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Expression of ACE2 in airways: Implication for COVID-19 risk and disease management in patients with chronic inflammatory respiratory diseases

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Abstract

Coronavirus disease 2019 (COVID-19), caused by the novel severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), has been a rising international cause of morbidity and mortality. Angiotensin-converting enzyme 2 (ACE2) is identified as a key cell entry receptor for SARS-CoV-2 and suggested to be a limiting factor for viral entry at the initial infection stage. Recent studies have demonstrated that ACE2 expression is highly enriched in nasal epithelial cells and type II alveolar epithelial cells, highlighting the importance of respiratory tract as the primary target site of SARS-CoV-2. The expression of ACE2 in airway epithelial cells is tightly regulated by inflammatory milieu and environmental and internal stimuli. Very recently, ACE2 has been reported to have different expression levels in airways under distinct chronic inflammatory airway diseases, such as chronic obstructive pulmonary disease (COPD) and allergic asthma, which may associate with the COVID-19 risk and affect the management of primary airway diseases. In this review, we focus on the cutting-edge progress in distribution, expression, and regulation of ACE2 in respiratory system in physiological and pathological conditions, and their implication for the development of COVID-19. We also discuss the management of airway diseases, including asthma, COPD, allergic rhinitis, and rhinosinusitis in the era of COVID-19.

KEYWORDS
airway disease, angiotensin-converting enzyme 2, coronavirus disease 2019, management, severe acute respiratory syndrome coronavirus 2

1 | INTRODUCTION

The current outbreak of coronavirus disease 2019 (COVID-19), which was first reported in December 2019, spreads across the globe rapidly and has been declared as pandemic on 11 March 2020 by the World Health Organization.[1-4] COVID-19 is caused by a novel severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) and associates with substantial morbidity and mortality.[2-8] As of 19 July 2020, more than 14.0 million cases have been

confirmed and over 600,000 deaths. The clinical manifestations of COVID-19 are heterogeneous, ranging from asymptomatic to severe disease and even death.[9,10] Currently, no specific antiviral drug proved by randomized controlled trials or vaccine is available for the prevention and treatment of COVID-19.[8,10,11] A key challenge for clinicians is to identify risk and protective factors for COVID-19 to improve outcomes. A number of epidemiological studies have identified older age, male gender, black and minority ethnicity, and various comorbidities including hypertension,

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diabetes, cardiovascular disease, and chronic obstructive pulmonary disease (COPD) as the risk factors for COVID-19 (Table 1), associated with severe illness and death.[12-30] However, the mechanisms underpinning the contribution of these factors for the development of COVID-19 are not fully understood, which may be associated with the capacity of SARS-CoV-2 to invade host cells and infect an individual person under different conditions.

Severe acute respiratory syndrome coronavirus 2 belongs to the broad family of viruses known as coronaviruses and shares 79.6% sequence identity to SARS-CoV, the virus accounting for SARS pandemic in 2003.[31-33] Similar to SARS-CoV, SARS-CoV-2 has been discovered to enter cells via binding to angiotensin-converting enzyme 2 (ACE2).[32,34-36] The SARS-CoV-2 spike protein demonstrates at least 10 times higher affinity in binding ACE2 than does SARS-CoV.[37] High ACE2 expression on host cells increases the susceptibility to SARS-CoV-2, and blocking ACE2 signalling prevents the viral infection in vitro.[32,36,38] In humans, ACE2 expression has been found to be increased in the lung of smokers and patients with COPD, and those patients have been reported more likely to have severe COVID-19, implicating a central role of ACE2 in COVID-19 development.[13,19,39]

Respiratory tract is continuously exposed to a multitude of pollutants and inhaled pathogens, making them a primary target of SARS-CoV-2. It is supported by the recent discovery of enriched ACE2 expression in nasal epithelial cells and type II alveolar (AT2) cells.[40-42] Airway diseases, including allergic rhinitis (AR), sinusitis, asthma, and COPD, are highly prevalent world-wide, affecting up to 50% of the populations.[43-45] The current COVID-19 pandemic makes the interaction between primary inflammatory airway diseases and COVID-19 critical for mitigating COVID-19 risk and management of primary airway diseases. Do patients with inflammatory airway diseases have an altered susceptibility to COVID-19? Will those patients have a unique disease course if infected by SARS-CoV-2? Will COVID-19 affect the management of the primary airway diseases? Understanding the expression and function of ACE2 in airways in physiological and pathological conditions may give valuable hints to answer these questions. In this review, we summarized cutting-edge advances in the very fast-moving

TABLE 1 Risk-associated diseases or factors for COVID-19

Comorbidities	Hazard or odds ratios (95% confidence intervals) for infection or poor prognosis risk
Hypertension	For composite end-points (admission to intensive care unit, invasive ventilation, or death): 1.58 (1.07-2.32)[13]; For death in patients with severe illness: 1.77 (1.11-2.84)[16]
Cardiovascular diseases	For death: 1.17 (1.12-1.22) for patients with chronic heart disease[12]
Diabetes	For death: 1.95 (1.83-2.07) for patients with HbA1c ≥ 58 mmol/mol;[12] For composite end-points (admission to intensive care unit, invasive ventilation, or death): 1.59 (1.03-2.45)[13]
COPD	For composite end-points (admission to intensive care unit, invasive ventilation, or death): 2.68 (1.42-5.06)[13]; For severe illness: 5.69 (2.49-13.00).[20]
Smoking	For death: 1.19 (1.14-1.24) for former smokers;[12] For death in critically ill patients: 5.21 (1.39-19.52)[14]
Asthma	For death: 1.13 (1.01-1.26) for severe asthmatic patients with recent OCS use[12]; For severe illness: 1.48 (1.15-1.92) for non-allergic asthmatic patients[27]
Malignancy	For composite end-points (admission to intensive care unit, invasive ventilation, or death): 3.50 (1.60-7.64);[13] For death: 2.98 (1.76-5.06)[15]
Obesity	For severe illness: 3.40 (1.40-2.86) for patients with BMI ≥ 28.0[22] For death: 1.40 (1.30-1.52) for patients with BMI from 35.0 to 39.9 and 1.92 (1.72-2.13) for patients with BMI ≥ 40.0[12]
Other factors	
Male	For death: 1.59 (1.53-1.65) for males[12]; For death: 0.46 (0.35-0.60) for females[15]; For death in patients with severe illness: 1.72 (1.05-2.82) for males.[16]
Advanced age	For death: 2.40 (2.16-2.66) for patients aged from 60 to 69 y, 6.08 (5.52-6.69) for patients aged from 70 to 79 y, and 20.61 (18.72-22.70) for those aged ≥80 y[12]; For death: 5.06 (2.82-9.06) for patients aged from 50 to 64 y, 9.97 (5.64-17.63) for patients aged from 65 to 79 y, and 20.33 (10.76-38.44) for those aged ≥80 y[15]; For death in patients with severe illness: 1.72 (1.09-2.73) for patients aged ≥65 y[16]
Blood group A	For infection: 1.54 (1.12-2.10) for subjects with blood group A[30]; For infection: 0.65 (0.46-0.93) for subjects with blood group O[30]
Black and minority ethnicity	For death: 1.48 (1.30-1.69) for Black patients[12]; For death: 1.44 (1.32-1.58) for South Asian patients[12]
Air pollution	Incidence of COVID-19 cases positively correlated with poor air quality (NO_2, $PM_{2.5}$, and PM_{10} levels)[25]; Increase of 1 $\mu g/m^3$ in $PM_{2.5}$ associated with 8% increase in the COVID-19 death rate[26] (preprint)

Abbreviations: BMI, body mass index; COPD: chronic obstructive pulmonary disease; HbA1c, haemoglobin A1c; OCS, oral corticosteroids.

field of ACE2 study and highlighted the changes in ACE2 expression and its implication for COVID-19 risk and disease management in patients with common upper and lower inflammatory airway diseases, including AR, rhinosinusitis, asthma, and COPD.

2 | STRUCTURE AND FUNCTION OF ACE2

The 40 kb *ACE2* gene located on chromosome Xp22 was first described in 2000.[46,47] The encoded human ACE2 protein is a type I transmembrane glycoprotein, which consists of 805 amino acids.[46,47] ACE2 orientates outside with the N-terminus and anchors to the plasma membrane through a short intracellular C-terminal tail.[46-48] The highly conserved catalytic site of ACE2 faces the extracellular space, where it can metabolize circulating peptides.[46-48] Despite 40% sequence identical of the active catalytic site between ACE and ACE2, they have distinct substrate specificity.[46-48] ACE2 functions as a carboxypeptidase, cleaving a single residue from angiotensin (Ang) II to generate Ang (1-7) (Figure 1) and a single residue from

Ang I to yield Ang (1-9), whereas, as the peptidyl dipeptidase, ACE cleaves the decapeptide Ang I into an octapeptide Ang II.[49] Ang II is the key player in renin-angiotensin system (RAS), a hormone system that maintains blood pressure homeostasis and fluid and salt balance.[50] Ang II mediates vasoconstriction and thus contributes to the overactivation of RAS, which is associated with a spectrum of diseases including hypertension, heart failure, and renal disease.[50,51] Therefore, ACE2 plays a crucial role in maintaining the balance of RAS by countering the activities of Ang II. Furthermore, ACE2 also can convert Ang A into alamandine, a protector in the cardiovascular system.[49] The membrane-bound ACE2 can be cleaved by a disintegrin and metalloproteinase 17 to become the soluble form, which may function as a competitive interceptor of binding ligands of membrane ACE2.[52]

In lung, Ang II is able to induce bronchoconstriction, vasoconstriction, fibroproliferation, cytokine expression, and cell apoptosis, thus promoting tissue injury.[49] As a negative regulator of Ang II, ACE2 has been reported to protect the lung from injury (Figure 1).[49] In the acute lung injury/acute respiratory distress syndrome model, the lack
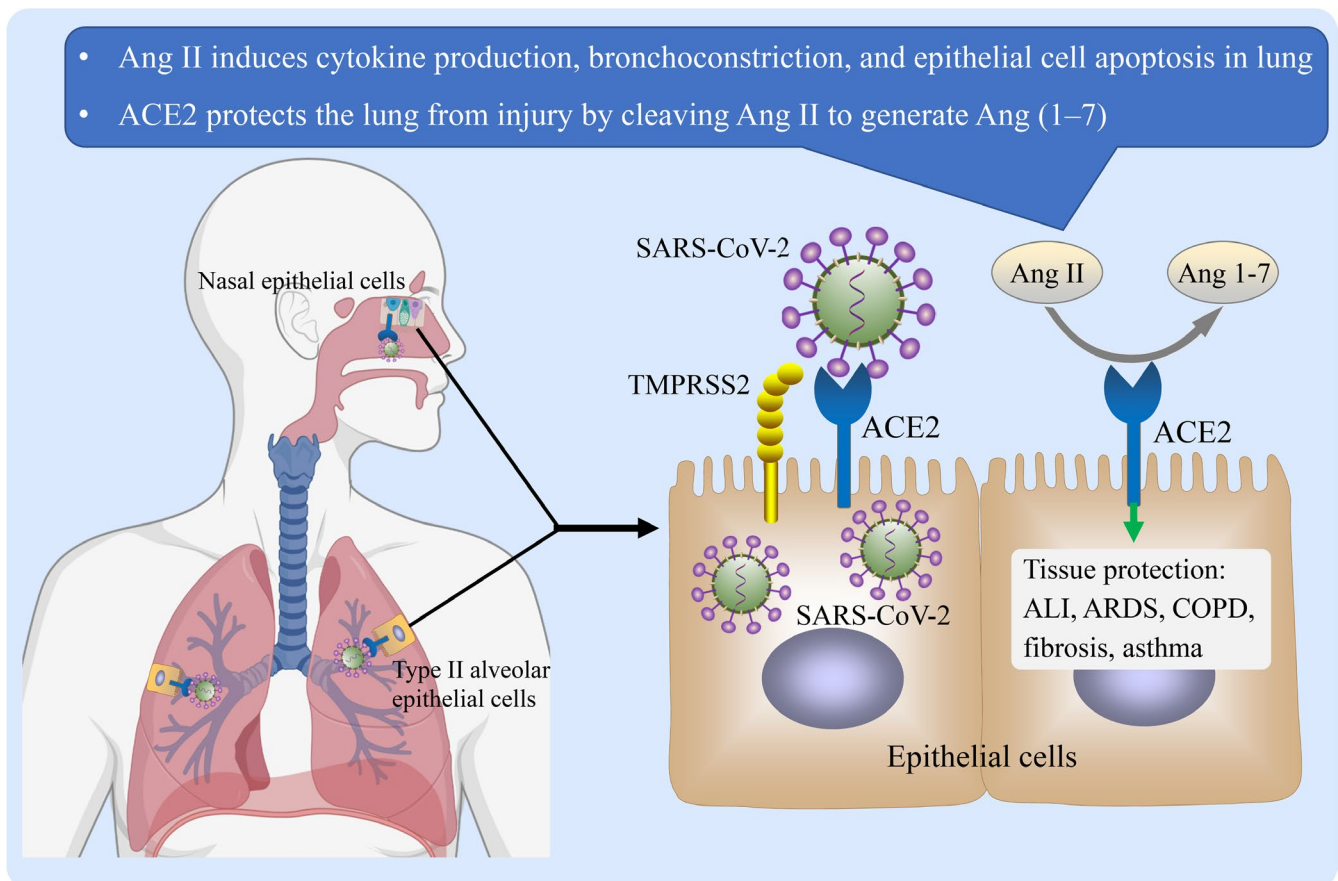
FIGURE 1 Expression and role of angiotensin-converting enzyme 2 (ACE2) in airways. ACE2 is expressed in airways, with a particularly higher expression level in nasal epithelial cells and type II alveolar epithelial cells in the lung. Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) binds to ACE2 expressed on human airway epithelial cells, and then, the serine protease TMPRSS2 cleaves and activates the spike protein of SARS-CoV-2, which ultimately facilities virus-cell fusion and cell entry. As a peptidase, ACE2 catalyses and inactivates angiotensin (Ang) II and produces the vasodilator peptide Ang (1-7). Ang II induces bronchoconstriction, vasoconstriction, fibroproliferation, cytokine expression, and cell apoptosis, thus promoting tissue injury. Accordingly, ACE2 has protective effects against tissue injury, including acute lung injury (ALI), acute respiratory distress syndrome (ARDS), fibrosis, chronic obstructive pulmonary disease (COPD), and allergic asthma

of ACE2 expression in the lung resulted in exaggerated lung oedema, massive neutrophil accumulation, and deteriorated lung function.[53] Vice versa, treatment with recombinant ACE2 protein protected mice from severe acute lung injury.[53] Transforming growth factor (TGF)-β1 is the most potent profibrotic cytokine and may act downstream of Ang II.[54] ACE2 overexpression significantly reduced TGF-β1 levels and demonstrated a protective effect against the development of bleomycin-induced fibrosis in murine models.[55] In the murine COPD model induced by cigarette smoking exposure, ACE2 overexpression in the lung significantly improved the lung function and pathological abnormalities.[56] In an ovalbumin-challenged mouse model of asthma, Ang (1-7) modulated ovalbumin-induced increases in total cell counts, eosinophils, and lymphocytes, and decreased goblet cell hyper/metaplasia.[57] These results support an important role of Ang (1-7) and ACE2 in reducing airway allergic inflammation (Figure 1).[57]

Besides as a peptidase to catalyse Ang II cleavage in the physiological condition, ACE2 has been identified as a functional receptor for the SARS-CoV and is important for the disease development after SARS-CoV infection.[31,53,58] ACE2 purified from Vero E6 cells was able to bind the S1 domain of the SARS-CoV spike protein in vitro.[31] ACE2 knockout mice showed reduced viral replication in the lungs in SARS-CoV infection.[59] In contrast, transgenic mice expressing human ACE2 were highly susceptible to SARS-CoV infection with more severe pulmonary lesions.[60] In addition, anti-ACE2 antibody and soluble ACE2 treatment inhibited SARS-CoV infection in mice.[58] Full-length genome sequencing revealed that SARS-CoV-2 shares 79.6% sequence identity to SARS-CoV.[32] SARS-CoV-2 has been found employing the same receptor as SARS-CoV, ACE2, for host cell entry (Figure 1).[32,34,35] To invade the host cells, there is a need for another player, the transmembrane protease TMPRSS2, a cellular serine protease, which cleaves and activates the SARS-CoV-2 spike protein and facilitates human cell entry.[38] The spike protein of SARS-CoV-2 also contains a furin-like cleavage site,[61-63] suggesting a potential role of furin and furin-like proteases in SARS-CoV-2 cell entry. Wrapp et al described a 3.5-angstrom-resolution structure of the SARS-CoV-2 trimeric spike protein by cryo-electron microscopy and found that SARS-CoV-2 spike protein binds to ACE2 at least 10 times more tightly than that of SARS-CoV,[37] which may explain the higher transmission efficiency of SARS-CoV-2 than SARS-CoV. Structurally, the receptor binding domain of the surface spike protein of SARS-CoV-2 is recognized by the extracellular peptidase domain of ACE2 mainly through the polar residues.[17,64]

3 | EXPRESSION OF ACE2 IN AIRWAYS

Airways consist of heterogeneous cell populations. The development of high-throughput single-cell RNA sequencing technique enables the robust profiling of the characteristics of specific cell types possible and offers an unbiased approach to study the expression of particular molecule in different cells.[65,66] By re-analysing of deposited public available single-cell RNA sequencing data

sets, researches have conducted timely studies of the expression of ACE2 and other genes relevant to SARS-CoV-2 cell entry in airways.

3.1 | Upper airways

Nasal cavity is the primary site of exposure to pollutants, airborne allergens, and inhaled pathogens and acts as the gateway to respiratory system. In patients with COVID-19, nasal swabs have yielded higher viral loads than throat swabs, underscoring the nasal epithelium as a portal for initial infection and transmission of SARS-CoV-2.[32] Indeed, ACE2 and TMPRSS2 protein expressions have been detected in nasal epithelium by immunohistochemistry.[67] Recently, by analysing single-cell RNA sequencing data of multiple tissues from healthy donors, Sungnak et al discovered an enriched ACE2 and TMPRSS2 mRNA expression in nasal epithelial cells, with the highest expression level observed in nasal secretory cells and ciliated cells (Figure 1).[40] Similarly, Ziegler et al observed significant enrichment of ACE2 mRNA expression in apical epithelial cells and, to a lesser extent, ciliated cells.[41] Consistently, the expression of ACE2 gene in goblet cells and ciliated cells was confirmed when analysing single-cell RNA sequencing data derived from air-liquid interface cultured nasal epithelial cells.[40] Notably, TMPRSS2 was found having a broader expression (28%) than ACE2 (4%) in nasal epithelial cells characterized by secretory phenotype,[41] suggesting that ACE2 may be the limiting factor for viral entry. In addition, TMPRSS2 is only expressed in a subset of ACE2-positive cells.[40] Thus, SARS-CoV-2 may use alternative facilitating mechanism to entry host cells. A potential substitute for TMPRSS2 is cathepsin B, another protease, which was found to be expressed in more than 70% of ACE2-positive cells.[40] ACE2 gene has been found to be coexpressed with genes involved in innate immunity in nasal epithelial cells, highlighting the important role of nasal epithelial cells in respiratory viral infection, spread and clearance.[40,41] In contrast, no significant ACE2 gene expression in immune cell populations, including T cells, dendritic cells, and mast cells, was discovered in nasal mucosal tissues.[40,41]

Anosmia is typical of viral rhinitis, and smell and taste disorder has been reported as one of the common symptoms of COVID-19 patients,[68-70] raising the possibility that SARS-CoV-2 may infect olfactory epithelium or sensory neurons. Indeed, high expression of ACE2 in epithelial cells of tongue has been reported based on the bulk and single-cell RNA sequencing of oral tissues.[71] Expression of ACE2 and TMPRSS2 was also detected in human and murine olfactory epithelium.[72-74] Interestingly, by analysing a recently published single-cell RNA sequencing data, Brann et al and Fodoulian et al found that sustentacular and olfactory stem cells, but not olfactory sensory neurons or neurons in olfactory bulb, expressed ACE2 and TMPRSS2 mRNA in humans (preprint).[73,74] In addition, enriched furin gene expression has been identified in olfactory epithelial gland cells, suggesting that other than TMPRSS2, furin may participate in helping SARS-CoV-2 cell entry.[41,73] The expression pattern of ACE2 protein in human olfactory

epithelium was verified by immunohistochemical staining of biopsied tissues (preprint).[73] These data indicate that SARS-CoV-2 infection of support cells in olfactory epithelium and olfactory bulb may disrupt the structure of olfactory epithelium and impair the function of olfactory sensory neurons or bulb neurons, ultimately resulting the disturbance in smell perception in COVID-19 patients.

3.2 | Lower airways

The lung is the most important target organ of SARS-CoV-2 infection. An analysis of single-cell RNA sequencing data derived from normal human lung tissues revealed that *ACE2* gene was expressed by 0.64% of cells in lung and 83% of ACE2-expressing cells were AT2 cells and 5% were type I alveolar cells (AT1) (Figure 1).[75] Nevertheless, bronchial airway epithelial cells, fibroblasts, endothelial cells, and macrophages had low expression of *ACE2*.[75] Consistently, the expression of *ACE2* in AT2, AT1, and ciliated cells was revealed by other studies in single-cell level.[40,41,76-79] In line with the single-cell RNA sequencing finding, by immunohistochemistry study, Bezara et al found that about 1% of surfactant protein C–positive AT2 cells were positive for ACE2 protein expression, whereas alveolar macrophages were negative for ACE2 staining (preprint).[80] Through investigating ACE2 protein expression in different anatomical regions, they found that apical ACE2 expression was rare and limited to ciliated cells in the trachea and bronchi (preprint).[80] In the submucosal glands of large airways, occasional serous cells and vessels near the acini were positive for ACE2, but ACE2 was regionally localized in ciliated cells in bronchioles (preprint).[80] Notably, regional distribution of ACE2 protein varied across different donors (preprint),[80] which possibly contributes to the difference in host susceptible to SARS-CoV-2 and variable clinical presentations of COVID-19. Similar to the finding in nasal epithelial cells, gene ontology enrichment analysis showed that the ACE2-expressing AT2 cells had high levels of multiple viral response-related genes, supporting the role of these cells in viral infection and control.[41] In addition, based on single-cell RNA sequencing data, *TMPRSS2* expression has been identified in various cell types of the lung, and the abundance was lower in club cells, ciliated epithelial cells, and AT1 cells than in AT2 cells.[40,41] Ziegler et al reported 34.2% of AT2 cells expressing *TMPRSS2*, 1.4% expressing *ACE2*, and 0.8% co-expressing *TMPRSS2* and *ACE2*.[41] Colocalization of ACE2 and TMPRSS2 could be observed on the apical membrane of AT2 cells by immunofluorescence staining (preprint).[80] These findings again suggest that ACE2 is likely the limiting factor for cell entry of SARS-CoV-2 and apical ACE2 and TMPRSS2 colocalization facilitates the cell entry.[40,41]

4 | REGULATION OF ACE2 EXPRESSION AND ASSOCIATED FACTORS

Coronavirus disease 2019 outbreak stirs the great research interest in ACE2 expression regulation in airway epithelial cells. The transcriptional and functional characteristics of ACE2 may be shaped by environmental stimuli, inflammatory cytokines, and medical treatments in different disease states.

## 4.1 | Interferon and viral infection

Antiviral interferons (IFNs) and subsequent IFN-induced chemokines and cytokines produced by virus-infected host cells are critical for effective antiviral response.[81] There are three types of IFNs: type I (IFN-α and IFN-β), type II (IFN-γ), and type III (IFN-λ), and each of them may have a non-redundant role in host defence and immunopathology, particularly at epithelial barriers.[82] Recently, Ziegler et al identified that *ACE2* represented an IFN-stimulated gene in human airway epithelial cells.[41] They found that *ACE2* gene expression was markedly up-regulated by IFN-α and to a lesser extent by IFN-γ in primary human nasal basal epithelial cells (Figure 2).[41] They further found signal transducer and activator of transcription (STAT) 1, STAT3, interferon regulatory factor (IRF) 8, and IRF1 binding sites in the transcription start site of *ACE2* in humans, supporting a role of IFNs in regulating *ACE2* expression.[41] In vivo, Sajuthi et al found a positive correlation between *ACE2* and the IFN response gene expression in nasal epithelial brushing cells collected from a large cohort of asthmatic and healthy children (preprint).[42] Upper airway viral infections are known to induce a robust IFN response.[83] In comparison with those from patients without viral infection, Ziegler et al found that *ACE2* mRNA expression was up-regulated in goblet cells and squamous cells in nasal wash samples from influenza virus–infected individuals.[41] Nevertheless, it should be noted that *ACE2* mRNA expression was only slightly elevated in nasal basal epithelial cells following IFN-α treatment in mouse.[41] Additionally, intranasal infection with murine gamma herpesvirus-68 failed to change the ACE2 expression in viral-transcript–positive cells or AT2 cells from the lungs of mice.[41] Therefore, a careful consideration of animal and cellular models will be required for assessing therapeutic interventions targeting the IFN system when studying ACE2-associated biology.

4.2 | Type 2 cytokine, allergen challenge, and eosinophil

Recently, several studies reported that IL-13 down-regulated *ACE2* mRNA expression in human nasal and bronchial epithelial cells cultured with an air-liquid interface method (Figure 2).[42,84,85] However, Ziegler et al showed that IL-4 and IL-13 had no effect on *ACE2* mRNA expression in human nasal basal epithelial cells when cultured submerged.[41] It also should be noted that the data regarding the regulation of ACE2 expression at protein level are still lacking.

Allergen challenge can initiate and perpetuate airway type 2 (T2) inflammation.[86] Jackson et al have found that allergic sensitization was inversely related to *ACE2* mRNA expression in nasal epithelium in asthmatic children.[84] In addition, significant negative
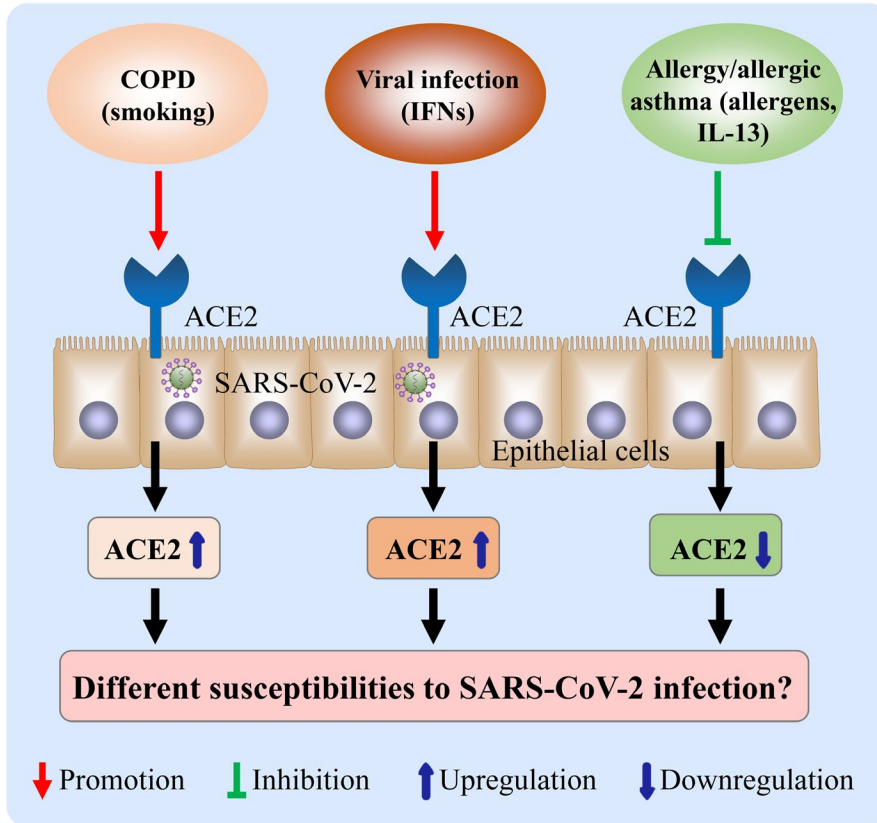
FIGURE 2 Regulation of angiotensin-converting enzyme 2 (ACE2) expression in airway epithelial cells. Tobacco exposure promotes ACE2 expression in airway epithelial cells, which may associate with increased ACE2 expression in the lung of patients with chronic obstructive pulmonary disease (COPD). Interferon (IFN)-α and IFN-γ promote ACE2 expression in airway epithelial cells. Allergens and interleukin (IL)-13 inhibit ACE2 expression in airway epithelial cells, may account for the lower ACE2 expression in patients with allergic asthma. Varied ACE2 in distinct chronic inflammatory airway diseases may contribute to different susceptibilities to severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) infection

correlations between *ACE2* mRNA expression and T2 biomarkers including the number of positive allergen-specific immunoglobulin (Ig)E tests, total IgE, fractional exhaled nitric oxide, and nasal epithelial IL-13 expression were found by them, although most correlation coefficients were low.[84] Furthermore, Jackson et al found that nasal cat allergen led to a significant reduction in *ACE2* mRNA expression in nasal brush samples in adult AR patients allergic to cat.[84] Consistently, segmental allergen bronchoprovocation to dust mite, ragweed, or cat significantly reduced *ACE2* mRNA expression in bronchial brushing cells in adult patients with mild asthma.[84] Therefore, respiratory allergen exposures and T2 inflammation decrease *ACE2* expression in both upper and lower airways (Figure 2).

Although eosinophilia is a hallmark of allergic respiratory inflammation,[87] accumulating evidence indicates an antiviral activity of eosinophils.[87] Eosinopenia has been noted in patients with COVID-19.[7,88] Patients with fewer peripheral blood eosinophils displayed worse radiographic aggravation and longer course of disease compared to those with normal eosinophil counts.[88] Peripheral blood eosinophil levels have been reported to be gradually increased in recovered patients before discharge, accompanied by the improved clinical status.[88] These data suggest that peripheral blood eosinophil count may be an indicator for diagnosis, clinical status monitor, and outcome prediction of COVID-19. Furthermore, Camiolo et al observed an increased *ACE2* gene expression in bronchial epithelial cells in asthmatic patients with low peripheral blood eosinophil counts compared with those with high

eosinophil counts,[89] suggesting a negative effect of eosinophil on *ACE2* expression.

Allergy is an immune response to allergen stimulation that is characterized by elevated T2 cytokines and eosinophilic inflammation. These above findings raise the possibility that allergy might be a protective factor for COVID-19. In a single-centre retrospective study with a small sample size (110 COVID-19 patients) in China, Shi et al observed a lower rate of comorbid allergy in patients with COVID-19 in comparison with the prevalence of allergic diseases in the general population.[90] When excluding patients with other underlying diseases and stratifying COVID-19 patients into those with (n = 21) and without allergy history (n = 44), they found that patients with allergy demonstrated lower proportions of bilateral lung lesions on chest computed tomography scanning and severe illness and higher circulating total T cell counts than those without allergy.[90] However, the relationship between allergy and COVID-19 obviously needs to be evaluated and validated in larger cohorts and those with different genetic and socio-economic backgrounds.

4.3 | Smoking

Several epidemiological studies reported that cigarette smoking exposure associated with increased risk for COVID-19 and current and former smokers was likely to develop severe COVID-19 than never smokers.[3,13,91,92] By comprehensively analysing transcriptomic and microarray data sets, Cai et al found a markedly higher *ACE2* mRNA

expression in ever smokers compared with never smokers in both airway epithelial cells and lung tissues in COPD and non-COPD subjects.[19] Brake et al observed an enhanced ACE2 protein expression in resected lung tissues from smokers with COPD and smokers with normal lung function when compared to heathy non-smoking individuals, with more prominent increase in smokers with COPD compared with smokers with normal lung function.[93] Leung et al found that current smokers had a significantly higher *ACE2* mRNA expression in bronchial bushing cells than never smoker.[39] In addition, smoking status defined by never, former, and current smokers was significantly associated with *ACE2* gene expression in large airway epithelium and lung tissues.[19] Therefore, these consistent results strongly support that tobacco exposure significant up-regulates *ACE2* gene expression in airways (Figure 2). However, further studies are needed to explore the mechanisms underlying tobacco-induced up-regulation of ACE2 pulmonary expression. In addition, electronic cigarette consumption has significantly increased, whether electronic cigarettes have effects on *ACE2* expression remains to be determined.

4.4 | Glucocorticoid and biologics

Intranasal and inhaled corticosteroids serve as the cornerstone for the treatment of inflammatory airway disorders such as AR, chronic rhinosinusitis (CRS), COPD, and asthma.[45,94] Specific concerns have been raised in relation to steroid therapy and possible risk of adverse outcomes in COVID-19. Whether baseline corticosteroid treatment has an effect on *ACE2* expression in airways and whether it even modifies the risk to SARS-CoV-2 infection remain open questions. Jackson et al reported that nasal corticosteroid use at the time of nasal sampling was not associated with alteration in *ACE2* mRNA expression in nasal epithelial cells in asthmatic children.[84] We have recently found that dexamethasone treatment did not change *ACE2* mRNA expression in human nasal epithelial cells in vitro (under review). However, Peters et al recently reported that use of inhaled corticosteroids was associated with lower expression of *ACE2* in sputum cells, which comprised a mix of multiple cell types, including structural airway cells (eg epithelial cells and squamous cells) and immune cells (eg macrophages and granulocytes).[95]

Additional questions of concern relate to the effect of biologics on COVID-19 risk. Omalizumab (an anti-IgE antibody), mepolizumab and reslizumab (blocking IL-5), benralizumab (blocking the IL-5 receptor), and dupilumab (blocking IL-4Rα) reduce asthma-related exacerbation and have been approved for the treatment of severe asthma.[96] In addition, dupilumab has recently been approved for the treatment of CRS with nasal polyps in the United States and Europe.[97] Currently, there is no evidence-based study addressing the effect of biologics on the risk of COVID-19 and ACE2 expression. However, given the finding of down-regulation of *ACE2* gene expression in airway epithelial cells by T2 cytokines, it is possible that reversal of T2 skewed allergic and eosinophilic inflammation by these biologics may potentially increase the risk

of SARS-CoV-2 infection by up-regulating ACE2 expression in airways. Very recently, Förster-Ruhrmann et al reported a case of 53-years-old female patient with refractory CRS with nasal polyps and treated with dupilumab, and this patient presented a light course of COVID-19.[98] Obviously, more studies are needed to clarify the impact of biologics on COVID-19 in patients with chronic inflammatory airway diseases.

4.5 | Other associated factors

A number of studies demonstrated a lower risk of SARS-CoV-2 infection in children compared with adults.[99] Older age has been independently associated with increased mortality of COVID-19 after adjusting for comorbidities (Table 1).[12,14] In a cohort of 305 individuals aged 4–60 years, Bunyavanich et al found that *ACE2* gene expression levels in nasal epithelial cells were the lowest in younger children and increased with age.[100] This age-dependent *ACE2* gene expression may account for the different risks and mortality of SARS-CoV-2 infection in different age groups.

Several studies have observed an increased susceptibility and mortality of SARS-CoV-2 infection in males compared with females (Table 1),[2,12,13,15] suggesting a gender-related expression of *ACE2*. Indeed, as an X chromosome–encoded gene, renal *ACE2* expression has been reported to be significantly down-regulated by oestrogens.[101] In addition to the direct effect on *ACE2* expression, sex hormone may also modify the immune response to viral infection. For examples, plasmacytoid dendritic cells from adult females produced more IFN-α than those from adult males in response to virus infection.[102] In addition, emerging evidence has implicated an association between ethnicity and incidence or outcome of COVID-19. By analysing large data sets (17 278 392 adults) in the UK, Williamson et al found that, after adjustment for other factors, Black and South Asian people were at a higher risk of death compared with people with White ethnicity.[12] In addition, Black and minority ethnicity people have been found to be associated with increased SARS-CoV-2 infection and poor clinical outcome.[23,24] The causes for ethnic disparities for COVID-19 development are unclear and may be related to different socio-economic and cultural background, lifestyle, genetic predisposition, and ACE2 expression.

5 | AIRWAY DISEASE AND COVID-19

5.1 | Upper airway disease

AR and CRS represented the two most common upper airway diseases. Previous studies reported low incidence of AR in COVID-19 patients, ranging from 0% to 1.8% in China.[7,103] However, those results generated were relied on the medical records, and may underestimate the incidences of upper airway comorbidities since they are usually not well considered under actual emergency situation.[7,103] Recently, we retrospectively studied a cohort of 1172

discharged COVID-19 patients by integrating patients' medical records and telephone questionnaire and found that the prevalence of AR in COVID-19 patients was 9.8%, which was comparable to that in general populations in Wuhan, China (9.7%) (preprint).[104,105] Additionally, we did not find an association between AR comorbidity and disease severity in COVID-19 patients, suggesting that comorbidity of AR may not modify the disease development and expression of COVID-19 (under review). In contrast to allergic asthma,[84] there was no difference in ACE2 mRNA and protein expressions between AR patients and healthy controls (under review). As for CRS, reduced *ACE2* gene expression was found in nasal epithelial cells form nasal polyp tissues compared with that in nasal epithelial cells from inferior turbinate tissues.[41] Nevertheless, mounting evidence demonstrates a significant heterogeneity in inflammatory patterns (endotypes) of CRS.[106] Type 1 (T1), T2, and type 17 (T3) endotype and mixed endotype have been reported for CRS.[106,107] It would be interesting to investigate whether ACE2 expression will vary across different CRS endotypes. Very recently, Chhiba et al reported that COVID-19 patients with rhinosinusitis showed a lower risk of hospitalization than those without rhinosinusitis.[108] However, Chhiba et al did not differentiate between the acute and CRS in their study. In addition, whether distinct CRS endotypes relate to different clinical expressions of COVID-19 also remains to be studied.

5.2 | Lower airway disease

Although asthmatic patients are at risk of more severe outcome after common cold virus infection than people without asthma,[109] asthma has unexpectedly not been identified as a significant risk factor for severe COVID-19.[5,7,110] Low prevalence of asthma (0%-0.9%) was observed in patients with COVID-19 in several studies in China.[13,16,91,111] In a US cohort including 1526 patients with COVID-19, Chhiba et al recorded a relative high prevalence of asthma (14%); however, there was no significant difference in hospitalization rate or mortality between patients with and without asthma.[108] Notably, asthma is a heterogeneous disease with varying levels of severity and distinct endotypes. In a large cohort (17 278 392 adults) study in the UK, severe asthma (with oral steroid treatment) was identified as a risk factor associated with COVID-19 death.[12] Allergic asthma is the most common type of asthma triggered by inhaled allergens. Jackson et al have recently reported reduced *ACE2* gene expression in nasal and bronchial epithelial cells after allergen challenge in AR and allergic asthmatic patients (Figure 2).[84] In addition, Kimura et al found that *ACE2* mRNA expression was reduced in bronchial epithelial cells in asthmatic patients with high T2 inflammation.[85] By analysing *ACE2* gene expression in bronchial epithelial cells in asthmatic patients with different endotypes, Camiolo et al identified a positive correlation between *ACE2* expression and scores of T1 gene expression and a negative correlation between *ACE2* expression and scores of T2 gene expression.[89] These data suggest an association between asthma endotypes and COVID-19, which was supported by

the finding that T2-low asthmatic patients demonstrated characteristics corresponding to risk factors for severe COVID-19, including male sex and history of hypertension.[89] Taken together, although allergic asthma appears to be a protective factor for COVID-19 (Figure 2), the associations between different phenotypic and endotypic asthma and COVID-19 remain to be defined.

Among the airway inflammatory diseases, COPD is the most commonly reported comorbidity in COVID-19 patients and the prevalence ranged from 1.5% to 5%.[13,91,112] Previous epidemiological studies have associated the COPD comorbidity with the severe illness and fatalities in COVID-19 patients.[13,20] Guan et al reported that severe COVID-19 cases had higher frequencies of COPD comorbidity than non-severe cases.[13] Leung et al reported that ACE2 protein expression in the bronchial epithelial cells was significantly increased in COPD versus non-COPD subjects.[39] Smoking is the primary aetiological factor for COPD, and evidence has shown increased ACE2 mRNA expression in smokers than in non-smokers.[19] Interestingly, Leung et al found that ACE2 expression was still increased in bronchial epithelial cells in COPD than in non-COPD subjects after adjusting the smoking status.[39] Therefore, it is likely that additional factors beyond smoking can modulate ACE2 expression in COPD patients.

6 | IMPLICATION FOR TREATMENT

Current interim guidance from World Health Organization on clinical management of COVID-19 advises against the administration of systemic corticosteroids.[113] For patients with primary chronic inflammatory airway diseases, several organizations suggest the patients with chronic airway diseases who are stable with inhaled or oral corticosteroid should continue their treatment.[113-117] The Allergic Rhinitis and its Impact of Asthma-European Academy of Allergology and Clinical Immunology statement advises that for patients with COVID-19 infection, intranasal corticosteroid treatment should be continued at the recommended dose for AR, since more sneezing after stopping means more spreading of the SARS-CoV-2.[114] The Global InNitiative for Asthma suggests that patients with asthma should not stop their prescribed inhaled corticosteroid during COVID-19 pandemic, as stopping corticosteroids often leads to the potentially dangerous worsening of asthma.[115] Very recently, in a retrospective study of 1,526 COVID-19 patients, Chhiba et al found that the ongoing use of intranasal corticosteroid was not associated with increased risk of COVID-19–related hospitalization.[108] Both the American College of Allergy, Asthma & Immunology (ACAAI) and the American Academy of Allergy, Asthma & Immunology (AAAAI) also recommend patients with asthma to continue to use their maintenance medications even during the pandemic.[116] In addition, balancing the risk of losing disease control and the lack of evidence or expectation of increased infectivity or mortality, ACAAI and AAAAI recommend to continue administration of biologic agents.[116] The Global Initiative for Chronic Obstructive Lung Disease also advises that

COPD patients should maintain their regular therapy, including corticosteroid administration, during the COVID-19 pandemic.[117] Collectively, although glucocorticoids and biologics targeting T2 inflammation may modify the ACE2 expression in airways, there is no direct evidence that they will change the risk for COVID-19. Considering the risk of exacerbation of primary airway diseases after stopping baseline treatment and subsequently increased potential risk to COVID-19, the baseline treatments are suggested to be continued for patients with chronic inflammatory airway disorders.

Cardiovascular diseases including hypertension and heart failure are the leading causes of death globally.[118] As the first-choice drugs in cardiovascular diseases, ACE inhibitors and angiotensin receptor blockers (ARBs) are thought to up-regulate ACE2 expression, thus may increase the risk of COVID-19.[118] Therefore, concerns have been raised regarding the use of ACE inhibitors and ARBs in the context of COVID-19 pandemic. However, by analysing the gene expression of *ACE2* and *TMPRSS2* in 1,051 lung tissue samples, Milne et al found that ACE inhibitor use reduced *ACE2* and TMPRSS2 gene expression, and ARBs use did not alter *ACE2* and *TMPRSS2* gene expression in the lung.[119] Several epidemiological studies demonstrated that the use of ACE inhibitors or ARBs was not associated with the increased risk or mortality of COVID-19.[118,120-122] The American College of Cardiology, the American Heart Association, the Heart Failure Society of America, and the European Society of Cardiology suggest that ACE inhibitors and ARBs should not be discontinued for patients with cardiovascular diseases.[123,124]

Antivirus drugs such as chloroquine, hydroxychloroquine, remdesivir, and lopinavir have been recommended for the treatment of COVID-19, although none of them has been thoroughly proved by randomized controlled trials.[11,125] There are big controversies regarding their therapeutic effects on COVID-19. Since *ACE2* is likely an IFN-stimulated gene and may be induced by viral infection,[41] antiviral treatment may reduce the ACE2 expression in airways in patients with COVID-19. Of note, as the Ang II converting enzyme, ACE2 protects lung from a variety of injuries.[49] The approaches targeting ACE2 for treating COVID-19 should be carefully considered in relation to the protective role of ACE2 in lung physiology and pathology.

In summary, enriched ACE2 expression in nasal epithelial cells and AT2 cells indicates that nose and lung are susceptible to the SARS-CoV-2 infection, thus representing the primary target organ of SARS-CoV-2. Expression of ACE2 in airways is tightly regulated by inflammatory milieu, such as IFNs and T2 cytokines, and environmental and internal stimuli, such as allergens and viruses. Altered ACE2 expression in chronic inflammatory airway diseases may modify COVID-19 risk, which however needs to be proved by further epidemiological and functional studies. Potential approaches targeting ACE2 for COVID-19 prevention and therapy should be developed under careful consideration regarding the multiple roles of ACE2 in lung physiology and pathology.

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