasal epithelial cells. Analysis of gene expression data from data set GSE19187 reveals inverse correlations between *ACE2* with the T2-driven genes *CLCA1* **(A)**, *POSTN* **(B)**, and *SERPINB2* **(C)**, with *SERPINB2* trending toward significance. r_s, Spearman rank correlation coefficient.

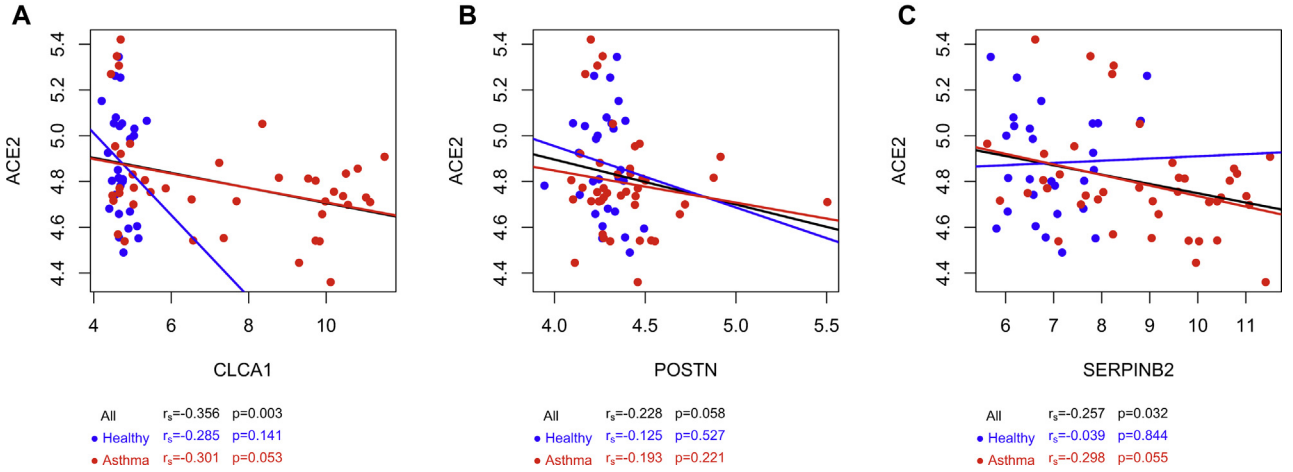

FIG E2. *ACE2* and type 2 (T2)-driven genes are inversely correlated in airway epithelial cells. In data set GSE4302 from adult participants with asthma and healthy controls, *ACE2* is inversely correlated with *CLCA1* (**A**), *POSTN* (**B**), and *SERPINB2* (**C**) when asthma and control groups are combined, with *POSTN* trending toward significance. r_s: Spearman rank correlation coefficient.

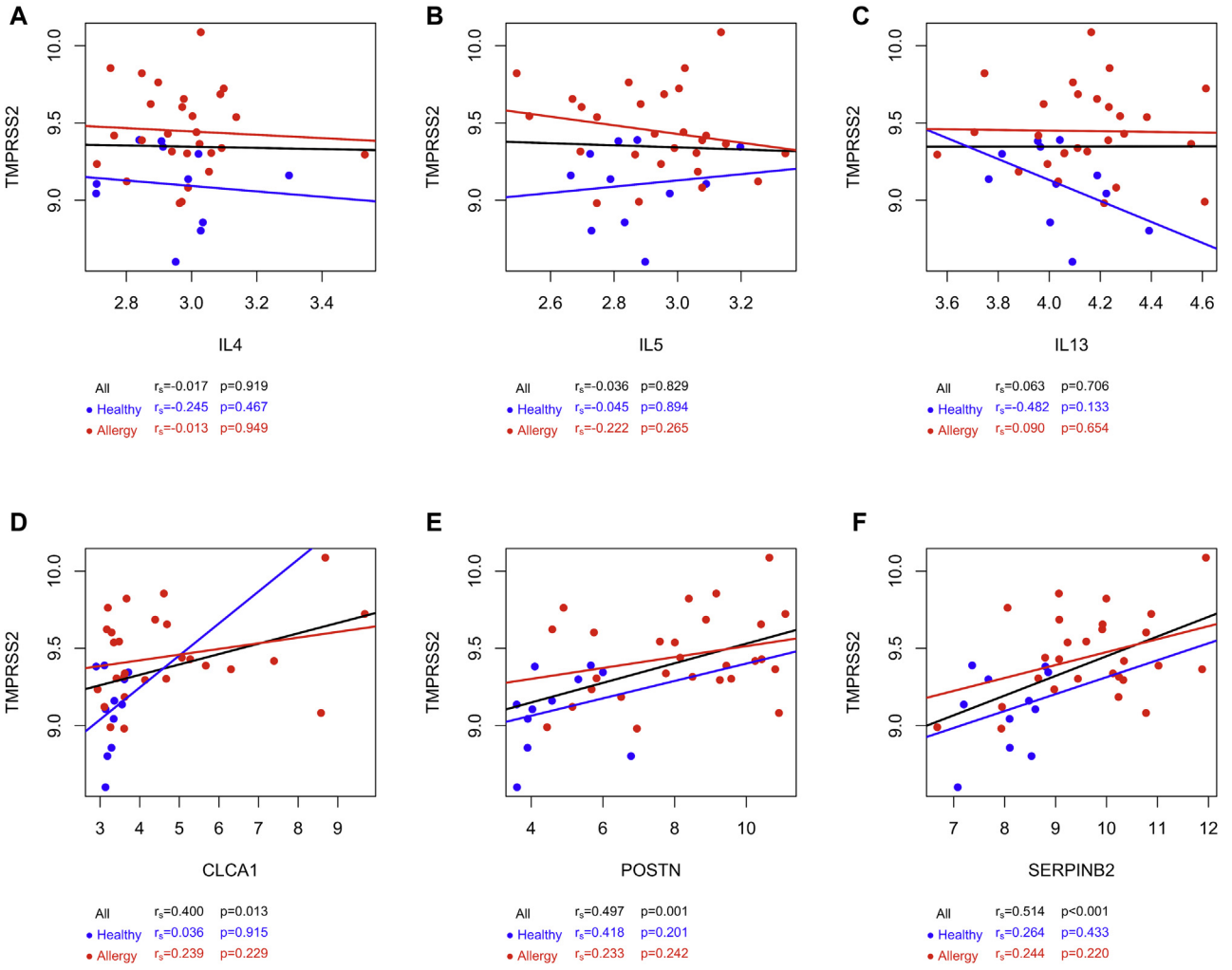

FIG E3. *TMPRSS2* expression is significantly correlated with type 2 (T2)-related genes in nasal epithelial cells. When the data set GSE19187 is used, significantly positive correlations between *TMPRSS2* and the T2-driven signature genes *CLCA1*, *POSTN*, and *SERPINB2* are present, but no such correlations are present with the T2 cytokines *IL-4*, *IL-5*, and *IL-13*. r_s, Spearman rank correlation coefficient.

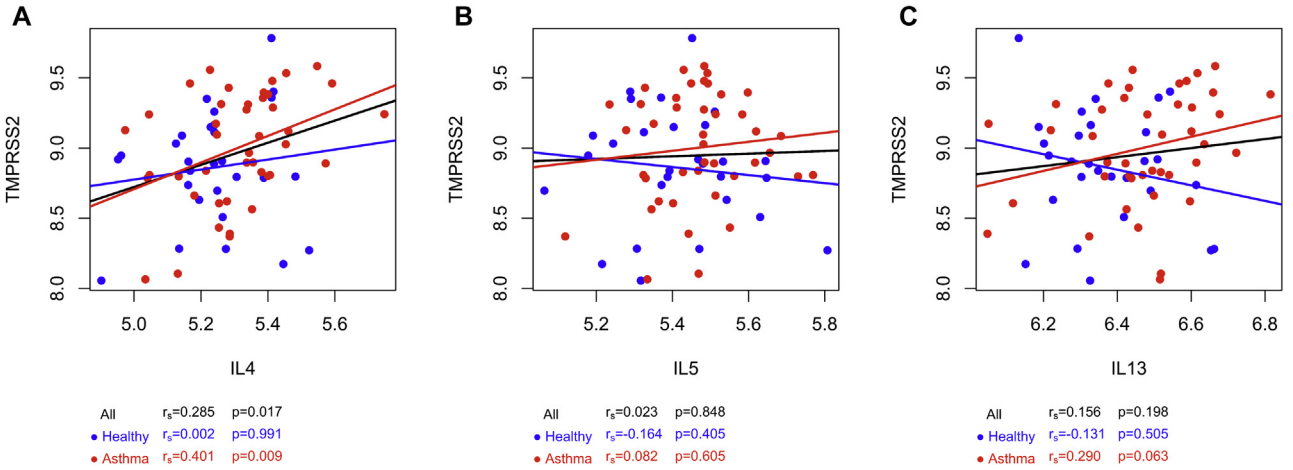

FIG E4. Airway epithelial *TMPRSS2* expression is significantly positively correlated with *IL-4* expression. Data from GSE4302 revealed a significant correlation between airway epithelial *TMPRSS2* expression and *IL-4* when both groups are combined and for asthma only **(A)**. **B** and **C,** This relationship is not demonstrated for *IL-5* and *IL-13*. r_s, Spearman rank correlation coefficient.

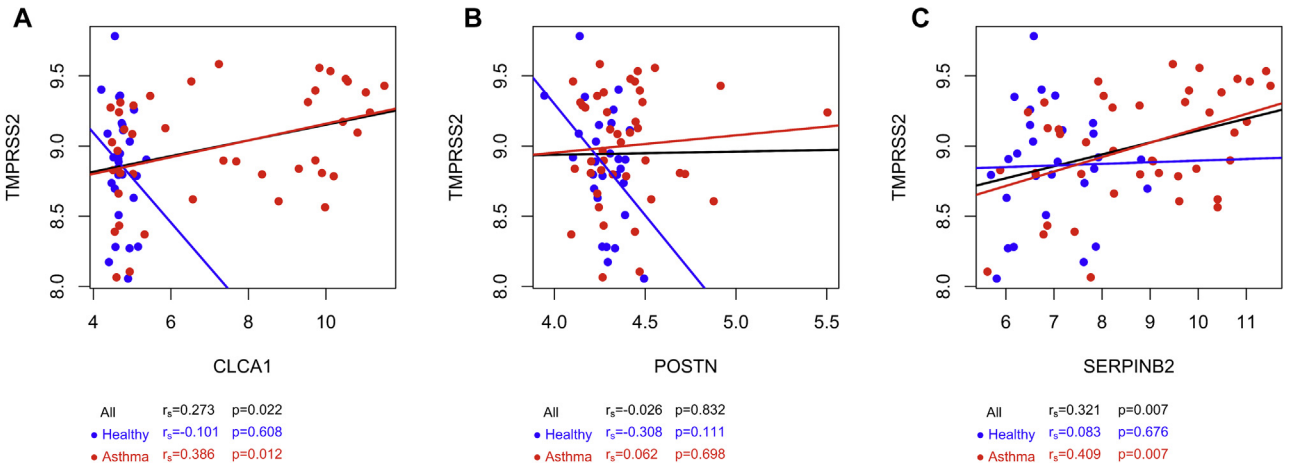

FIG E5. *TMPRSS2* and type 2 (T2)-driven genes are significantly positively correlated in airway epithelial cells. In data set GSE4302, airway epithelial cell *TMPRSS2* expression is significantly positively correlated with expression of *CLCA1* (**A**) and *SERPINB2* (**C**) but not with expression of *POSTN* (**B**) when asthma and healthy control groups are combined. r_s, Spearman rank correlation coefficient.

TABLE E1. Participants in GSE19187

Characteristic	Control	Isolated rhinitis	Rhinitis with controlled asthma	Rhinitis with uncontrolled asthma
Sample size (n)	11	14	7	6
Dust mite allergy	No	Yes	Yes	Yes
Mean age, y, ± SD	12 ± 3	11 ± 3	12 ± 3	9 ± 1
T2-low–to–T2-high ratio	11:0	6:8	2:5	1:5

For additional information, please see Giovannini-Chami et al.[E1]
T2, Type 2.

TABLE E2. Participants in GSE4302 study

Characteristic	Healthy	Asthma	
		T2-low	T2-high
Sample size (n)	28	20	22
Mean age, y, ± SD	36 ± 9	36 ± 11	37 ± 12

For additional information, see Woodruff et al.[E2]
T2, Type 2.