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# The paradoxical problem with COVID-19 ocular infection: Moderate clinical manifestation and potential infection risk

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ABSTRACT

Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), which induced mainly the respiratory damage also caused ocular surface symptoms. However, the detailed description of ocular manifestations, severity fluctuations in confirmed COVID-19 adult patients still lacked. We analyzed onset clinical symptoms and duration, ocular symptoms, needs for medication, outcomes in 28 conjunctivitis patients who were extracted from 3198 COVID-19 patients hospitalized in Huoshenshan Hospital and Taikangtongji Hospital, Wuhan, China. The expression levels of ACE2, TMPRSS2, ANPEP, DPP4, NRP1 on fetal and adult ocular surface and mouse lacrimal glands were assessed by single cell seq analysis. Our results indicated that conjunctivitis was a rare and self-limited complication in adults with COVID-19 while the existence of coronavirus receptors on human ocular surface and mouse lacrimal glands indicated the risk of SARS-CoV-2 infection. Our research firstly examined SARS-CoV-2 receptors, including the new discovered one, NRP1, on the fetal ocular surface and in the mouse lacrimal glands.



## 1. Introduction

Coronavirus disease 2019 (COVID-19), which is caused by severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), has quickly expanded into a pandemic, raising attention for the coronavirus family again after the severe acute respiratory syndrome (SARS) outbreak in 2003. Although according to the data obtained until now, coronavirus is transmitted mainly through respiratory tracts among human beings, the role of ocular transmission was also emphasized in recent coronavirus research. Among 7 types of human coronaviruses identified, 3 have been reported to be identified in ocular secretions, including human coronavirus NL63 (HCoV-NL63), severe acute respiratory syndrome coronavirus (SARS-CoV) and SARS-CoV-2 [1–3]. Additionally, the cellular entry receptors of several types of human coronavirus have been found on the ocular surface according to the published researches [4]. However, whether the lacrimal gland is another important harbor for SARS-CoV-2 replication in eyes is still unknown and systematic studies of conjunctivitis characteristics in COVID-19 patients are lacking.

To directly study this mystery, we conducted a large-scale retrospective survey of the incidence, clinical presentations and clinical correlations of conjunctivitis and SARS-CoV-2 detected among 3059 COVID-19 patients hospitalized in Huoshenshan Hospital, Wuhan, China and 139 COVID-19 patients hospitalized in Taikangtongji Hospital, Wuhan, China, along with single-cell RNA Seq analysis in human cornea public datasets. In animal model study, the expression of several types of coronavirus receptors in mouse lacrimal glands was also tested using single-cell RNA Seq analysis, exploring another possible pathway for coronavirus to be transmitted through ocular pathways.

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## 2. Materials and methods

### 2.1. Ethics approval

This study was approved by the Medical Ethical Committee of Wuhan Huoshenshan Hospital of China, and written informed consent was obtained from each patient.

### 2.2. Patient samples

A total of 28 COVID-19 patients with conjunctivitis symptoms were selected for this research. Among these 28 patients, 24 of them were hospitalized in Huoshenshan Hospital, Wuhan, China and the other 4 were hospitalized in Taikangtongji Hospital, Wuhan, China. The diagnosis of COVID-19 was confirmed by specific chest computed tomography (CT) image changes and/or positive reverse transcription polymerase chain reaction (RT-PCR) results of SARS-CoV-2. Due to the rapidly worsening pandemic which resulted in shortage of medical crews in Wuhan, China, the conjunctival swab tests were not performed on COVID-19 patients with conjunctivitis. The degree of severity of each patient was determined according to the clinical classification criterion of Diagnosis and Treatment Protocol for Novel Coronavirus Pneumonia released by the National Health Commission (7th trail version) in China on March 3, 2020. Patients' clinical and laboratory data were collected from the hospital electronic medical records.

### 2.3. Single cell RNA seq data analysis

Two samples of mouse developing lacrimal glands were downloaded from the GSE100106 which were performed at two time points: E16, when lacrimal glands have undergone an initial round of epithelial branching to create future acinar and ductal structures, and P4, when structural features of acini and ducts become recognizable [5]. The single cell sequence RNA dataset of human adult cornea-conjunctivat issues excised from 4 human donor eyes (51, 75, 81 and 86 years old) and 13 developing human eyes (10–21 post-conception weeks) were obtained from the website portal (http://covid19ocularsurface.org/) [4]. The Seurat R package (version 4.0.2) was used to normalize data via "LogNormalize" method [4]. Both fetal and adult ocular surface samples from human eyes and samples from P4 and E16 mice lacrimal glands were combined by the Seurat standard integration approach, respectively. Datasets were integrated based on 2000 genes selected by "FindVariableFeatures". Cells from human corneal tissues and mouse lacrimal glands were then both clustered using a resolution of 0.05. Markers for each cluster were identified by "FindMarkers" function, and only those with adjusted P values < 0.05 and |logFC| > 1 were regarded as marker genes. Cell types were assigned to the clusters using genes lists according to the gene lists and the data on the website (http://biocc.hrbmu.edu.cn/CellMarker/index.jsp). Uniform manifold approximation and projection (UMAP) was used to visualize the clustering results. "Dotplot" function was used to visualize the selected four genes' expression levels in different clusters, including ACE2, TMPRSS2, DPP4 and ANPEP.

### 2.4. Statistical analysis

Calculations were performed using the Prism software version 7 (Graphpad). Comparison of the mean for conjunctivitis course among patients with different COVID-19 severities and sexes was done with *t* test. The length of the clinical courses between patients with conjunctivitis and those without were also compared using *t* test. The normality of the data had been calculated using the test of Kolmogorov simirnov before performing t tests. The efficacy of several ocular medications used on these patients was compared using one-way ANOVA, and the Brown-Forsythe test. The tests with a p value less than 0.05 was considered statistically significant.

## 3. Result

### 3.1. Young patients with moderate COVID-19 were more likely to have conjunctivitis

Conjunctivitis was a rare clinical complication that occurred in 28 out of 3198 COVID-19 patients (0.9%). This rate found in our study is consistent with the prevalence reported in previously published research [6]. There were 13 males and 15 females included in our conjunctivitis research, accounting for 0.8% of males (1634) and 1% of females (1564) recorded, respectively (Table 1). According to the data collected, patients below 40 years who acquired ocular symptoms (1%) were slightly higher than those over 40 years old (0.9%) (Fig. 1A, Table 1). The prevalence of conjunctivitis in moderate COVID-19 patients (1579) and severe COVID-19 patients (1456) were 1.1% (17) and 0.8% (11), respectively (Table 1). No conjunctival symptoms were observed in critical group. At baseline, most patients with conjunctivitis had no underlying diseases (14 [50%]). Among the remaining patients with underlying diseases, hypertension (8 [28.6%]) was the most common one, followed by other 6 diseases, including glaucoma (1 [3.6%]), nephritis (1 [3.6%]), autoimmune anemia (1 [3.6%]), diabetes (1 [3.6%]), hepatitis B (1 [3.6%]), and cerebral infarction (1 [3.6%]). (Fig. 1B).

### 3.2. Conjunctivitis – not a common manifestation in COVID-19 period

Symptoms and signs of conjunctivitis among these 28 COVID-19 patients include: itching (19 [67.9%]), chemosis (3 [10.7%]), conjunctival hyperemia (2 [7.1%]), orbital pain (2 [7.1%]), epiphora (1 [7.1%]) and exudation (1 [7.1%]) (Fig. 1C). The mean days post-onset (dpo) of conjunctival symptoms was 24.9 ± 14.8 days (Supplementary Fig. 1). The dpo of conjunctivitis has no gender difference between male and female patients (Male: 25.7 ± 4.9 days, Female: 24.1 ± 3.2 days, p = 0.78) but varies between moderate group and severe group which was significant (Moderate: 20.2 ± 2.3 days, Severe: 32.1 ± 5.7 days, p = 0.04), with longer incubation period in severe patients (Supplementary Fig. 1). Notably, there was one patient has eye itch as the first symptom for his SARS-CoV-2 infection. The length of conjunctivitis course varied from 1 day (21 [75%]) to 6 days (1 [10.7%]) (Fig. 1D).

To further investigate the clinical relationship between severity of SARS-CoV-2 infection and conjunctival manifestations, the body temperature and blood oxygen saturation (SpO2) during the whole course of conjunctivitis were also analyzed. For the 7 patients whose conjunctivitis course ranged from 2 days to 6 days, body temperatures were all under 37.3 °C while the values of SpO2 were no fewer than 93%, with no significant changes observed (Fig. 2A, B). Most patients with conjunctivitis were afebrile with body temperature lower than 37.3 °C. Only one patient had fever with temperature of 38.5 °C (Fig. 2C) while the values of SpO2 were all above 92% (Fig. 2D). The respiratory symptoms of this patient worsened along with his ocular symptom onset on the same day, he complained more of short of breath, dry cough and orbital pain. Interestingly, one of the afebrile patients still was found to have pulmonary aggravation shown by CT scan who also presented discomfort in both eyes on the same day.

Among our 28 patients, 10 of them had their SARS-CoV-2 specific antibody tested during the hospitalization. Nine patients were found to have high levels of IgM and IgG, except one patient, whose IgM and IgG were both below standard value. (Fig. 2E). The excep-

**Table 1**
Percentage of conjunctivitis patients in enrolled COVID-19 patients.

| | Conjunctivitis | Huoshenshan Hospital | Taikangtongji Hospital | Percentage |
|---|---|---|---|---|
| Total | 28 | 3059 | 139 | 0.9% |
| Male | 13 | 1560 | 74 | 0.8% |
| Female | 15 | 1499 | 65 | 1% |
| Moderate | 17 | 1483 | 96 | 1% |
| Severe | 11 | 1418 | 38 | 0.8% |
| Critical | 0 | 158 | 5 | 0 |
| Age≦40 | 4 | 412 | 5 | 1% |
| Age > 40 | 24 | 2647 | 134 | 0.9% |

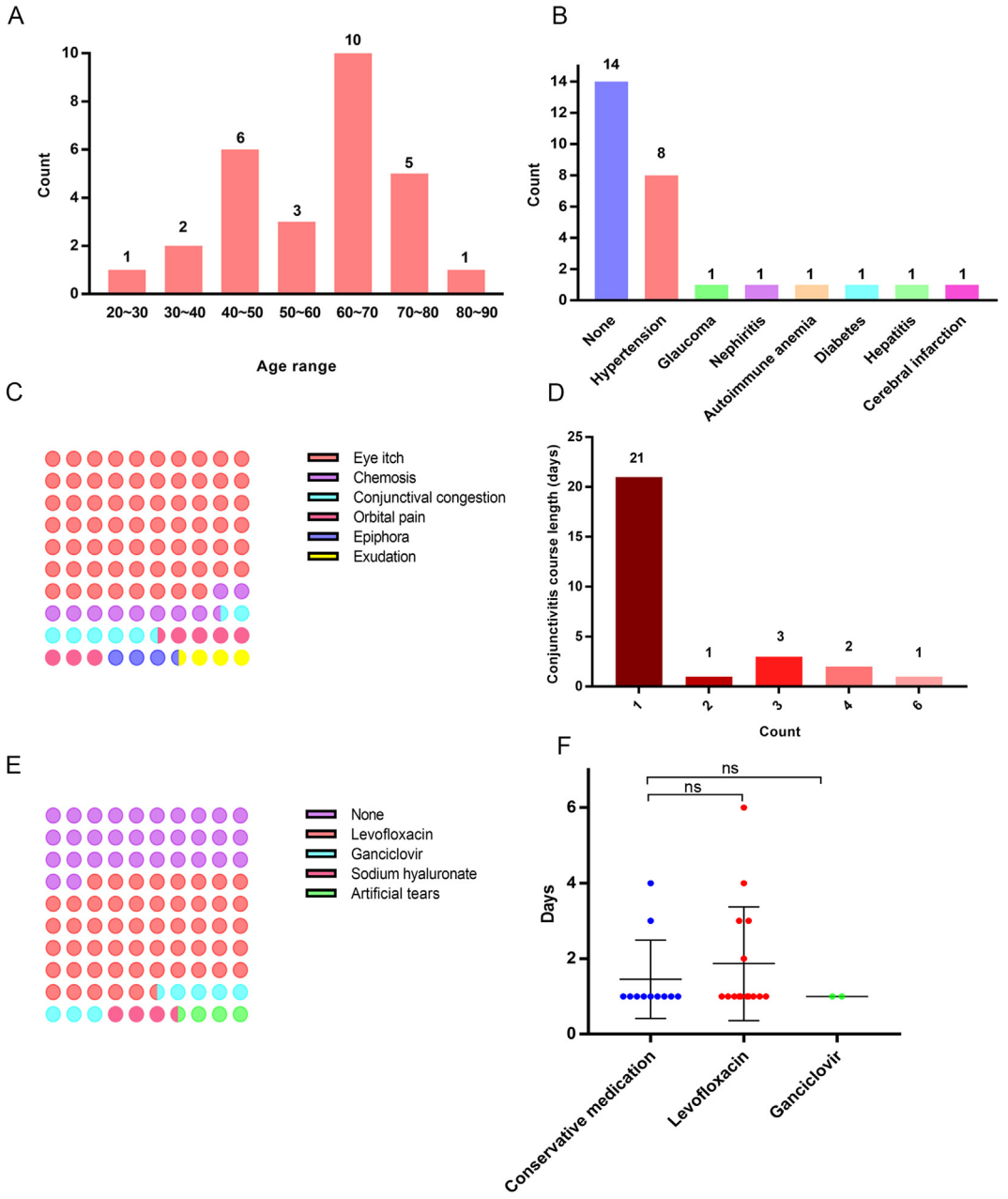


**Fig. 1.** Baseline information and clinical characteristics of 28 COVID-19 patients with conjunctivitis. A, The age distribution of 28 COVID-19 patients with conjunctivitis. B, The underlying diseases of 28 COVID-19 patients with conjunctivitis. C, Ocular symptoms and signs presented during SARS-CoV-2 infection. D, The distribution of conjunctivitis course length in 28 COVID-19 patients with conjunctivitis. E, Treatment of conjunctivitis among 28 COVID-19 patients with conjunctivitis since ocular symptoms onset. F, The comparison of conjunctivitis course length using different drug treatments in 28 COVID-19 patients with conjunctivitis. Ns, stands for no statistical significance.

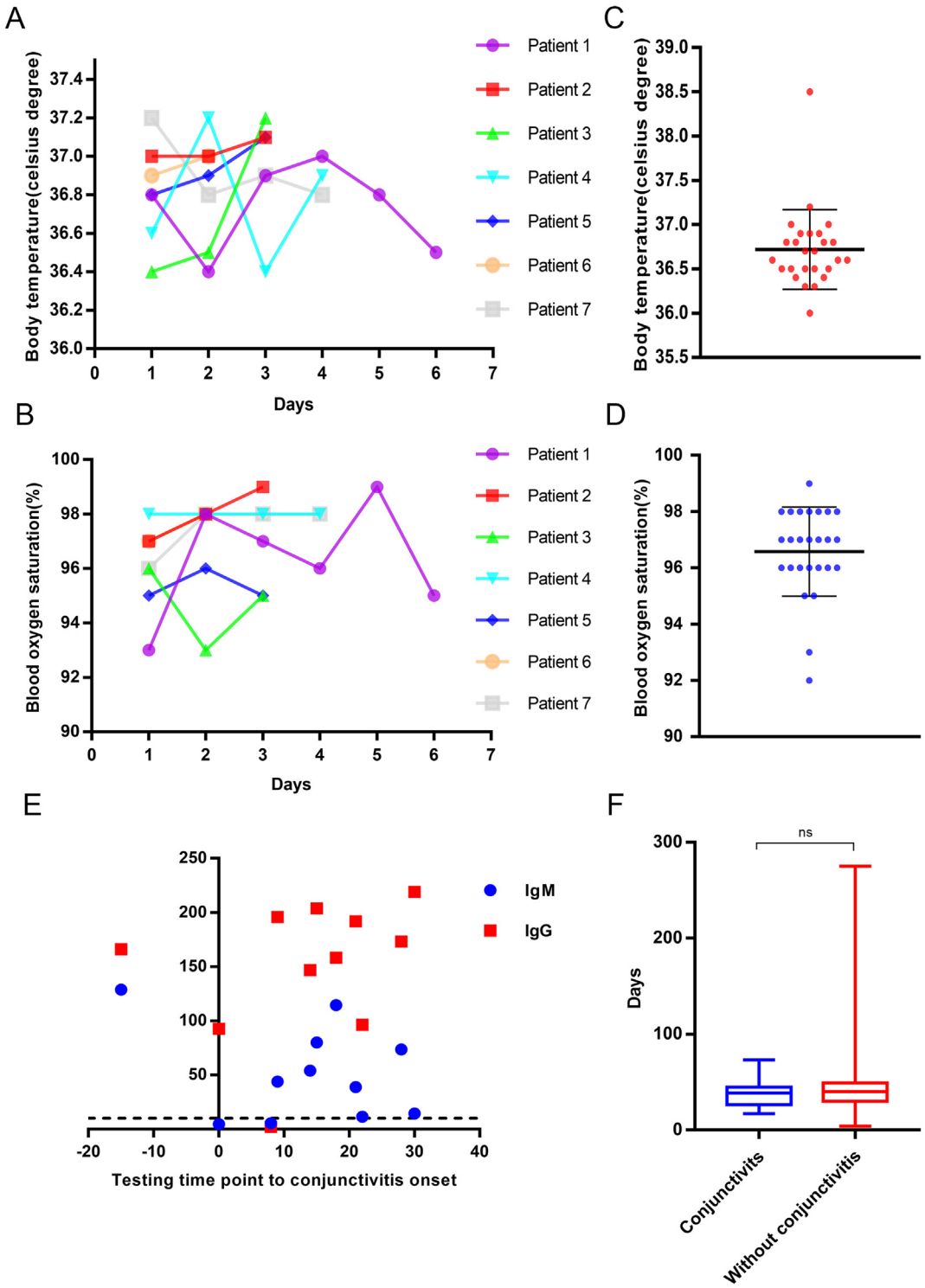


**Fig. 2.** The relationship between severity of COVID-19 and conjunctivitis onset. A, The change of body temperature among 7 patients whose conjunctivitis courses were over 1 day. B, The change of $SpO_2$ among 7 patients. C, The body temperature of 28 COVID-19 patients with conjunctivitis on the onset day of ocular symptoms. D, The $SpO_2$ of 28 COVID-19 patients with conjunctivitis on the onset day of ocular symptoms. E, The value of SARS-CoV-2 specific IgM and IgG of 11 COVID-19 patients with conjunctivitis. The value of pots over the dotted line was higher than normal. F, The comparison of COVID-19 course between patients with conjunctivitis and without. Ns, stands for no statistical significance.

tional patient was an 82 years old female whose body temperature was around 36.5 °C while SpO2 was around 99% during the whole COVID-19 course, no specific symptoms and signs were found during the whole disease course.

### 3.3. Conjunctivitis in COVID-19 patients – self-limited disease

Regarding the treatment of conjunctivitis, 15 patients (53.6%) received levofloxacin, 2 (7.1%) received ganciclovir, 1 (3.6%) had

sodium hyaluronate, 1 (3.6%) had artificial tears, and 9 patients (32.1%) were observed without treatments (Fig. 1E). All drugs for the treatment of ocular symptoms were in the form of eye drops. The conjunctivitis course didn't vary with different treatments (p = 0.6) (Fig. 1F). Compared with non-conjunctivitis patients, the whole clinical course of COVID-19 in patients with conjunctivitis showed no differences (p = 0.6) (Fig. 2F). Moreover, all conjunctivitis patients involved in this study had a favorable prognosis and were discharged from their hospitals. However, there were 5 patients taking cortisone during the ocular symptoms course while 4 of them experienced only 1 day of eye itching and another one had conjunctival oversecretion for 3 days. All 18 patients received traditional Chinese antiviral drugs during their whole stays at hospitals. Different combined antiviral therapies, including Arbidol (3), Cephalosporins, Quinolones, Macrolides and Recombinant human Interferon were applied in 5 patients whose ocular symptoms lasted only 1 day. These antiviral treatments might shorten the length of ocular discomfort and relieve the ocular symptoms.

### *3.4. Expression of SARS-CoV-2 entry receptors in the human ocular surface and mouse lacrimal glands*

As an important cellular entry receptor of SARS-CoV-2, angiotensin converting enzyme 2 (ACE2) expression was highly detected in the conjunctival epithelium, also on corneal epithelium and limbal cells (Fig. 3A, B, C) [7]. Transmembrane protease serine 2 (TMPRSS2) was expressed in fewer cells and was detected mainly in the conjunctival cells, serving as a crucial serine protease in SARS-CoV-2 infection (Fig. 3A, B, C) [7]. Comparing adult and fetal single-cell RNA seq analysis results, the expression of TMPRSS2 in fetal ocular surface was higher than adults' which may ascribe to the different sample sizes provided (Fig. 3B). However, the result was consistent with the higher incidence of ocular surface symptoms in children with COVID-19 [8]. Acting as a host factor for promoting SARS-CoV-2 infection, Neuropilin-1 (NRP1) was not detected in the ocular surface, which may due to the limited single cell RNA seq data published online [9,10]. Considering dipeptidyl peptidase 4 (DPP4) and aminopeptidase N (ANPEP) were identified as the cellular entry receptors of middle east respiratory syndrome coronavirus (MERS-CoV) and human coronavirus 229E (HCoV-229E), respectively, their expression patterns were also analyzed [11,12]. ANPEP expressed far more abundantly in the adult ocular surface than the fetal surface and the expression of DPP4 was quite low in both of them which may explain the few reports of MERS inducing ocular surface involvement under such a pandemic (Fig. 3B). The higher expression of ANPEP on the adult ocular surface may be able to explain why elderly were more susceptible to HCoV-229E than children and infants observed in previous report

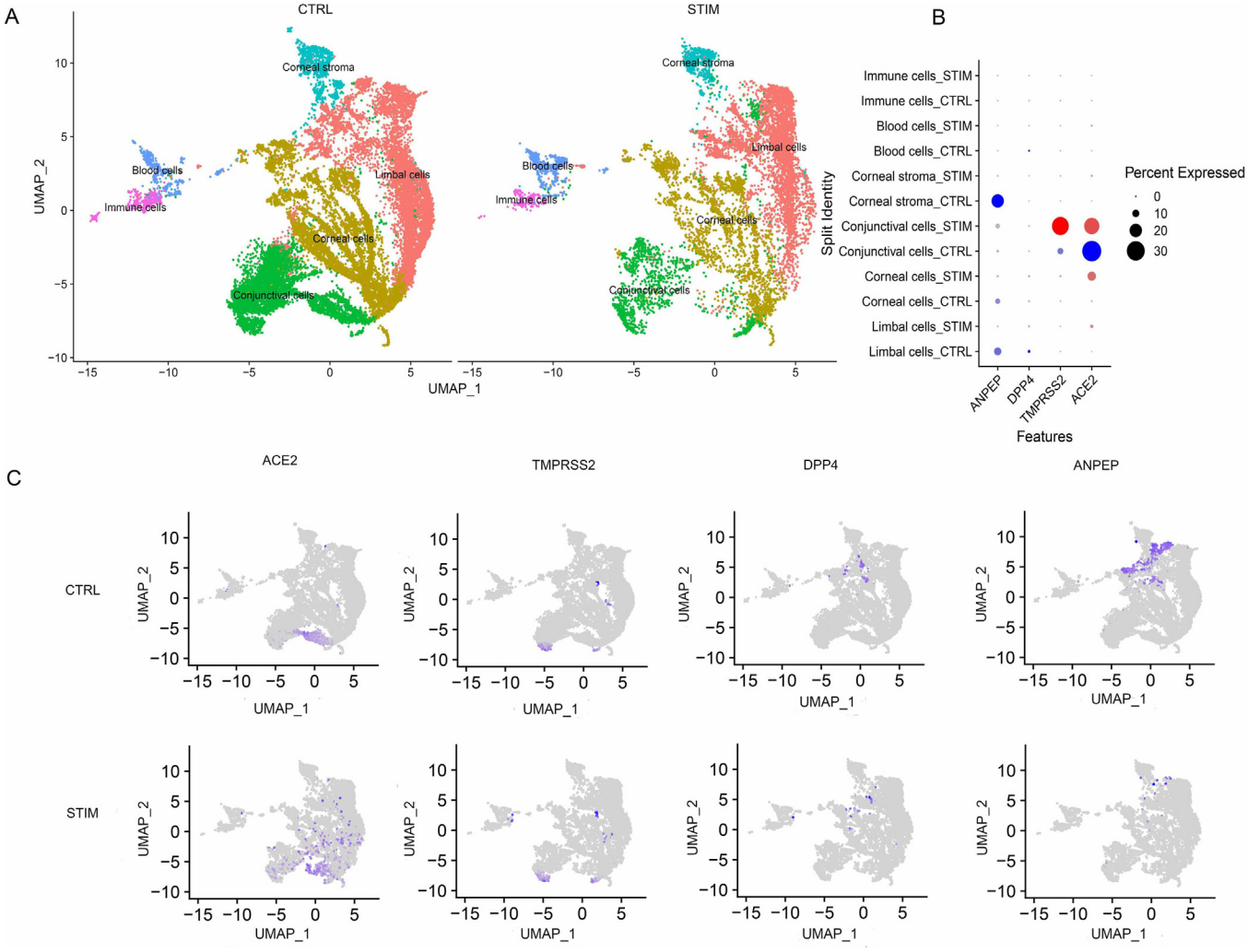


**Fig. 3.** Single-cell RNA seq analysis of human cornea-conjunctiva tissues of adults and fetuses. A, The UMAP cluster map shows the clustering of different cells in the cornea-conjunctiva tissues. B, Differences in percentage of expression of ACE2, TMPRSS2, ANPEP and DPP4 in cornea- conjunctiva cells of adults and fetuses. C, Expression and distribution of ACE2, TMPRSS2, ANPEP and DPP4 in different cell clusters in the cornea-conjunctiva tissues. CTRL, adult cornea-conjunctiva tissues; STIM, fetal cornea-conjunctiva tissues.

[13]. In the end, we proposed that HCoV-229E, SARS-CoV and SARS-CoV-2 possessed the ability to infect human beings through ocular routes.

To deduce the underlying mechanism of SARS-CoV-2 reserved in the lacrimal gland, the expression of Ace2, Nrp1 and Tmprss2 in mouse lacrimal glands was assessed. Ace2 and Tmprss2 expressed in the lacrimal gland at a low rate (Fig. 4), which may be due to the small sample size. However, whether or not these two crucial receptors expressed in the nasolacrimal ducts remains unclear due to the lack of nasolacrimal duct tissue dataset online. In contrast, Nrp1 expressed much more abundantly in the lacrimal glands which facilitates SARS-CoV-2 infectivity (Fig. 4) [9,10]. In addition, both Dpp4 and Anpep were expressed in lacrimal glands (Fig. 4), providing the theoretical basis for these two kinds of coronaviruses to harbor in lacrimal gland and replicate.

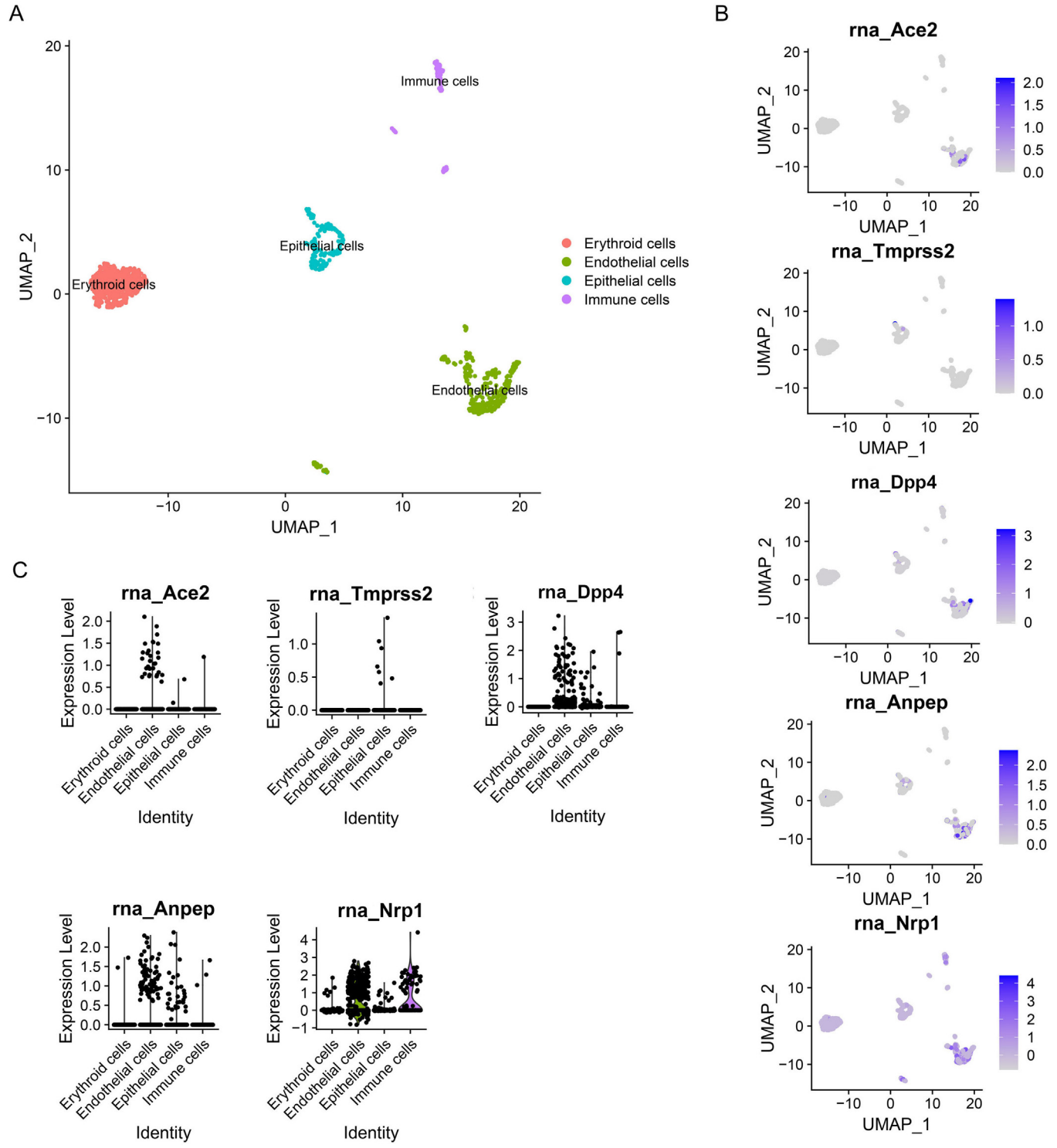


**Fig. 4.** Single-cell RNA seq analysis of mouse lacrimal glands. A, The UMAP cluster map shows the clustering of different types of cells in the lacrimal glands. B,C, Expression and distribution of Ace2, Tmprss2, Anpep, Dpp4 and Nrp1 in different cell clusters in the lacrimal glands.

## 4. Discussion

Unlike signs and symptoms of other systems, ocular involvement and manifestations are quite rare as found in our clinic data. It seemed that ocular involvement in COVID-19 did not affect the disease severity, nor the course length. The alterations of the sense of taste and smell revealed the neuroinvasiveness of SARS-CoV-2 which may also induce ocular symptoms, including dry eyes [14]. However, ocular symptoms were prominent in patients with other respiratory virus infection, such as avian influenza virus H7, adenovirus 37, measles, and coronaviruses (Supplementary Table 1) [15]. Notably, people wearing glasses were less likely to infect SARS-CoV-2 which suggested virus might be able to invade the body through ocular routes [16]. The nasolacrimal duct serves as an anatomical bridge connecting ocular surface and respiratory tract, permitting the virus to get access to respiratory tissues from ocular surface by tears. Research in the rhesus macaques has revealed that SARS-CoV-2 can be transported from the ocular surface to the respiratory tract by the tear flow, which may explain the positive RT-PCR results in tears in the early course of disease while it turns to negative results in the later period [17]. SARS-CoV-2 has been reported to be detected in the conjunctival swabs obtained from COVID-19 patients with or without ocular involvement [3,18]. However, SARS-CoV-2 positivity in patients with conjunctivitis was not high. The traditional collecting method of tears using Schirmer strips or disposable sampling swabs may limit the volume of tears been collected. Moreover, the viral load in conjunctival swabs was significantly lower than in nasopharyngeal swabs which may be explained by the natural barrage of eye structures, such as eyelids and eyelashes [19]. The presence of virus in conjunctival secretions may be transient so that tear analysis may not reveal the presence of SARS-CoV-2 [20,21]. Our single-cell RNA seq analysis didn't show receptor expression in details due to the limited data resources published online [4]. However, other research has demonstrated that the expression of ACE2 and TMPRSS2 in human ocular surface does exist, though lower than other organs, which still enables SARS-CoV-2 to bind with ocular epithelial cells and replicate on the ocular surface under specific circumstances [4,22]. A research reported the incidence of ocular surface symptoms in COVID-19 children was 22.7% (49 out of 216 patients) which was higher than in adults [8]. The symptoms included conjunctival discharge, eye rubbing, conjunctival congestion, ocular pain, tearing and eyelid swelling, which was also consistent with symptoms reported in our data. The higher incidence conjunctivitis among pediatric patients was consistent with the higher expression of TMPRSS2 and ACE2 on fetal ocular surface than adults. According to the clinical data analysis, the presence of conjunctivitis during viral infection was not common in COVID-19, as compared with other respiratory viruses infection [4,22,8]. Since the ocular symptoms did not affect the progression of COVID-19, ocular infection might not play an important role in patients' recovery and prognosis either. However, whether ocular route was a potential infection route during SARS-CoV-2 epidemic is still controversial while whether the antiviral components in tears prevent SARS-CoV-2 from binding with the receptors on the ocular surface is still unknown.

SARS-CoV-2 had been reported to be detected in tears by RT-PCR, highlighting the possibility of virus survival in human lacrimal gland [3]. SARS-CoV-2 uses ACE2 and TMPRSS2 as receptors to facilitate viral entry into cells, who both expressed in lacrimal glands, together with DPP4 and ANPEP, increasing its ability to survive or even replicate in the lacrimal glands. An embryonic study found that Tmprss2 enriched in non-neural ectoderm (NNE) in mice, from which cornea, conjunctiva, lacrimal gland, and nasolacrimal duct evolved [23]. Though the distribution of Ace2 and Tmprss2 in nasolacrimal ducts has not been reported, current evidence indicates that it is possible. Similar to mice, whether human lacrimal glands and nasolacrimal ducts possess ACE2 and TMPRSS2 or other coronavirus entry receptors is worth to be investigated since many kinds of coronavirus entry receptors are highly expressed through the respiratory tract, providing the theory of ocular transmission of coronavirus [24,25]. Further research will allow the collection of more detailed data to confirm the key component of ocular tropism of coronaviruses. We suggest that even though the ocular symptoms did not play a crucial role in COVID-19 progression, ocular surface was still an important potential infection route during SARS-CoV-2. Eye protection was thus needed to protect health workers from getting contaminant particles into their eyes.

Apart from SARS-CoV-2, there are many other respiratory viruses which have ocular tropism [15]. During SARS outbreak in Toronto, ocular surface exposure to contaminated body fluids and lack of eye protection were found to be associated with an increased risk of SARS-Co Vinfection [26]. While no ocular symptoms were reported during 2003 SARS outbreak, SARS-CoV was reported to be detected in three tear samples during the early phase of disease [2]. However, most of other studies failed to detect the virus in tears nor from conjunctival swabs collected from SARS patients. Similarly, virus was also rarely found in those samples in SARS-CoV-2 infected patients. In regard of COVID-19, the importance of ocular transmission route has been highlighted during the disease investigation. The virus receptor distribution on ocular surface may provide a new insight into the risk of ocular transmission. Besides respiratory viruses, the antigen and virus-specific antibody of several other families of viruses have also been reported to be detected on ocular surface, including human immunodeficiency virus (HIV), ebolavirus (EBOV) and human papillomavirus (HPV) (Supplementary Table 1) [27–29,31–53]. Especially EBOV, which has been demonstrated to infect rhesus monkeys through conjunctival exposure [30]. Moreover, EBOV can still be detected in a conjunctival swab obtained from patients 10 days after the clearance of viremia while the viral RNA was still detectable for up to 10 weeks after death in the conjunctiva, suggesting that the ocular route played an extremely important role in human-to-human transmission of EBOV [28]. While efforts to illustrate the exact ocular transmission route mechanism are still underway, it's still compelling to stress on eye protection against virus.

## Author contributions

J.W., J.W.S. and Y.Y. conducted data analysis and wrote the manuscript; P.C.W., T.L., Y.J.G. and J.Z. contributed the data collection; W.D., Y.H., P.R.Z., Q.Y.W., W.W.L. and Andrew Chen provided advice during the manuscript writing; C.Y.X. and X.Y.X. conceived the study.

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## Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

## Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.csbj.2021.01.039.

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