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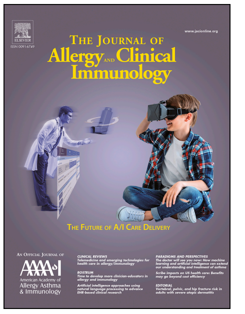

Association of Respiratory Allergy, Asthma and Expression of the SARS-CoV-2 Receptor, *ACE2*

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PII: S0091-6749(20)30551-0

DOI: https://doi.org/10.1016/j.jaci.2020.04.009

Reference: YMAI 14507

To appear in: *Journal of Allergy and Clinical Immunology*

Received Date: 7 April 2020

Revised Date: 15 April 2020

Accepted Date: 16 April 2020

Please cite this article as: Jackson DJ, Busse WW, Bacharier LB, Kattan M, O'Connor GT, Wood RA, Visness CM, Durham SR, Larson D, Esnault S, Ober C, Gergen PJ, Becker P, Togias A, Gern JE, Altman MC, Association of Respiratory Allergy, Asthma and Expression of the SARS-CoV-2 Receptor, *ACE2*, *Journal of Allergy and Clinical Immunology* (2020), doi: https://doi.org/10.1016/j.jaci.2020.04.009.

Association of Respiratory Allergy, Asthma and Expression of the SARS-CoV-2 Receptor, *ACE2*

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Word Count: 1140

Capsule Summary:
Underlying respiratory allergy and experimental allergen exposure reduce the expression of the SARS-CoV-2 receptor, *ACE2,* which could lead to reduced COVID-19 susceptibility.

Key Words: SARS-CoV-2, COVID-19, asthma, respiratory allergy, allergic sensitization, receptor, *ACE2* expression

Funding:
Funded by NIH/NIAID: UM1AI114271 & UM1AI109565 and NIH/NHLBI: RO1HL12384

Dr. Jackson reports grants from NIH/NIAID/NHLBI and GlaxoSmithKline, personal fees for DSMB from Pfizer and for consulting from Novartis, Sanofi-Regeneron, GlaxoSmithKline, Vifor Pharma and Astra Zeneca.
Dr. Busse reports grants from NIH/NIAID/NHLBI, during the conduct of the study; personal fees from Novartis, Sandoz, Regeneron, AstraZeneca, GlaxoSmithKline, Genentech, Teva, Elsevier, Arrowhead, resTORbio, Med Learning Group, Practicing Clinicians Exchange, Boston Scientific, Medscape.
Dr. Bacharier reports grant support from NIH/NIAID/NHLBI, Sanofi and Vectura, and personal fees from GlaxoSmithKline, Genentech, Novartis, Merck, DBV Technologies, Teva, Boehringer Ingelheim, AstraZeneca, WebMD/Medscape, Sanofi, Regeneron, Vectura, and Circassia.
Dr. Wood receives grant support from the NIH, Astellas, Aimmune, DBV, Sanofi, and Regeneron, and royalties from Up To Date.
Dr. Gern reports grants from NIH, personal fees and stock options from Meissa Vaccines Inc., personal fees from AstraZeneca and Ena Therapeutics and a patent on methods for production of rhinoviruses.
Dr. Altman reports personal fees for consulting from Regeneron.
Drs. Becker, Durham, Esnault, Gergen, Kattan, Larson, Ober, O'Connor, Togias, and Visness report no conflicts of interest.

Conflict of Interest

Dr. Jackson reports grants from NIH/NIAID/NHLBI and GlaxoSmithKline, personal fees for DSMB from Pfizer and for consulting from Novartis, Sanofi-Regeneron, GlaxoSmithKline, Vifor Pharma and Astra Zeneca.
Dr. Busse reports grants from NIH/NIAID/NHLBI, during the conduct of the study; personal fees from Novartis, Sandoz, Regeneron, AstraZeneca, GlaxoSmithKline, Genentech, Teva, Elsevier, Arrowhead, resTORbio, Med Learning Group, Practicing Clinicians Exchange, Boston Scientific, Medscape.
Dr. Bacharier reports grant support from NIH/NIAID/NHLBI, Sanofi and Vectura, and personal fees from GlaxoSmithKline, Genentech, Novartis, Merck, DBV Technologies, Teva, Boehringer Ingelheim, AstraZeneca, WebMD/Medscape, Sanofi, Regeneron, Vectura, and Circassia.
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To the Editor:

The novel coronavirus SARS-CoV-2 (COVID-19) was recognized in December 2019 as a cause of severe pneumonia and has now led to a global pandemic.(1) Respiratory illnesses caused by COVID-19 cover a range of severity. The identification of risk and protective factors for disease severity from COVID-19 is critical to direct development of new treatments and infection prevention strategies. Early large case series have identified a number of risk factors for severe disease including older age, hypertension, diabetes, cardiovascular disease, tobacco exposure and COPD.(2) The Center for Disease Control (CDC) lists asthma as a risk factor for severe COVID-19 illness, which is logical given that many respiratory viruses have been well established to cause more serious illnesses in those with chronic airway diseases such as asthma. However, asthma and respiratory allergy have not been identified as significant risk factors for severe COVID-19 illness in case series from China.(2) These preliminary reports led us to question whether we could identify features of allergy and/or asthma that could be associated with potential for reduced COVID-19 illness severity.

SARS-CoV-2 uses angiotensin-converting enzyme-2 (ACE2) as its cellular receptor, as do SARS-CoV and coronavirus NL63.(1) Higher ACE2 expression increases *in vitro* susceptibility to SARS-CoV,(3) and studies examining factors that impact *ACE2* gene expression have revealed its upregulation is associated with smoking, diabetes, and hypertension, all associated with increased COVID-19 illness severity.(4)

We hypothesized that one potential explanation for the unexpected observation that asthma and other allergic diseases may not be a risk factor for severe COVID-19 disease is a reduced *ACE2* gene expression in airway cells and thus decreased susceptibility to infection. To test this hypothesis, we examined whether asthma and respiratory allergy are associated with reduced *ACE2* expression in airway cells from three different cohorts of children and adults. In all three studies, total RNA was extracted from nasal or lower airway epithelial brush samples with RNA-sequencing performed independently for each study as previously described and provided in detail in the online supplement.(5) Differential expression of *ACE2* was assessed using a weighted linear mixed effects model (limma) appropriate for RNA-seq data and empirical Bayes method.

Children at high risk for asthma based upon parental histories and living in urban neighborhoods were enrolled prenatally and followed prospectively in the Urban Environment and Childhood Asthma (URECA) cohort and 318 had nasal epithelial brushes obtained at 11 years of age. Prevalence of asthma was assessed at 10 years of age and atopic status was defined by allergic sensitization trajectories [no/minimal, low, medium, and high] as previously described.(6) Additional type 2 biomarkers, including fractional exhaled nitric oxide (FeNO), peripheral blood eosinophils, and total IgE, were evaluated using standard methods. In URECA, allergic sensitization was inversely related to *ACE2* expression in nasal epithelium regardless of asthma status (Figure 1A). Within children with asthma, moderate allergic sensitization (fold change (FC)=0.70, p=4.2E-3) and high allergic sensitization (FC=0.54, p=6.4E-5) were associated with progressively greater reductions in *ACE2* compared to children with asthma but no/minimal allergic sensitization (Figure 1B). *ACE2* expression was also significantly inversely associated with type 2 biomarkers (Supplementary Table 1) including the number of positive allergen-specific IgE tests (beta coefficient -0.089, p=3.1E-5), total IgE (beta coefficient -0.31, p=5.1E-6), FeNO (beta coefficient -0.45, p=3.4E-3), and nasal epithelial *IL13* expression (beta coefficient -0.123, p=8.6E-5). *ACE2* expression was not significantly correlated with peripheral blood eosinophils (beta coefficient -0.13, p=0.07). Although male sex has been associated with increased COVID-19 illness severity(2), no sex-based differences in *ACE2* expression were found in URECA. Of note, 10 participants reported nasal corticosteroid use at the time of nasal sampling and it was not associated with alterations in *ACE2* expression.

We also evaluated 24 adult participants with allergic rhinitis to cat, without asthma symptoms in the prior year, who were enrolled in a study where they underwent nasal cat allergen challenge (NAC) and exposure to cat allergen through an environmental exposure chamber (EEC) as previously described.(5) Pre/post-allergen challenge nasal brush samples were obtained. Allergen exposure by both NAC and EEC led to significant reductions in *ACE2* expression (Figure 2A; NAC: FC=0.81, p=2.4E-3; EEC: FC=0.79, p=1.6E-3).

An additional cohort of 23 adult participants with mild asthma, not treated with asthma controller therapy, underwent segmental allergen bronchoprovocation to dust mite, ragweed, or cat, as previously described.(7) Pre/post-allergen challenge bronchial brushings were obtained and demonstrated significantly reduced *ACE2* expression in lower airway epithelium post-allergen challenge (Figure 2B: FC 0.64, p=0.01).

From in vitro models obtained from Gene Expression Omnibus, we assessed the effects of IL-13, a type 2 cytokine strongly related to allergic asthma, on *ACE2* expression in differentiated airway epithelial cells. IL-13 significantly reduced *ACE2* expression (Supplemental Figure 1) in both nasal (FC=0.44 p=5.8E-4) and bronchial epithelium (FC=0.80, p=5.1E-3).

Viral respiratory infections are the most common trigger of severe asthma exacerbations in children and adults. Unexpectedly, large epidemiological studies of the COVID-19 pandemic in China did not identify asthma as a risk factor of severe COVID-19 related illnesses.(2) Here, we report that respiratory allergy and controlled allergen exposures are each associated with significant reductions in *ACE2* expression. *ACE2* expression was lowest in those with both high levels of allergic sensitization and asthma. Importantly, non-atopic asthma was not associated with reduced *ACE2* expression. Given that ACE2 serves as the receptor for SARS-CoV-2, our findings suggest a potential mechanism of reduced COVID-19 severity in patients with respiratory allergies. However, it is likely that additional factors beyond *ACE2* expression modulate the response to COVID-19 in allergic individuals, and elucidation of these factors may also provide important insights into COVID-19 disease pathogenesis.

Strengths of our study include carefully phenotyped cohorts of children and adults. Further, the allergen challenge studies included both upper and lower airway samples, with each demonstrating a consistent impact on *ACE2* expression. Limitations include lack of clinical information to directly link *ACE2* expression to SARS-CoV-2 infection and illness severity in our study populations. In addition, we do not have data on the ACE2 protein levels to confirm the gene expression data, though previous work suggests a direct association between *ACE2* mRNA levels and ACE2 protein levels in the lung.(8)

It is important to note that early data in the US suggest a higher rate of asthma in patients hospitalized for severe COVID-19 illness, but do not specify whether asthma was allergic or not, an important differentiation that relates to our findings, nor the potential presence of other co-morbidities, such as obesity, that have been identified as risk factors for COVID-19 illness.(9) Future studies focused on respiratory allergy, asthma and, perhaps, other allergic disorders are needed to provide greater understanding of the impact of underlying allergy on COVID-19 susceptibility and disease severity. The modulation of *ACE2* expression by type 2 inflammatory processes suggests the need to comprehensively evaluate the role of type 2 immune regulation in COVID-19 pathogenesis. Further elucidation of these relationships could identify novel therapeutic strategies to more effectively control this pandemic.

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Figure Legends

Figure 1. *ACE2* expression is decreased in the nasal epithelium of children with allergic sensitization and allergic asthma.
(A) *ACE2* expression levels in nasal brush samples from 11 year old children in the URECA cohort according to asthma diagnosis by age 10, dichotomized as No(-) or Yes(+), and IgE sensitization trajectory at age 10, dichotomized as not/minimally IgE sensitized (-) or IgE sensitized (+), showing lower levels of *ACE2* in children with atopy and atopic asthma. **(B)** *ACE2* expression in URECA children with asthma, subdivided according to the degree of IgE sensitization, demonstrating progressively lower levels of *ACE2* according to the degree of IgE sensitization among children with asthma. Children with both asthma and the highest IgE sensitization had the lowest levels of *ACE2* expression. Expression levels are log2 transformed, shown are median values (horizontal), interquartile ranges (boxes), and 1.5 IQR (whiskers). The printed fold

changes (FC) are for the non-log2-transformed expression values to aid in interpretation of the effect sizes.

Figure 2. *ACE2* expression is decreased in nasal and bronchial epithelium of allergic individuals after allergen challenge.
(A) *ACE2* expression was significantly decreased in nasal brush samples from adults in the cohort with allergic rhinitis and cat allergen sensitization both 8 hours after a cat allergen NAC, and 8 hours after the second day of a cat allergen EEC (n=24). **(B)** *ACE2* was significantly decreased in bronchial epithelial brush samples from adults with allergic asthma 48 hours after a segmental bronchial allergen challenge (n=23). Expression levels are log2 transformed, shown are median values (horizontal), interquartile ranges (boxes), and 1.5 IQR (whiskers). The printed fold changes (FC) are for the non-log2-transformed expression values to aid in interpretation of the effect sizes.

Figure 1

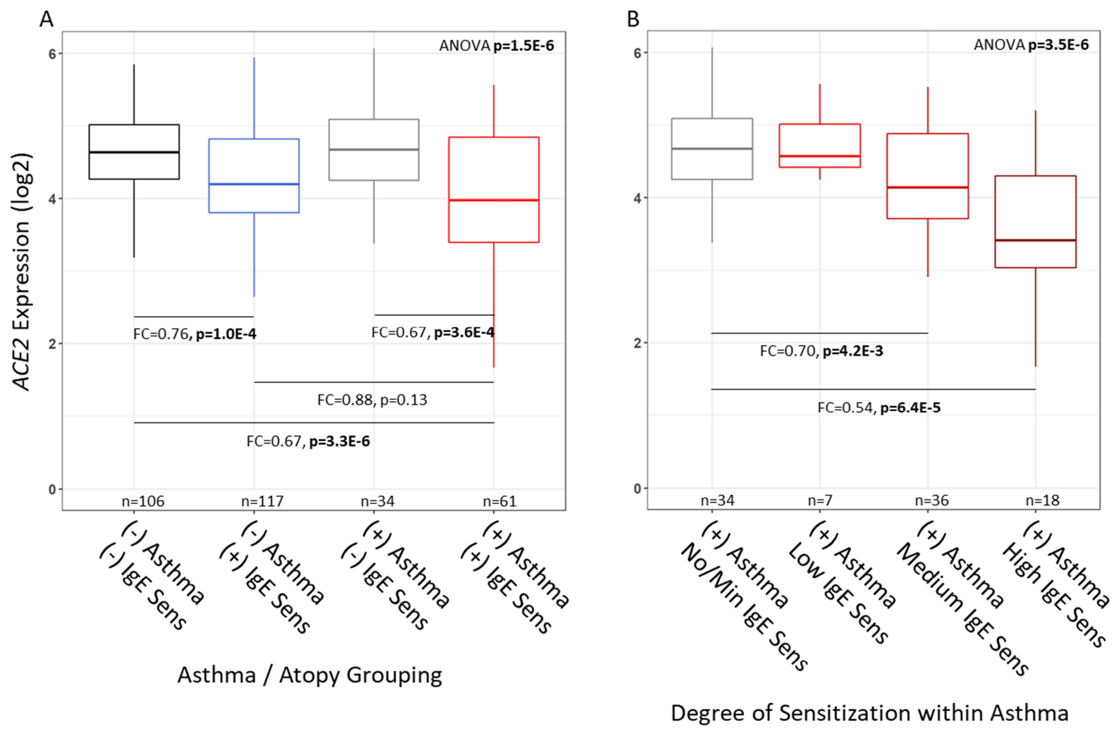
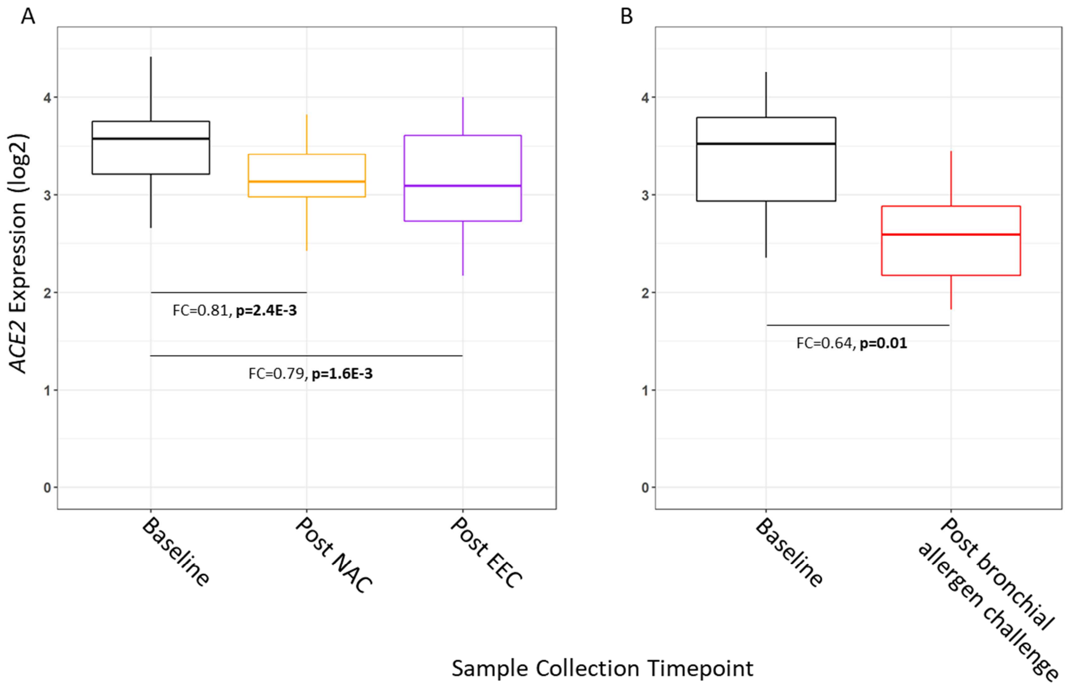

A
ANOVA p=1.5E-6
ACE2 Expression (log2)
FC=0.76, p=1.0E-4
FC=0.67, p=3.6E-4
FC=0.88, p=0.13
FC=0.67, p=3.3E-6
n=106
n=117
n=34
n=61
(-) Asthma (-) IgE Sens
(-) Asthma (+) IgE Sens
(+) Asthma (-) IgE Sens
(+) Asthma (+) IgE Sens
Asthma / Atopy Grouping
B
ANOVA p=3.5E-6
FC=0.70, p=4.2E-3
FC=0.54, p=6.4E-5
n=34
n=7
n=36
n=18
(+) Asthma No/Min IgE Sens
(+) Asthma Low IgE Sens
(+) Asthma Medium IgE Sens
(+) Asthma High IgE Sens
Degree of Sensitization within Asthma

Figure 2


A
B
ACE2 Expression (log2)
0
1
2
3
4
FC=0.81, p=2.4E-3
FC=0.79, p=1.6E-3
Baseline
Post NAC
Post EEC
FC=0.64, p=0.01
Baseline
Post bronchial allergen challenge
Sample Collection Timepoint

Supplementary Methods:

In all three studies, total RNA was extracted from epithelial brush samples preserved in RLT buffer (Qiagen, MD, USA). Samples were thawed, vortexed, and quick-spun, and the supernatant transferred to fresh tubes. The samples were then spun through a Qiashredder column (Qiagen) and extracted using RNeasy mini kits (Qiagen) with 25 ul elution volumes following the manufacturer's protocol. In the cat allergy upper airway challenge study, sequencing libraries were constructed from total RNA using TruSeq RNA Sample Preparation Kits v2 (Illumina). In the URECA and adult asthma studies, sequencing libraries were constructed from total RNA using SMART-Seq v4 Ultra Low Input RNA Kit (Takara). For each study, libraries were clustered onto a flowcell using a cBOT amplification system with a HiSeq SR v4 Cluster Kit (Illumina). Single-read sequencing was carried out on a HiSeq2500 sequencer (Illumina), using a HiSeq SBS v4 Kit to generate 58-base reads, with a target of approximately 10 million reads per sample. Sample for each study was processed and sequenced independently.

Reads were processed using workflows managed on the Galaxy platform. Reads were trimmed by 1 base at the 3' end, and then trimmed from both ends until base calls had a minimum quality score of at least 30 (Galaxy FASTQ Trimmer tool v1.0.0). FastqMcf (v1.1.2) was used to remove any remaining adapter sequence. To align the trimmed reads, we used the STAR aligner with the GRCh38 reference genome and gene annotations from ensembl release 91. Gene counts were generated using HTSeq-count (v0.4.1). For quality control, samples were kept that had counts >1 million, percent of reads aligned >80% and median CV coverage <1. Genes were filtered to include those that had a trimmed mean of M values (TMM) normalization count of at least 1 in at least 10% of libraries and were classified as protein coding using BioMart(1). Counts were transformed to log2 counts per million along with observations level weights using voomWithQualityWeights from the limma R package(2) to create a weighted gene expression matrix suitable for downstream analyses.

Differential expression of *ACE2* was assessed independently in each dataset using a weighted linear mixed effects model (limma) appropriate for RNA-seq data and empirical Bayes method(2, 3). Mixed-effects linear regression models were used including relevant clinical or technical variables (for URECA, cytologically determined cell percentages in the brush and the clinical site; for the upper airway challenge study, processing batch; for the adult asthma study no fixed effects were included) and a random effect of participant in both of the airway challenge studies. p-values <0.05 were considered statistically significant.

We searched NCBI's Gene Expression Omnibus for the terms "IL13" and "epithelial" subset to organism *homo sapiens.(4)* From this we identified two studies investigating the effects of IL-13 stimulation on human airway epithelial cells grown at air liquid interface that had repeated measures in the IL-13 stimulation and unstimulated groups. GSE110799 has the study design: "Human nasal epithelial cells isolated from nasal turbinates were cultured in air-liquid interface (ALI) until the full differentiation was complete. Differentiated cells at ALI-D47 were incubated with 100 ng/mL of IL-13 for 3 days.” GSE37693 has the study design: “RNA was isolated from primary culture airway epithelial cells grown at air-liquid interface, treated with or without IL-13 for 21 days”.(5) Differential expression analysis was performed using GEO2R, which performs voom and limma(2, 3) in the NCBI GEO browser.

Supplementary Table 1: Association of T2 biomarkers & nasal brush *ACE2* Expression in the URECA cohort.

Biomarker	**Association with *ACE2* Expression (β coefficient)**	**(p-value)**
# of positive allergen-specific IgE	-0.089	3.1E-5
Total IgE	-0.31	5.1E-6
Fractional exhaled nitric oxide (FeNO)	-0.45	3.4E-3
Blood eosinophils	-0.13	0.07
Nasal epithelial *IL13* expression	-0.123	8.6E-5

Supplementary Figure 1. IL-13 stimulation decreases *ACE2* expression in nasal and bronchial epithelium.
IL-13 stimulation of airway epithelial cells grown in an air liquid interface decreased *ACE2* expression in **(A)** nasal epithelium (FC=0.44, p-value=5.8E-4; n=2 per condition) and **(B)** bronchial epithelium (FC=0.80, p-value=5.1E-3; n=4 per condition). Shown are mean expression levels (red) and individual points representing biological replicates.

Supplementary References

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Supplementary Figure 1