Gendered differences in relative ACE2 expression in the nasal epithelium*

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To the Editor:

There is a well-established relationship between gender and olfactory dysfunction (OD). A highly referenced 1991 study of patients presenting to a smell and taste center found that female patients (34.8%) more frequently complained of dysosmia than males (28.9%)[1]. More specifically, a recent meta-analysis of 23 studies including anosmia and dysgeusia in COVID-19 patients consistently found among all included studies that COVID-19 anosmia is more common in females than males[2]. The aim of this study was to assess whether a gendered difference in relative expression of ACE2 (angiotensin-converting enzyme 2) receptors in the nasal epithelium is a source of this difference in COVID-19-related olfactory loss.

Studies published near the beginning of the COVID-19 outbreak established that the ACE2 receptor is the receptor for SARS-CoV-2, with the prospect of using this information to treat COVID-19 infection[3]. Given its effect on SARS-CoV-2 and related viruses, the relative expression of ACE2 in the nasal epithelium between women and men may serve as a partial explanation for the difference in OD.

We aimed to ascertain the gendered difference in relative ACE2 expression in the nasal epithelium as a partial explanation for the variance in post-viral olfactory loss between female and male patients. We hypothesized that, given ACE2's involvement in COVID-19 and its presence in non-neuronal olfactory cells, healthy women may express greater levels of ACE2 than men, making them more susceptible to post-COVID-19 anosmia.

Samples from nasal epithelium were obtained from 36 subjects (23 female, 13 male) ranging in age from 21-36 years old. Nasal endoscopy was performed to assess the condition of the subjects' sinuses and nasal passages. Nasal swabs of the test subjects were obtained from the antero-medial surface of the cranial portion of the middle turbinate of the nostril that the participant reported as less obstructed, where olfactory receptor neurons have been shown to be present[4]. Relative mRNA ACE2 expression levels in relation to two reference genes (peptidyl-prolyl isomerase A [PPIA] and TATA-box binding protein [TBP]) in the nasal samples were calculated by the $2^{-\Delta\Delta CT}$ method and used for statistical comparison between male and female subjects by Student's t-test.

Our data provides significant evidence ($t = 2.69$; $p = 0.006$) that females express a 1.75-fold greater proportion of ACE2/PPIA mRNA in their nasal epithelium than males. For further validation, there is similarly strong evidence ($t = 3.01$; $p = 0.002$) of 1.73-fold greater relative expression of ACE2/TBP mRNA levels among females in comparison to males. Overall, this data indicates that females with typical olfactory function express relatively more ACE2 receptor mRNA (in comparison to reference genes) in their nasal epithelium than males (Figure 1).

The presence of ACE2 receptors on support cells in the nasal epithelium emphasizes the role of ACE2 in the clinical presentation of COVID-19 anosmia. Sustentacular cells, as well as other non-neuronal olfactory cells, express ACE2 at much higher levels than OSNs or olfactory bulb neurons[5, 6]. By infecting the sustentacular cells, SARS-CoV-2 could reduce the support system surrounding OSNs and negatively impact olfaction. COVID-19-related anosmia tends to be acute[7], which is consistent with the timeline of damage to supporting cells but not OSNs themselves. Thereby, increased expression of ACE2 receptors in the nasal cavity among women can explain the elevated levels of COVID-19-related OD in comparison to men.

Our results are potentially biased by the small sample size, particularly of males (13 males, 23 females). Moreover, sex-based differences in OD have been shown in upper respiratory infectious olfactory loss but not in numerous other common diagnoses[8]. Cumulatively, this suggests that the physiological explanation for the gendered difference in anosmia may be specific to post-viral anosmia. Further studies in post-viral anosmia and ACE2 receptors with larger sample sizes are warranted to validate this hypothesis. This could have implications for the treatment of post-viral anosmia and dysosmia.

When considered in tandem, increased expression of ACE2 receptor mRNA in the nasal epithelium (Figure 1) and increased

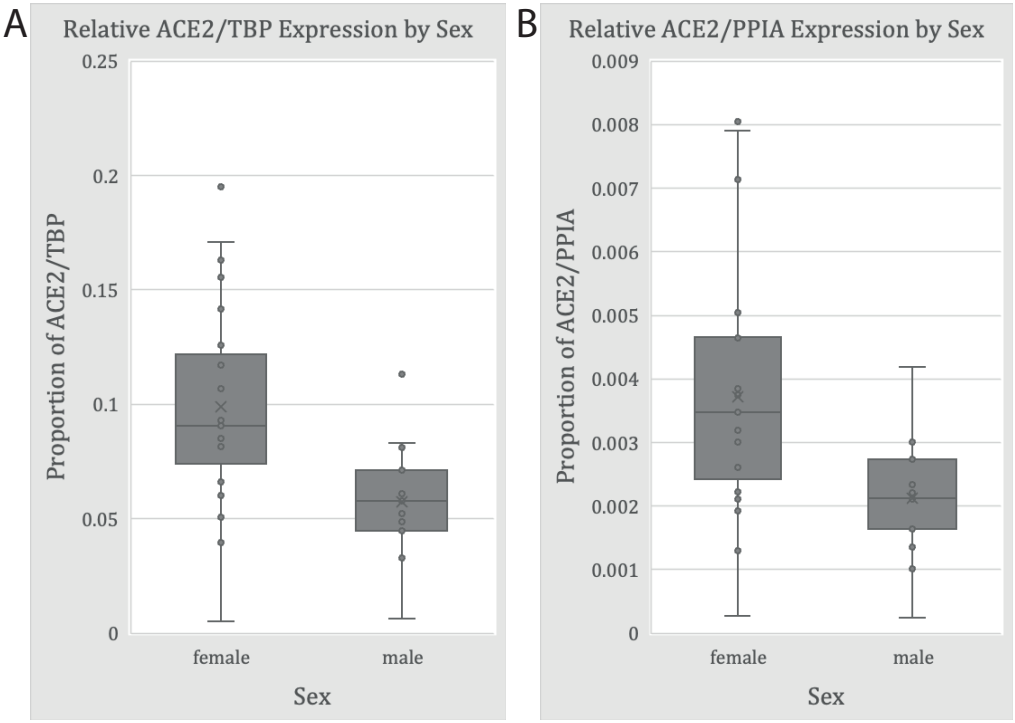

Figure 1. Box-and-whisker plots of ACE2 expression relative to reference genes (a) TBP and (b) PPIA by sex, with inlaid data points.

rates of acute post-COVID-19 OD in females[2] provides evidence that elevated relative ACE2 expression in females is the culprit for the sex difference in post-COVID-19 anosmia. Given conflicting evidence in studies of the same scale[9], these findings should be reinvestigated with a larger sample. Future studies should consider sampling from the olfactory cleft directly to increase confidence in their results of gene expression in the nasal epithelium.

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Authorship contribution

JHJ: data analysis, writing the manuscript, review of the manuscript; AJ: collection of data, review of the manuscript; SF: conceptualization, collection of data, data analysis, review of the manuscript; TH: conceptualization, collection of data, data analysis, review of the manuscript.

Conflict of interest

No conflicts of interest exists.

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Table 1. Average (± 1 SD) Identification and Threshold scores from "Sniffin' Sticks" tests performed on subjects, in comparison with normative scores for the corresponding age group from Oleszkiewicz et al. [10].

	Males	Females	Combined
Identification score (± standard deviation) vs. Normative for 21-30 y.o. [10]	14.15 (± 1.46) 13.62 (± 1.72) [10]	14.17 (± 1.34) 13.61 (± 1.97) [10]	14.17 (± 1.36) 13.62 (± 1.86) [10]
Threshold score (± standard deviation) vs. Normative for 21-30 y.o. [10]	7.71 (± 1.31) 9.11 (± 2.96) [10]	7.92 (± 1.51) 9.35 (± 3.00) [10]	7.85 (± 1.43) 9.25 (± 2.98) [10]

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This manuscript contains online supplementary material

SUPPLEMENTARY MATERIAL

Methods

Samples of nasal epithelium were obtained from 36 subjects (23 female, 13 male) ranging in age from 21-36 years old. Nasal endoscopy was performed to assess the condition of the subjects' sinuses and nasal passages. Nasal swabs of the test subjects by the use of FLOQBrushs (Copan Flock Technologies SRL, Brescia, Italy) were obtained from the middle turbinate of the nostril that the participant reported as clearer. mRNA expression levels of the target gene ACE2 and of two reference genes (peptidylprolyl isomerase A [PPIA] and TATA-box binding protein [TBP]) in the nasal epithelium samples were determined by quantitative PCR on a LightCycler 480 System (Roche Diagnostics, Basel, Switzerland). Relative ACE2 expression levels in relation to PPIA and TBP were calculated by the $2^{-\Delta\Delta CT}$ method and used for statistical comparison between male and female subjects by Student's t-test.

The tips of the brushes were stored in RNAlater (Thermo Fisher Scientific, Waltham, MA) at -20°C until further processing. Thawed brush tips were incubated in QIAzol Lysis Reagent (Qiagen, Hilden, Germany) for 30 min at 4°C. After being lysed, RNA from the nasal epithelium samples was isolated using the RNeasy Lipid Tissue Mini Kit (Qiagen). After assessment of RNA quantity and quality by spectrophotometric analysis and automated electrophoresis (2100 Bioanalyzer; Agilent) a total of 11 µl of the RNA solution was subjected to cDNA synthesis with SuperScript III Reverse Transcriptase (Thermo Fisher Scientific, Waltham, MA). This was followed by a preamplification step applying the TaqMan PreAmp Master Mix and gene-specific Taqman Gene Expression assays (ACE2: Hs00222343_m1, PPIA: Hs99999904_m1, TBP: Hs00427620_m1, all from Thermo Fisher Scientific).

All subjects were healthy, in the sense that they had not contracted COVID-19 and had typical olfactory function, which was assessed using the Threshold and Identification parts of the "Sniffin' Sticks" test [1]. As shown in Table 1, identification scores for the subjects ($\mu = 14.17$; $\sigma = 1.36$) were in-line with the normative scores for the corresponding age group [2]. (All of the test subjects were between 21-30 years old, except for one 36 y.o.) Threshold scores for the group ($\mu = 7.85$; $\sigma = 1.43$) were slightly lower than the normative values for the corresponding age groups, but still within one standard deviation. Both scores remain in-line with their respective groups when stratifying by sex [2]. None of the participants had active upper respiratory tract infection symptoms. Two participants had pollen allergies but were tested during the symptom-free period. This analysis helps validate that our results are not atypical of the general population, and thus that, as groups, our sample is representative of people with typical olfactory function.

Our findings could potentially be generalized to post-viral anosmia, not just in regard to COVID-19. ACE2 receptor was also previously linked with SARS-CoV-1, the virus responsible for the SARS outbreak of 2003[3]. Given recent research suggesting that other viruses such as the influenza A virus also have a relationship with ACE2 receptors[4], it is possible that variance in relative ACE2 receptor expression between males and females may explain differences in post-viral anosmia preceding COVID-19.

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