Letters

RESEARCH LETTER

Nasal Gene Expression of Angiotensin-Converting Enzyme 2 in Children and Adults

Children account for less than 2% of identified cases of coronavirus disease 2019 (COVID-19).[1,2] It is hypothesized that the lower risk among children is due to differential expression of angiotensin-converting enzyme 2 (ACE2),[3] the receptor that severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) uses for host entry.[4] We investigated *ACE2* gene expression in the nasal epithelium of children and adults.

Editorial

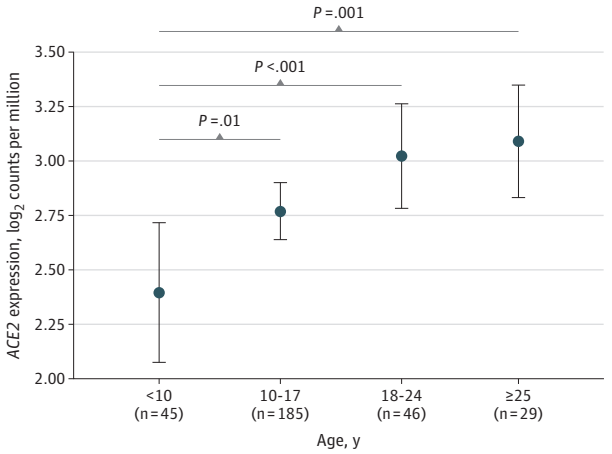
Methods | We conducted a retrospective examination of nasal epithelium from individuals aged 4 to 60 years encountered within the Mount Sinai Health System, New York, New York, during 2015-2018. Samples were collected from individuals with and without asthma for research on nasal biomarkers of asthma. The study was approved by the Mount Sinai institutional review board. Written informed consent was obtained from participants (or their parents for minors). Nasal epithelium was collected using a cytology brush that was immediately placed in RNA stabilization fluid and stored at −80 °C. RNA was isolated within 6 months. RNA samples were checked for quality and sequenced as a single batch in 2018. Sequence data processing included sequence alignment and normalization of gene expression counts across genes and samples.

Given the role of ACE2 in SARS-CoV-2 host entry,[4] *ACE2* gene expression was the focus of this study. Linear regression models with and without adjustment for covariates (sex and asthma) were built with *ACE2* gene expression in $\log_2$ counts per million as the dependent variable and age group as the independent variable using R software, version 3.6.0 (R Foundation). Age was categorized into the following groups reflecting developmental life stages: younger children (aged <10 years), older children (aged 10-17 years), young adults (aged 18-24 years), and adults (aged ≥25 years). Two-sided tests and a significance threshold of $P \leq .05$ were used. Trend pattern was evaluated using polynomial orthogonal contrasts.

Results | The cohort of 305 individuals aged 4 to 60 years was balanced with regard to sex (48.9% male). Because the cohort had been recruited to study biomarkers of asthma, 49.8% had asthma.

We found age-dependent *ACE2* gene expression in nasal epithelium (**Figure**). *ACE2* gene expression was lowest (mean $\log_2$ counts per million, 2.40; 95% CI, 2.07-2.72) in younger children (n = 45) and increased with age, with mean $\log_2$ counts per million of 2.77 (95% CI, 2.64-2.90) for older children (n = 185), 3.02 (95% CI, 2.78-3.26) for young adults (n = 46), and 3.09 (95% CI, 2.83-3.35) for adults (n = 29).

Figure. Nasal Gene Expression of *ACE2* in Different Age Groups



Data are means (data points) and 95% confidence intervals (error bars) for angiotensin-converting enzyme 2 (*ACE2*) gene expression in younger children (aged <10 years), older children (aged 10-17 years), young adults (aged 18-24 years), and adults (aged ≥25 years). Gene counts are shown as logarithmic ($\log_2$) counts per million. *P* values are from linear regression modeling in which *ACE2* gene expression in $\log_2$ counts per million was the dependent variable and age group was the independent variable.

Table. β Coefficients for Age Group From Unadjusted and Adjusted Linear Regression Models[a]

	β Coefficient (95% CI)[c]	
Age group, y[b]	Unadjusted model	Adjusted model[d]
10-17	0.37 (0.08-0.67)	0.30 (0.01-0.59)
18-24	0.63 (0.26-1.00)	0.49 (0.13-0.86)
≥25	0.69 (0.27-1.11)	0.52 (0.09-0.94)

[a] Angiotensin-converting enzyme 2 gene expression in $\log_2$ counts per million was the dependent variable and age group was the independent variable.

[b] Children younger than 10 years were the reference age group.

[c] β Coefficients indicate the difference in *ACE2* gene expression (in $\log_2$ counts per million) between a given age group and the group of children younger than 10 years.

[d] Adjusted for sex and asthma.

Linear regression with *ACE2* gene expression as the dependent variable and age group as the independent variable showed that compared with younger children, *ACE2* gene expression was significantly higher in older children ($P = .01$), young adults ($P < .001$), and adults ($P = .001$) (Figure). As the distributions of sex and asthma varied among the age groups, a linear regression model adjusted for sex and asthma was built that also showed significant adjusted associations ($P \leq .05$) between *ACE2* expression and age group. Regression (β) coefficients for age groups from the unadjusted and adjusted models are shown in the **Table**. These regression coefficients indicate the difference in *ACE2* expression (in $\log_2$ counts per million) between a given age group and the group of children

younger than 10 years. Tests for trend using polynomial orthogonal contrasts indicated a significant linear trend for change in *ACE2* expression with advancing age group ($P \leq .05$).

Discussion | The results from this study show age-dependent expression of *ACE2* in nasal epithelium, the first point of contact for SARS-CoV-2 and the human body. Covariate-adjusted models showed that the positive association between *ACE2* gene expression and age was independent of sex and asthma. Lower *ACE2* expression in children relative to adults may help explain why COVID-19 is less prevalent in children.[3] A limitation of this study is that the sample did not include individuals older than 60 years.

Few studies have examined the relationship between ACE2 in the airway and age. A study of bronchoalveolar lavage fluid from 92 patients with acute respiratory distress syndrome reported no association between ACE2 protein activity and age,[5] but epithelial gene expression was not examined, and ACE2 protein may be variably shed into bronchoalveolar lavage fluid. Furthermore, the lung and nasal environments are distinct, with known differences in gene expression.[6] This study provides novel results on *ACE2* gene expression in nasal epithelium and its relationship with age.

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Acquisition, analysis, or interpretation of data: Bunyavanich, Do.
Drafting of the manuscript: Bunyavanich.
Critical revision of the manuscript for important intellectual content: All authors.
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