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Type 2 Immunity and Age Modify Gene Expression of COVID19 Receptors in Eosinophilic Gastrointestinal Disorders

Austin W.T. **Chiang** Ph.D.[1**] ,Loan D. **Duong**, B.S.[2**], , Tetsuo **Shoda**, M.D., Ph.D.[3], Quan M. **Nhu**, M.D., Ph.D.[1,4], Melanie **Ruffner**, M.D., Ph.D.[5], Takeo **Hara**, M.D., Ph.D.[5], Bailey **Aaron**, B.S.[5], Erik **Joplin**, B.S.[2], Mario **Manresa**, Ph.D.[1], J. Pablo **Abonia**, M.D.[3], Evan **Dellon** M.D., M.P.H.[6], Ikuo **Hirano**, M.D.[7], Nirmala **Gonsalves**, M.D.[7], Sandeep **Gupta** M.D.[8], Glenn **Furuta**, M.D.[9], Marc E. **Rothenberg**, M.D., Ph.D.[3], Nathan E. **Lewis**, Ph.D.[2], Amanda B. **Muir***, M.D.[5], Seema S. **Aceves**, M.D., Ph.D.[1,10,*#] and the CEGIR Investigator Group[11]

***Co-senior authors**
****Co-first authors**
#Corresponding author

[1] Department of Bioengineering, Department of Pediatrics, University of California, [2]Division of Allergy, Immunology, Department of Pediatrics, University of California, San Diego, Rady Children's Hospital, San Diego, [3]Division of Allergy, Immunology, Department of Pediatrics, Cincinnati Children's Hospital Medical Center, University of Cincinnati, [4]Division of Gastroenterology and Hepatology, Scripps Clinic, [5]Department of Pediatrics, Division of Gastroenterology, Children's Hospital of Philadelphia, [6]Center for Esophageal Diseases and Swallowing, Division of Gastroenterology and Hepatology, University of North Carolina School of Medicine, Chapel Hill, NC, [7]Division of Gastroenterology, Department of Medicine, Feinberg School of Medicine, Northwestern University, Division of Gastroenterology, Indiana University School of Medicine, Riley Hospital for Children, [9]Digestive Health Institute, Section of Pediatric Gastroenterology, Hepatology and Nutrition, Children's Hospital Colorado, Gastrointestinal Eosinophilic Disease Program, Mucosal Inflammation Program, University of Colorado School of Medicine, Aurora, CO, [10]Division of Allergy, Immunology, Department of Medicine, University of California, San Diego, [11]Appendix 1

***Correspondence:**
Professor Seema S. Aceves, MD, PhD
Division of Allergy Immunology
University of California San Diego,
9500 Gilman Drive MC-0760
La Jolla, CA 92093
858-966-5961 (phone); 858-966-6360 (fax); saceves@health.ucsd.edu
Amanda Muir, MD

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Division of Gastroenterology, Department of Pediatrics
3615 Civic Center Blvd
Philadelphia, PA 19104
(267) 426-7223 (phone); (267) 426-7814 (fax); muira@email.chop.edu

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Abstract
Infection with SARS-CoV-2 can lead to COVID-19. The gastrointestinal tract is now an appreciated portal of infection. SARS-CoV-2 enters host cells via angiotensin converting enzyme-2 (ACE2) and the serine protease TMPRSS2. Eosinophilic gastrointestinal disorders are inflammatory conditions caused by chronic type 2 (T2) inflammation. However, the effects of the T2 atopic inflammatory milieu on SARS-COV-2 viral entry gene expression in the GI tract is poorly understood.
We analyzed tissue ACE2 and TMPRSS2 gene expression in pediatric eosinophilic esophagitis (EoE), eosinophilic gastritis (EG) and in normal adult esophagi using publicly available RNA sequencing datasets. Similar to findings evaluating the airway, there was no difference in tissue ACE2/TMPRSS2 expression in EoE or EG when compared to control non-EoE/EG esophagus/stomach. ACE2 gene expression was significantly lower in esophagi from children with or without EoE and from adults with EoE as compared to normal adult esophagi. Type 2 immunity and pediatric age could be protective for infection by SARS-CoV-2 in the gastrointestinal tract due to decreased expression of ACE2.

Key words: ACE2; atopy; esophagus; gastrointestinal; inflammation; Renin angiotensin system; SARS-CoV-2; COVID-19; Pediatric

What is known

- Coronavirus induced disease-2019 (COVID-19) has rapidly spread around the world, infecting millions of people and causing both respiratory and gastrointestinal symptoms.
- ACE2 and TMPRSS2 are the major portals of entry that allow SAR-CoV2 entry at epithelial barriers.
- The gastrointestinal tract is a potential nidus of SARS-CoV-2 infection with high levels of ACE2 expression in the esophagus reported.

What is new

- Normal adult esophagi have higher expression of ACE2 compared to adults with EoE.
- Children with and without EoE have lower levels of esopahgeal ACE2 gene expression compared with normal adult esophagi.
- Adults and children with EoE express less TMPRSS2 compared to non-EoE adults.

Abbreviations:

ACE1, 2: angiotensin converting enzyme-1, -2
AGT: angiotensinogen
AGTR1, 2: angiotensin receptor-1, -2
ANG: angiotensinogen
COVID-19: coronavirus induced disease-2019
DEG: differentially expressed gene
ds: double stranded
EG: eosinophilic gastritis
EGID: eosinophilic gastrointestinal disorder
EoE: eosinophilic esophagitis
GI: gastrointestinal
R-A system: renin angiotensin system
REN: renin
RNAseq: RNA sequencing
SARS-CoV-2: severe acute respiratory syndrome coronavirus-2
ss: single stranded
TMPRSS2: transmembrane serine protease 2
TPM: transcripts per million
FPKM: fragments per kilobase of exon model per million reads mapped

Introduction

Since December 2019, a fast-moving global pandemic, coronavirus induced disease-2019 (COVID-19), has ensued with over 1 million deaths worldwide.[1] Severe acute respiratory syndrome coronavirus-2 (SARS-CoV-2) enters host cells via ACE2, a member of the renin angiotensin system (R-A system), and the serine protease, TMPRSS2.[2] New evidence demonstrates that SARS-CoV-2 can infect and be harbored in the gastrointestinal (GI) tract.[3]

While T2 inflammatory atopic diseases are common, they do not necessarily associate with worsening COVID-19 risk. [4,5] Jackson et al demonstrated that ACE2 expression in nasal airway epithelium was significantly decreased in atopic asthmatics compared to non-atopic asthmatics. Children also are relatively protected, with substantially lower morbidity and mortality rates from COVID-19. [6,7] Eosinophilic gastrointestinal disorders, especially EoE and EG, are increasing in prevalence and caused by T2 bowel inflammation. The current clinical data demonstrate that patients with EGIDs do not have worse COVID-19 and, despite the ability of SARSCoV-2 to infect the esophagus[3], there are no reports of COVID-19 infection in EoE. It is not currently clear if T2 inflammation associated with EGIDs could decrease the ability of SARSCoV-2 to infect the esophagus, stomach or bowel. However, given the increasing prevalence of EGIDs, this is a salient question.[8]

While ACE2 classically works in the renin-angiotensin (R-A) system to regulate blood pressure, it is also an important mediator of inflammation (Figure 1A).[9] The differential expression of ACE2 and the R-A system by age, organ system, and in the presence of inflammation has become of heightened interest due to the COVID-19 pandemic. ACE1 conversion of angiotensinogen to angiotensin 2 and its subsequent binding to angiotensin receptors-1 and -2 (AGTR1, 2) leads to inflammation. ACE2 mediated conversion of angiotensin 2 to angiotensin 1-7 results in anti-inflammatory effects. For this reason, we aimed to understand the expression of not only ACE2 but also R-A system genes in the tissue of EoE and EG patients as compared to non-inflamed control subjects. We further evaluated TMPRSS2 expression in these patient populations since spike protein cleavage by TMPRSS2 is required for ACE2 mediated cell entry of SARSCoV-2[2].

Herein, we study the influence of age and EoE or EG disease state on ACE-2, TMPRSS-2, and R-A system gene expression using RNA sequencing data from patients who were, in part, from the multicenter Consortium for Eosinophilic Gastrointestinal Research (CEGIR). We hypothesize that the pediatric esophagus may be less vulnerable than the adult esophagus to infection by SARSCoV-2 and that T2 inflammation associated with EGIDs can influence esophageal ACE-2 and TMPRSS2 expression.

Methods

Datasets

Publicly available RNA-Seq data were used for esophageal biopsy specimens of children with EoE (n=7) or control subjects (n=6) (GSE58640), gastric biopsy specimens from patients with eosinophilic gastritis (EG) (n=9) or control (n=12) subjects (EGID express: https://egidexpress.research. cchmc.org/data/). Patients who were designated as

controls had no pathologic findings on their endoscopies. The available RNA-Seq data were stored in different formats such as read count, TPM, and FPKM. This hinders the integrative analysis of the RNA-Seq data. To ensure fair comparisons across different datasets, we converted RNA-Seq count data (GSE58640) to transcripts per million (TPM). Eight total genes were analyzed in the RNAseq dataset. The esophagus of normal adults transcriptomic data were downloaded from GTEx Portal (https://gtexportal.org/home/datasets). Specifically, the gene read counts of the RNA-Seq GTEx version 8 data set (GTEx_Analysis_2017-06-05_v8_RNASeQCv1.1.9_gene_tpm.gct.gz) were downloaded. The sample annotations were also downloaded from the GTEx Portal (https://gtexportal.org/home/histologyPage) by selecting the Tissue = "Esophagus - Mucosa". Data was further selected to include normal esophageal mucosa with no histologic abnormalities from people ages 20-59 (n=184).

Atopy was defined as in the original publications.[10,11] In the EG and EoE cohorts, atopy was defined as the presence of asthma, allergic rhinitis, atopic dermatitis, and/or food allergy.

Quantification

For the raw data of esophageal biopsy specimens of patients with EoE or control subjects (GSE58640), RNA-Seq quality was assessed using *FastQC*. Adapter sequences and low-quality bases were trimmed using *Trimmomatic*. Sequence alignment was performed using *STAR* against the Human genome (GRCH.38; GCF_000001405.38) with the default parameters. The expression of each gene was quantified using *HTSeq* and converted the count data to transcripts per million (TPM) for further analysis.

Statistics

Differential gene expression was performed between different pairwise disease states comparisons for the 8 genes (ACE, ACE2, AGT, AGTR1, AGTR2, MAS1, REN, and TMPRSS2) of the renin angiotensin pathway using the limma package in R[12]. in which the p values were derived for all genes. The p values were further adjusted to multiple testing for each gene using false discovery rate (FDR) method by the Benjamini-Hochberg[13]. Genes with an FDR adjusted p value < 0.05 were defined as differential expressed genes. For the comparisons of ACE2 and TMPRSS2 genes across different conditions, we applied the Wilcoxon rank-sum test on their gene expressions (TPM) in the studied conditions. Genes were considered as statistically significant differential expression at $p < 0.05$.

Results

RNA sequencing data from children with EoE (mean age=6 years, range 3-10 years old) or non-inflamed control esophagus (mean age=13 years, range 2-17 years old) and EG patients (mean age=16 years, range 7-31 years old) or non-inflamed control stomach (mean age=15 years, range 8-28 years old) was compared for R-A system and TMPRSS2 gene expression. Comparative transcriptomics of active EoE and EG tissue demonstrated that EoE and EG patients had similar ACE2 and TMPRSS2 expression when compared with non-inflamed esophagi or stomach from control subjects (Figure 1B,C). Of the R-A system genes, REN expression was significantly lower in patients with active EoE (Figure 1B). Within the R-A system, AGTR1 and REN were differentially expressed and downregulated in active EG

as compared to non-inflamed stomach from control subjects (Figure 1C). On single gene pairwise comparison, EoE patients with concurrent atopy (n=8) had significantly lower esophageal expression of TMPRSS2 as compared with non-atopic EoE patients (n=2) ($p<.05$) however this was not significant using multiple gene comparisons, likely due to the small sample size (Figure 1D).

COVID-19 is a milder disease in children, perhaps due to differences in the expression level of SARS-CoV-2 entry proteins.[7] Therefore, we evaluated expression levels of viral receptors in the context of age. Since control patients with non-inflamed esophagi have symptoms that warrant endoscopy and since our dataset contained only pediatric control subjects, we utilized RNA sequence data from normal adult esophagi. Normal pediatric samples are not available in this database. We compared the esophageal expression of ACE2 and TMPRSS2 in normal adults as compared to non-EoE children. ACE2 was expressed at significantly higher levels in the adult normal, as compared with the pediatric control, esophagus (Figure 2A).

To assess the expression of ACE2 and TMPRSS2 in the context T2 eosinophilic inflammation as compared to a histologically normal esophagus, we compared ACE2 and TMPRSS2 gene expression in the esophagus of normal and EoE adults. We also compared the expression level of ACE2 and TMPRSS2 in children with EoE to that in the normal adult esophagus. ACE2 was expressed at significantly higher levels in the esophagus of normal adults as compared with adult or pediatric EoE patients (Figure 2B and C). Interestingly, esophageal specimens from both adult and pediatric EoE subjects also expressed significantly less TMPRSS2 as compared to normal adults (Figure 2B and C). Together, these data support the hypothesis that the esophagus from children may be relatively protected from SARS-CoV-2 GI infectivity due to lower ACE2 expression and that esophageal Th2 type inflammation in children or adults may be particularly protective for infection.

Discussion

While the respiratory tract has been considered the main nidus for viral entry, the GI tract may also be a primary entry portal for COVID-19.[14] Both the ileum and esophagus are considered "high risk" for SARS-CoV-2 infection based on the level of ACE2 transcript. [15,16] Herein, we provide the first analysis the expression of ACE2, R-A system and TMRPSS2 genes in EoE and EG versus control esophagi and stomach, using RNAseq data. We have found that age and eosinophilic T2 inflammation ~~both~~ influence the expression of the SARS-COV-2 viral genes required for viral entry.

These results should be evaluated in organ specific and atopic specific contexts. While Radzikowksa et al found no difference in ACE2 expression between bronchial biopsies of normal and asthmatic patients, when looked at in the context of the atopic state, Jackson et al found lower levels of expression of ACE2 in allergic asthmatic adults and the negative correlation of ACE2 expression with IL-13 and IgE levels[4,17]. Similarly, we show here that pediatric EoE subjects had no overall differences in ACE2/TMPRSS2 expression compared to non-inflamed control esophagi but that adult and pediatric EoE patients had significantly lower ACE2 and TMPRSS2 expression compared to normal tissue. It is possible that the distinct phenotypes of EoE[18] and asthma may also lead to differential risk of infection.

The most serious consequence of COVID19 is severe life-threatening pulmonary edema and acute respiratory distress syndrome (ARDS). During ARDS type lung injury, the R-A system acts to increase capillary permeability contributing to pulmonary edema. ACE2, on the other hand, acts to negatively regulate the effects of the classical R-A system and induces an anti-inflammatory effects.[19] While ACE2 is seemingly protective against severe ARDS in the setting of lung injury, it is also the gateway by which SARS-CoV-2 enters the mucosal surface.

We acknowledge that a limitation of this work is that it evaluates only RNA expression. Future work should evaluate ACE2/TMPRSS2 protein-level expression in these populations as well as the severity and acquisition rate of SARS-CoV2 in EGID patients across ages. While we have tried to control for differing methodologies across studies, a direct comparison of pediatric and adult gastrointestinal tissue would provide superior results. In addition, our EGID sample sizes were small and normal tissue RNA sequence data was available only in adults.

In conclusion, we have demonstrated the ACE2/TMPRSS2 has limited gene expression in the GI tract and provided potential mechanisms whereby young age and EoE-associated Th2 inflammation could confer advantages for lower infection rates from COVID19 by decreasing the expression of its receptor and protease. These insights may be of utility when designing novel SARS-CoV-2 therapies and understanding the clinical course and risks of COVID19.

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References

1. Guan W-J, Ni Z-Y, Hu Y, et al. Clinical Characteristics of Coronavirus Disease 2019 in China. *N Engl J Med*. 2020;382(18):1708-1720

2. Hoffmann M, Kleine-Weber H, Schroeder S, et al. SARS-CoV-2 Cell Entry Depends on ACE2 and TMPRSS2 and Is Blocked by a Clinically Proven Protease Inhibitor. *Cell*. 2020;181(2):271-280.e8.

3. Ng SC, Tilg H. COVID-19 and the gastrointestinal tract: more than meets the eye. *Gut*. 2020;69(6):973-974.

4. Jackson DJ, Busse WW, Bacharier LB, et al. Association of respiratory allergy, asthma, and expression of the SARS-CoV-2 receptor ACE2. *J Allergy Clin Immunol* 2020;146(1):203-206.e3

5. Savarino E, Lorenzon G, Ghisa M, et al. Lack of complications in patients with eosinophilic gastrointestinal diseases during SARS-CoV-2 outbreak. *J Allergy Clin Immunol Pract*. 2020;8(8):2790-2792.e2791.

6. Cruz AT, Zeichner SL. COVID-19 in Children: Initial Characterization of the Pediatric Disease. *Pediatrics*. 2020;145(6):e20200834

7. Lingappan K, Karmouty-Quintana H, Davies J, Akkanti B, Harting MT. Understanding the age divide in COVID-19: why are children overwhelmingly spared? *Am J Physiol Lung Cell Mol Physiol*. 2020;319(1):L39-L44.

8. Gonsalves N. Eosinophilic Gastrointestinal Disorders. *Clin Rev Allergy Immunol*. 2019;57(2):272-285.

9. Gaddam RR, Chambers S, Bhatia M. ACE and ACE2 in inflammation: a tale of two enzymes. *Inflamm Allergy Drug Targets*. 2014;13(4):224-234.

10. Sherrill JD, Kiran KC, Blanchard C, et al. Analysis and expansion of the eosinophilic esophagitis transcriptome by RNA sequencing. *Genes Immun*. 2014;15(6):361-369.

11. Shoda T, Wen T, Caldwell JM, et al. Molecular, endoscopic, histologic, and circulating biomarker-based diagnosis of eosinophilic gastritis: Multi-site study. *J Allergy Clin Immunol*. 2020;145(1):255-269.

12. Ritchie ME, Phipson B, Wu D, et al. limma powers differential expression analyses for RNA-sequencing and microarray studies. *Nucleic Acids Res*. 2015;43(7):e47-e47.

13. Benjamini Y, Hochberg Y. Controlling the false discovery rate: a practical and powerful approach to multiple testing. *J Royal Stat Soc Ser*. 57(1):289-300.

14. Lin L, Jiang X, Zhang Z, et al. Gastrointestinal symptoms of 95 cases with SARS-CoV-2 infection. *Gut*. 2020;69(6):997-1001.

15. Zou X, Chen K, Zou J, Han P, Hao J, Han Z. Single-cell RNA-seq data analysis on the receptor ACE2 expression reveals the potential risk of different human organs vulnerable to 2019-nCoV infection. *Front Med*. 2020;14(2):185-192.

16. Deinhardt-Emmer S, Wittschieber D, Sanft J, et al. Early postmortem mapping of

SARS-CoV-2 RNA in patients with COVID-19 and correlation to tissue damage. *bioRxiv*. 2020;395:2020.07.01.182550.

17. Radzikowska U, Ding M, Tan G, et al. Distribution of ACE2, CD147, CD26, and other SARS-CoV-2 associated molecules in tissues and immune cells in health and in asthma, COPD, obesity, hypertension, and COVID-19 risk factors. *Allergy*. 2020;2000688(360):e3325.

18. Atkins D, Furuta GT, Liacouras CA, Spergel JM. Eosinophilic esophagitis phenotypes: Ready for prime time? *Pediatr Allergy Immunol*. 2017;28(4):312-319.

19. Jia HP, Look DC, Shi L, et al. ACE2 receptor expression and severe acute respiratory syndrome coronavirus infection depend on differentiation of human airway epithelia. *J Virol*. 2005;79(23):14614-14621.

Figure 1. Expression of R-A system genes and in EGIDs. (A) EGIDs and associated Th2 inflammation as well as age effect on TMPRSS2/ACE2 expression. ACE1 (ACE) conversion of angiotensin I to angiotensin II causes inflammation and hypertension. ACE2 cleavage of angiotensin II to angiotensin 1-7 and binding to Mas receptor (MAS1) decreases blood pressure lowering and inflammation. Angiotensinogen (AGT) is cleaved by renin (REN) and non-renin EoE-associated proteases, chymase (CMA1) and kallikrein 1 (KLK1) (dashed line). (**B-D).** R-A system and TMPRSS2 transcriptomes in EoE (B) and EG (C) biopsies. (TPM: transcripts per million)

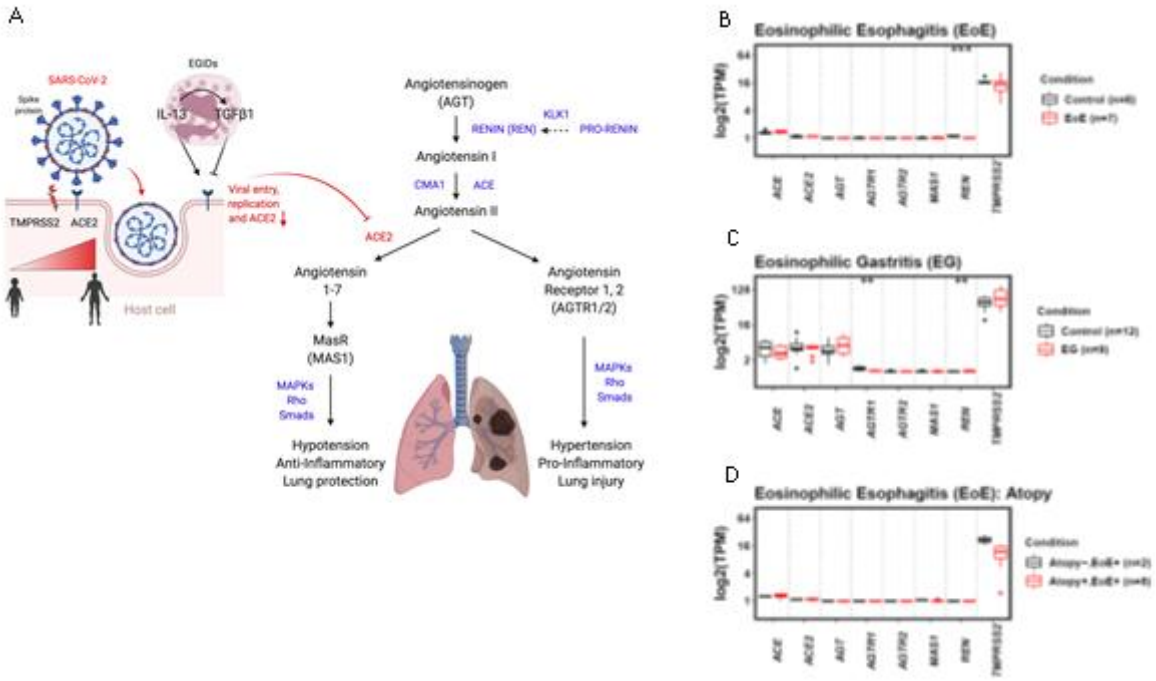

Figure 2: Age and EoE status effect ACE2/TMPRSS2 gene expression. Expression of SARS-CoV2 receptors in (A) normal adult versus pediatric non-inflamed controls, (B) normal adult vs. pediatric EoE, and (C) normal adult vs. adult EoE.