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# Higher pharyngeal epithelial gene expression of angiotensin-converting Enzyme-2 in patients with upper respiratory infection



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## ABSTRACT

We analyzed the expression of ACE2 in the pharyngeal epithelium and examined its relationship with clinical features and serological parameters in patients with upper respiratory infection (URI). The expression level of the ACE2 gene was significantly higher in patients with URI (n = 125) than in healthy control (HC) individuals (n = 52) ( $p < 0.0001$ ). The ACE2 gene expression level was significantly and positively correlated with age ( $r=0.1799$ ,  $p = 0.0447$ ) and body temperature ( $r=0.1927$ ,  $p = 0.0427$ ), which may help explain increasing coinfections with SARS-CoV-2 and other respiratory pathogens.

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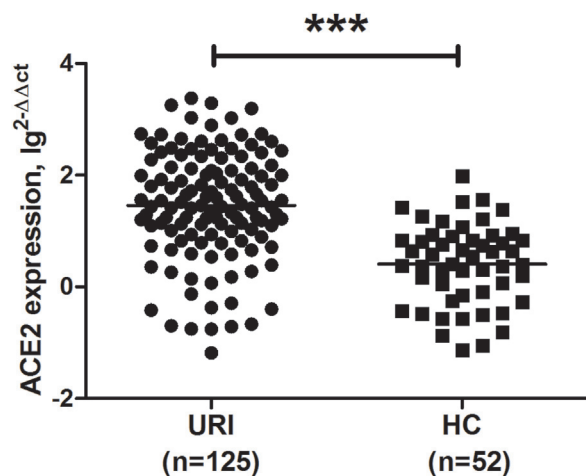
## Introduction

Coinfection with severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) and other respiratory pathogens has been reported recently (Khaddour et al., 2020). It is hypothesized that the higher risk of coinfection among patients with coronavirus disease 2019 (COVID-19) is due to the differential expression of angiotensin-converting enzyme 2 (ACE2), the receptor that SARS-CoV-2 uses for host entry. We analyzed ACE2 gene expression in the pharyngeal epithelium of patients with upper respiratory infection (URI) and healthy individuals.

## Methods

We conducted a retrospective examination of the pharyngeal epithelium from individuals treated at the Third Affiliated Hospital, Southern Medical University, China from March to May 2020. Samples were collected from 125 patients with URI and 52 age- and gender-matched healthy individuals with no infection; all participants tested negative for SARS-CoV-2. Written informed consent was obtained from all participants. Pharyngeal epithelium from throat swabs was collected and immediately

placed in RNA stabilization fluid. Total RNA was extracted from the pharyngeal epithelium and stored at  $-80^{\circ}\text{C}$ . Real-time transcription-polymerase chain reaction analysis (RT-PCR) was used to determine the expression of the ACE2 gene. The relative expression



**Figure 1. The increased levels of ACE2 gene in pharyngeal epithelium of patients with URI.** Data are means and 95% confidence intervals (error bars) for ACE2 gene expression in the pharyngeal epithelium of URI patients and HC individuals, \*\*\* ( $p < 0.0001$ ). Data were log-transformed by taking the base 10 logarithm to account for the skewness. After the above processing, the treated data were normal distributed and had constant variances.

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**Table 1**  
Association of ACE2 gene with clinical pathological parameters of patients with URI.

Clinical parameters	Data	Relative ACE2 mRNA expression	
	(Mean ± SD)	r	p
Age (years)	33.69 ± 15.88	0.1799	0.0447
Body temperature (°C)	37.69 ± 0.82	0.1927	0.0427
CRP <sup>a</sup> (mg/L)	30.48 ± 54.90	0.0281	0.7824
SAA <sup>b</sup> (mg/L)	98.39 ± 84.78	−0.1760	0.2649
Leucocyte count (*10 <sup>9</sup> /L)	7.14 ± 8.5	0.1651	0.2902
Lymphocyte count (*10 <sup>9</sup> /L)	1.62 ± 0.86	0.0443	0.6986
Neutrophil count (*10 <sup>9</sup> /L)	9.12 ± 5.50	0.1178	0.4201

<sup>a</sup> CRP: C-reaction protein;

<sup>b</sup> SAA: Serum amyloid protein A.

level of ACE2 was normalized to the internal control GAPDH expression and calculated by the comparative C<sub>T</sub> ( $\Delta\Delta C_T$ ) method. Primers, targeting ACE2 and GAPDH were used: ACE2, forward: 5'-ACAGTCCACACTTGCCCAAAT-3', reverse: 5'-TGAGAGCACTGAA-GACCCATT-3'; GAPDH, forward: 5'-CTGGGCTACTGAGCACC-3', reverse: 5'-AAGTGGCTGTTGAGGGCAATG-3'. All data were statistically analyzed using Graph Pad Prism5 (version 5.0) software. ACE2 mRNA levels were log-transformed by taking the base 10 logarithm to account for the skewed distribution. Quantitative data are expressed as the mean ± SD. Data with a Gaussian distribution were analyzed using an unpaired *t*-test or one-way analysis of variance (ANOVA). Spearman's correlation test was performed to assess the correlation of the ACE2 gene and clinical variables. *P* values less than 0.05 were considered statistically significant.

Considering the evidence that ACE2 is the main host cell receptor of SARS-CoV-2 and plays a crucial role in the entry of virus into the cell to cause the final infection, ACE2 gene expression was the focus of this study.

## Results

The participant cohort consisted of 177 individuals aged 1–95. Among participants with URI (125) the median age was 33.69 ± 15.88, 57.6% were male, and 65.6% experienced fever with a body temperature over 37.4°C. For the healthy control (HC) participants (52) the median age was 38.63 ± 16.06 and 61.54% were male. The expression level of the ACE2 gene was significantly higher in patients with URI (*n* = 125) than in HC individuals (*n* = 52) (*p* < 0.0001, Figure 1). There was a significant, positive correlation between ACE2 gene expression level and age (*r* = 0.1799, *p* = 0.0447), and with body temperature (*r* = 0.1927, *p* = 0.0427) (Table 1).

## Discussion

In this study, we demonstrated that the ACE2 gene level was significantly upregulated in the pharyngeal epithelium of patients with URI. We also found that the ACE2 gene level was significantly and positively correlated with both age and body temperature. To our knowledge, there are only a few reports demonstrating that the ACE2 gene level in the pharyngeal epithelium is increased in patients with URI. A recent study reported that ACE2 is an interferon-stimulated gene in human airway epithelial cells, which may help explain increasing coinfections with SARS-CoV-2 and other respiratory pathogens (Ziegler et al., 2020). Significantly, upregulation of other receptors after upper airway infection has been reported, including Platelet-activating factor receptor (PAFr), suggesting a multi-layered response after infection (Shukla et al., 2016; Bhalla et al., 2020).

There have been increasing reports about coinfection with severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2)

and other respiratory pathogens. The China-Japan Friendship Hospital reported a case of coinfection with influenza A virus and SARS-CoV-2 (Wu et al., 2020). A recent study reported that 5 out of 115 COVID-19 patients were also diagnosed with influenza (Ding et al., 2020). This raises the concern that there may be mixed infections of seasonal influenza and the novel coronavirus. Measures should be taken to enhance the respiratory infectious diseases surveillance systems and avoid lethal secondary infections.

This study provides novel results on ACE2 gene expression in the pharyngeal epithelium and its relationship with URI disease. Future exploration will shed more light on the molecular mechanism on how ACE2 is upregulated in URI patients.

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## Potential conflicts of interest

None reported.

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This manuscript has been released as a pre-print at Medrxiv[7]. Miss. Mingjiao Zhang and Miss. Lingyan Du contribute equally to this work. While completing this work, they are engaging in nucleic acid screening of SARS-CoV-2. Their primary research interests are infectious diseases. We thank our colleagues from the department of laboratory medicine, The Third Affiliated Hospital, Southern Medical University, for their kind help.

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