Distinct disease severity between children and older adults with COVID-19: Impacts of ACE2 expression, distribution, and lung progenitor cells

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Summary: Compared to children, ACE2 positive cells in the older adults are generally reduced and mainly distributed in lower pulmonary tract. The lung progenitor cells are also decreased. These risks may impact disease severity and recovery from pneumonia caused by SARS-Cov-2 infection in older patients.

Abstract

Background: Children and older adults with coronavirus disease 2019 (COVID-19) display a distinct spectrum of disease severity yet the risk factors aren't well understood. We sought to examine the expression pattern of angiotensin-converting enzyme 2 (ACE2), the cell-entry receptor for severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), and the role of lung progenitor cells in children and older patients.

Methods: We retrospectively analysed clinical features in a cohort of 299 patients with COVID-19. The expression and distribution of ACE2 and lung progenitor cells were systematically examined using a combination of public single-cell RNA-seq datasets, lung biopsies, and *ex vivo* infection of lung tissues with SARS-CoV-2 pseudovirus in children and older adults. We also followed up patients who had recovered from COVID-19.

Results: Compared with children, older patients (> 50 yrs.) were more likely to develop into serious pneumonia with reduced lymphocytes and aberrant inflammatory response (p = 0.001). The expression level of *ACE2* and lung progenitor cell markers were generally decreased in older patients. Notably, ACE2 positive cells were mainly distributed in the alveolar region, including SFTPC positive cells, but rarely in airway regions in the older adults (p < 0.01). The follow-up of discharged patients revealed a prolonged recovery from pneumonia in the older (p < 0.025).

Conclusion: Compared to children, ACE2 positive cells are generally decreased in older adults and mainly presented in the lower pulmonary tract. The lung progenitor cells are also decreased. These risk factors may impact disease severity and recovery from pneumonia caused by SARS-Cov-2 infection in older patients.

Keywords COVID-19, disease severity, patients' ages, ACE2 expression and distribution, lung progenitor cells.

Introduction

The pandemic of Coronavirus Disease 2019 (COVID-19) due to the severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) infection has affected over 73 million people over 1,630,000 of whom have died in the last 10 months (https://coronavirus.jhu.edu).[1] Currently, many therapeutic strategies are being explored in ongoing clinical trials, including antiviral drugs, biological response modifiers, and RAAS inhibitors. Ultimately, the development of effective vaccines is key to combat COVID-19 and appropriate measures are being taken [2, 3] Unfortunately, the understanding of how SARS-CoV-2 infection-provoked lung injury is not clear. Epidemiological studies indicate a broad spectrum of disease severity in children and older adults. Compared with the older adults, children and young adults generally display much milder symptoms with a lower mortality (< 1%), whereas mortality can be as high as 15% in older adults (> 50 yrs.).[4] The underlying factors for this variance in disease severity remain elusive.[5, 6] Lung is a main target of SARS-CoV-2. Pathological changes after SARS-CoV-2 infection include extensive necrotizing bronchiolitis and severe alveolitis.[7] Lung repair is associated with lung progenitor cells-modulated regeneration,[8] and ACE2 is the cell-entry receptor of SARS-CoV-2.[9] Nonetheless, little is known about the differences in clinical features, laboratory parameters, profiles of *ACE2*, and lung progenitor cells between infected children and adults. Several reports suggest that *ACE2* expression increases with age, particularly in adults who smoke or have lung cancer.[10, 11] The increased *ACE2* expression in adult lungs is thought to be a risk factor for the consequences of COVID-19.[12] Nevertheless, there is no direct evidence to support this assumption. In this study, we retrospectively analysed the clinical characteristics of 173 children and 126 adult patients with COVID-19. Meanwhile, we examined *ACE2* expression, distribution, and lung progenitor cells in lung biopsy samples from 26 children and 24 adults.

Methods

Study Design and Patients

Patients with COVID-19 were enrolled from four hospitals in China, including Taihe Hospital of Hubei University of Medicine, the Third People's Hospital of Shenzhen, Wuhan Children's Hospital and Guangzhou Women and Children's Medical Centre. Patients who fulfilled the following criteria were included: (1) diagnosed with laboratory-confirmed COVID-19 according to the WHO guideline [13] and the recommendation of the National Health Commission of the People's Republic of China (NHC).[14]; (2) age $\leq$ 80 years; (3) written informed consent was obtained. Briefly, SARS-CoV-2 was detected by quantitative polymerase chain reaction (qPCR) with samples from nasopharyngeal swabs. Infection was defined as at least two positive test results. Disease severity was classified into five levels from asymptomatic to critically ill according to the recommendation of NHC and WHO guidelines.[14] The medical history of adult patients was summarized in Supplementary Table 1. Clinical data, laboratory parameters, radiological findings were acquired from electronic medical records. The data collection forms were reviewed independently by 3 researchers. This study was approved by the Institutional Review Board of the four hospitals respectively. Written informed consent was obtained from patients and/or guardians before data collection.

Pseudovirus generation and infection

The coding-sequence of spike protein (SARS-CoV-2, QHR63250) was cloned into pVAX-1 plasmid upon verification of Sanger-sequencing, and transfected into 293T cells with human immunodeficiency virus type I pNL4-3GFP$^+$ Env$^-$ Vpr$^-$ backbone to package GFP-expressed SARS-CoV-2 pseudovirus as previously described.[15] 48 hours later, pseudovirus-containing supernatant was collected and frozen at -150 °C. For *ex vivo* infection, lung tissue from children and older patients were cut into small tissue blocks (2-3 mm^3) and incubated with pseudovirus supernatant in 24 well tissue culture plates for 48 hours or 293T culture medium. Green fluorescent protein (GFP) and ACE2 were detected by IHF staining of frozen sections.

Immunostaining

Paraffin-embedded sections were deparaffinized and incubated with primary antibodies at 4°C overnight, and secondary antibodies at room temperature for 1h as described previously.[16] Pseudoviral-infected tissue was embedded for cryosection. After rehydration, tissue sections were incubated with primary antibodies (4°C overnight), secondary antibodies (1h, room temperature) and DAPI. To exclude non-specific fluorescent staining of ACE2, only second antibody IgG with fluorescent conjugation was simultaneously used in slides as negative control and the ACE2 immunostaining in kidney sections was set as a positive control.[17] Fifty lung tissue biopsy samples in which 26 are from children and 24 from adults, were used for immunostaining against ACE2. $ACE2^+$ cells were captured at different angles to generate 20 random views in a single lung slide for each patient. All slides with positive immunostaining signals were determined by two independent reviewers. The distribution of $ACE2^+$ cells was determined as the average cell number per 0.025 cm^2 lung tissue.[18] Antibodies are described in the supplementary materials.

Bioinformatic data of single-cell sequencing in lung cells.

Twelve single-cell RNA-sequencing datasets of healthy lung tissues from three studies were used to explore the co-expression of genes. Four samples, “LMEX0000001623” (Homo sapiens, 1-day old, 2,342 cells), “LMEX0000001624” (Homo sapiens, 1-day old, 2,000 cells), “LMEX0000001625” (Homo sapiens, 21-month old, 2,000 cells), and “LMEX0000001626” (Homo sapiens, 9-year old, 2,000 cells) were obtained from the LungMAP Consortium [U01HL122642] and downloaded from (www.lungmap.net) on May 4th, 2020.[19] Five samples, “Hum1” (Homo sapiens, 76-year old, 2,193 cells), “Hum2” (Homo sapiens, 88-years old, 2,997 cells), “098C” (Homo sapiens, 41-years old, 2,849 cells), “133C” (Homo sapiens, 32-years old, 2,074 cells), and “222C” (Homo sapiens, 65-years old, 2,825 cells) were from GEO accession number GSE133747.[20] Three samples, “Patient 1” (Homo

sapiens, 75-years old, 3,987 cells), "Patient 2" (Homo sapiens, 46-years old, 4,606 cells), and "Patient 3" (Homo sapiens, 51-years old, 3,714 cells) were obtained from the Human Lung Cell Atlas project.[21] The raw read counts of each gene in each cell were downloaded in either the "tab-delimited table", "Matrix Market" or "Seurat" format, and loaded and processed using R 3.6.1. We aggregated the reads from all cells in each sample and normalized the expression of each gene using TPM (Transcripts Per Million). The coefficients were calculated using the "GGally" and visualized using "ggplot2".

Statistical analysis

Kruskal-Wallis test was utilized to analyse the blood and laboratory biochemical measurements in COVID-19 patients. The distribution of age and clinical severity, follow-up data, and *ex vivo* infection data were examined by Chi-square test or Fisher's exact test. *ACE2* expression and distribution were analysed by students' *t* test or ANOVA. qPCR results were analysed using unpaired *t* test. Data are presented as mean ± SD, and $p < 0.05$ was considered significant. Statistical analysis was performed using SPSS software (version 20.0, IBM, Armonk, NY, USA).

Results

A different spectrum of disease severity in young and older patients with COVID-19

In this retrospective study, 299 patients with COVID-19 were enrolled from four different hospitals in China between January 17, 2020 and March 25, 2020 (Figure 1A), including 173 children and 126 adult patients (Supplementary Table 1). There was no remarkable difference between males and females on the distribution of ages (Chi-square test, $p = 0.995$; Figure 1B), whereas a significant relationship was revealed between disease severity and age (Figure 1B, Chi-square test, $p = 0.001$). First, patients aged over 50 yrs.-old were more likely to develop into severe (35.1%) and critical

(2.7%) pneumonia, whereas only 0.6% in severe and no critical pneumonia observed in patients aged below 16 yrs. Second, the older patients had more severe symptoms comparing to the counterpart of children patients, including fever, cough, and sore throat (Chi-square or Fisher's exact test, $p < 0.05$). Third, ICU admission was more common in older patients (Chi-square test, $p = 0.001$). Our results suggested older patients (> 50 yrs.) were more vulnerable to COVID-19 than younger adults and children.[4]

Compared with children, white blood cell, lymphocyte, and platelet count were significantly decreased in adult patients with COVID-19, while the number of neutrophils, neutrophil over lymphocyte ratio, high-sensitivity C-reactive protein (hs-CRP), prothrombin time (PT), fibrogen (FIB), and total bilirubin were increased (Figure 2, Kruskal-Wallis test, $p < 0.0001$). Moreover, compared with children, alanine aminotransferase, creatine, and creatine kinase significantly increased in adult patients (Figure 2). These laboratory findings indicated that adult patients were more sensitive to SARS-CoV-2 infection with multiple organs affected, which agreed with the previous report.[22]

The distinct expression and distribution of ACE2 in lungs of children and older patients

ACE2 is a cell-entry receptor for SARS-CoV-2 to invade the human respiratory system.[23] Recent studies indicate the high vulnerability of older adults to COVID-19 are possibly associated with ACE2 expression in the respiratory system.[11, 12, 22] To assess the role of *ACE2* in SARS-CoV-2 infection and the potential relation between *ACE2* expression and disease severity, we examined the spectrum of *ACE2* expression and distribution in the lung tissue of age-matched children and adults (Supplementary Table 2 and 3).

First, a histological examination was performed to ensure the quality of structures of bronchus and alveolus presented in patient's lung tissue sections (Figure 3A i, ii, vii, and viii). Compared with children's lung sections, shrivelled epithelial layer, atrophic muscle layer, and reduced ciliated cells

were presented in the bronchus of older adults, indicating bronchus degeneration with aging [24]. Second, lung tissue sections were subjected to immunostaining for human ACE2 using kidney as a positive control, and isotype IgG was used to exclude non-specific signals (Figure 3A iii-vii and x-xiv). The expression and distribution of ACE2 were determined in 26 lung samples from children (aged 2 months to 12 yrs.) and 24 lung samples from adults (aged 16 yrs. to 80 yrs.). The results revealed that ACE2 positive ($ACE2^+$) cells were sporadically distributed in all lung regions, including the bronchus (Br) and pulmonary alveolar (PA) areas (Figure 3A iii-v and x-xii). Due to increasing mortality in COVID-19 patients aged over 10 years,[4] samples were grouped as 0-10Y, 10-50Y, 50-60Y, 60-70Y and > 70Y based on patient ages, at least 3 patients' lung samples in each grouped age were subjected to analysis. 20 images were randomly captured from different view-fields of the slide for each sample.[18] Unexpectedly, our results indicated that $ACE2^+$ cells were highly enriched in lung tissues of children and gradually decreased with increased age. Of note, $ACE2^+$ cells were significantly decreased in older adults (50-70 yrs.) compared with children (Figure 3B i, One-way ANOVA, $p = 0.001$), but there was no remarkable difference between 10-50 yrs.-group and 0-10 yrs.-group (Figure 3B i, One-way ANOVA, $p > 0.05$). In a comparison of $ACE2^+$ cells between 26 children (< 16 yrs.) and 14 older adults (> 50 yrs.), it revealed significantly decreased $ACE2^+$ cell numbers in older adults (Figure 3B ii, Mann-Whitney test, $p < 0.0001$), in agreement with the reduced *ACE2* mRNA expression level detected in the lung biopsy samples from age-matched children and older adults (Figure 3B iii, Mann-Whitney test, $p = 0.05$, n = 5 respectively). These results indicate that the ACE2 expression level is irrelevant to the distinct spectrum of disease severity between children and older adults.

Because SARS-CoV-2 could infect $ACE2^+$ cells in the regions of bronchus and alveoli, we further examined the distribution of $ACE2^+$ cells only expressed in these regions in the lungs of children and older adults. We observed a reduction of $ACE2^+$ cells in the bronchial region with increased age but

no such reduction in the pulmonary alveolar region (Figure 3C i, Kruskal-Wallis test, p = 0.0005; Figure 3C ii, Kruskal-Wallis test, p = 0.0986), indicating that the alveolar region in older adults is at high-risk of SARS-CoV-2 infection. When compared with children (< 16 yrs.), $ACE2^+$ cell number was remarkably decreased in older adults (> 50 yrs.) in the bronchial region (Figure 3C iii, One-way ANOVA, p < 0.0001), but not obviously so in the alveolar region (Figure 3C iii). With degenerative changes of the bronchus in older individuals, $ACE2^+$ cells were mainly located in the alveolar region that may facilitate easy infection at the lower respiratory tract by SARS-CoV-2 and develop into severe pneumonia.

Profile of Lung progenitor cells in Children and Older Adults

Lung progenitor cells play a putative role in lung development, injury and repair following SARS-CoV-2 infection and influenza virus-induced respiratory failure.[25, 26] Recent studies indicate that *ACE2* is expressed in certain lung cells including lung progenitor cells at different stages of lung development.[19-21] To evaluate the expression profile of *ACE2* in lung progenitor cells that may contribute to the disease severity of COVID-19 in children and older adults, we analysed the co-expression of *ACE2*, *TMPRSS2*, and lung progenitor marker genes using three public single-cell RNA-seq datasets of human lungs, including 12 samples from 4 children, 3 young adults and 5 elder adults [19-21]. Pearson correlation coefficient (PCC) was used to predict the possibility of co-expressions among each pair of "*SFTPC*", "*PDPN*", "*ACE2*", "*TMPRSS2*", "*KRT5*", "*TP63*", "*SOX2*", "*NKX2-1*", "*SOX9*" and "*SCGB1A1*" based on their TPM in 12 samples of datasets in tissue level[27]. The results showed that most of lung progenitor genes were not strongly coupled with *ACE2* in lung tissues except for *SFTPC* and *PDPN* (Figure 4A i, PPC value >0.5). Second, we counted the percentage of cells with at least two reads in "*ACE2*", "TMPRSS2", and another progenitor

marker gene for each sample. 12 sets of samples were grouped together in a box plot to evaluate the co-expressions of *ACE2* and each lung progenitor gene in individual cells. The results suggested that *ACE2* was mainly expressed in the type II alveolar cell (AT II, $SFTPC^{+}$) with a high possibility (Figure 4A ii). Taken together, our data analysis suggests that *ACE2* is mainly detected in AT II cells but rarely in type I alveolar cell (AT I cells, $PDPN^{+}$) and other lung progenitor cells (*KRT5*, *TP63*, *SOX2*, *SOX9*, *SCGB1A1*, and *NKX2-1* expressed cells.).

Next, immunostaining was performed to confirm the bioinformatic analysis using paraffin-embedded lung tissue sections from children and older adults. In agreement with the above bioinformatic analysis, ACE2 was only detected in SFTPC positive cells but rarely KRT5, NKX2.1, SOX2, TP63, PDPN, or SCGB1A1 positive cells (Figure 4B). The SFTPC positive cells serve as AT II cells, while basal, bronchial, and bipotent progenitor cells ($KRT5^{+}$/$TP63^{+}$, $NKX2.1^{+}$/$SOX2^{+}$, $SCGB1A1^{+}$/$SFTPC^{+}$ or $PDPN^{+}$/$SFTPC^{+}$) can differentiate into bronchial or AT II cells during lung injury-repair or disease.[28-30] These results highlighted that these progenitor cells may not be main targets by SARS-CoV-2. Finally, the expression level of progenitor marker genes was examined in lung tissues of children and older adults by qPCR. Compared with children, dramatically reduced mRNA expression of lung progenitor markers was observed including *KRT5, KRT8*, *TP63*, *NKX2.1*, *SOX2*, *SOX9*, *ATNX1*, and *SCGB1A1* in lung tissues of older adults, indicating a weaker regenerative potential in older adults (Figure 4C, unpaired *t* test, $p < 0.05$).

Transmembrane Serine Protease 2 (TMPRSS2), the co-factor for viral entry upon SARS-CoV-2 infection, was abundantly expressed in a variety of cells including in human lung tissues. [31-35] We further examined the co-expression profiles of *TMPRSS2*, *ACE2*, and lung progenitor marker genes by using published single cell RNA sequencing datasets. In line with previous reports, [32-35] it revealed *TMPRSS2* was widely expressed in almost all lung cells (Supplementary Figure 1A). *TMPRSS2* was also detected in $ACE2^{+}$ cells although ACE2 positive cells are generally few in lung tissues (Figure

4A i, Supplementary Figure 1A and 1B). Importantly, our results indicated that *TMPRSS2* was widely expressed in human lung tissue, including bronchus, submucosa, and alveoli, and no significant difference between children and older adults (Supplementary Figure 1C i-iii). These data suggested TMPRSS2, different from ACE2 which was mainly expressed in AT II specific cells, was much more abundantly expressed in lung tissues.

***Ex vivo* infection with SARS-CoV-2 pseudovirus and follow-up of patients with COVID-19**

We used a spike driven GFP-expression SARS-CoV-2 pseudovirus to determine the capacity of SARS-CoV-2 infection in fresh lung tissues from children and older adults. These lung biopsies were obtained from three children (< 10 yrs.-old) and three older adults (> 50 yrs.-old) respectively during surgery for lung abscess disease and lung cancer. After 48 hours of SARS-CoV-2 infection at a concentration of 1×10^5 TU, we examined the infection capacity of SARS-CoV-2 in lung tissues by GFP signal. Only ACE2 but no GFP signal was detected in control lung tissue without SARS-CoV-2 infection (Figure 5A, i and v). In contrast, lung tissues infected with SARS-CoV-2 pseudovirus presented strong co-expression of GFP and ACE2 signals. In addition, GFP positive cells were exclusively found in $ACE2^+$ cells, and no GFP positive cell was found in ACE2 negative cells. This suggests that $ACE2^+$ cells are the main targets of SARS-CoV-2 in COVID-19. Importantly, no difference was found in the susceptibility of lung tissues to SARS-CoV-2 infection between children and the older (Figure 5A; Figure 5B, $p = 0.9$). Almost all $ACE2^+$ cells (red) were infected by SARS-CoV-2 pseudovirus (green) in lung tissues, suggesting children and older adults are similarly susceptible to SARS-CoV-2 infection.

To further evaluate the capacity of lung repair in children and older adults, a 2-month follow-up was conducted after discharge in 12 children (aged 2 months to 15 yrs.), 17 young adults (aged 18-50 yrs.), and 10 older patients (aged 52-75 yrs.) with moderate pneumonia. The data revealed a remarkable difference in their recovery capacity from pneumonia (Figure 5C-E, Chi-square test, $p = 0.0025$). Nine (9/12; 75%) children had completely recovered from pneumonia, while three (3/12;

25%) children were considered partially recover since the lesion in lung was not absorbed completely based on the radiological images. In contrast, only one (1/10; 10%) older patient recovered completely, while 9 (9/10; 90%) patients recovered partially with residual lung lesions. Four (4/17, 23.5%) patients in the young adults recovered completely, while 13 (13/17, 76.5%) patients recovered partially (Figure 5D, E). Notably, of the 10 older patients, one had evidence of lung fibrosis detected on a CT scan (Figure 5C).

Discussion

Among patients suffered from SARS-CoV-2 infection, *the older* are *more likely to* become severely and critically ill with higher mortality than children and young adults.[22] Older patients with comorbidities and acute respiratory distress syndrome (ARDS) are at risk of increased mortality.[36, 37] Nonetheless, little direct evidence was available about the spectrum of disease severity linked with the expression pattern of ACE2 and lung progenitor cells. ACE2 is the cell-entry receptor for SARS-CoV-2, and lung progenitor cells are critically involved in lung repair and regeneration after injury. We systematically assessed the profile of ACE2 and lung progenitor cells in children and older patients using a combination of clinical cohort analysis, public single-cell RNA-seq datasets, and lung biopsies. This study presents several key findings.

First, in patients who suffered from SARS-CoV-2, older patients (> 50 yrs.) were particularly vulnerable to COVID-19 (Figure 1). In those aged above 50 yrs., 35.1% and 2.7% developed into severe and critical pneumonia respectively, compared with children (< 16 yrs.-old) of whom only 0.6% presented with severe pneumonia and the others suffered a mild or moderate illness. This distinct variance in disease severity between children and older patients was also reflected by the laboratory parameters of inflammation (Figure 2).

Second, our study revealed the patterns of ACE2 expression and distribution in lungs were different in children and older patients. Higher expression levels of ACE2 were remarkably presented in children's lungs in our clinical samples compared with adult patients including those patients with smoking and cancer history (Supplementary Table 2 and 3, Figure 3). Our finding suggests that the ACE2 expression level is not sufficient to determine the higher vulnerability of COVID-19 in older patients. Recent reports indicated that ACE2 was highly expressed in the sinonasal cavity and pulmonary alveoli, proposing sites of ACE2 distribution were more important in disease transmission and disease severities in patients with COVID-19.[38] We therefore examined sites of ACE2 distribution and found a remarkable difference in the distribution of ACE2 between children and older adults. $ACE2^{+}$ cells were mainly located in the alveolar region and notably decreased at the bronchus in the older (Figure 3B). *ex vivo* infection of pseudoviral SARS-CoV-2 indicated the similar susceptibility of ACE2-expressed cells in the alveolus between children and older adults (Figure 5A-B).

Several contrasting findings exist in the literature on ACE2 and ages as well as cigarette exposure.[39, 40] Some prior bioinformatic studies indicated that the *ACE2* expression in the respiratory system was increased with ages, smoking or cancers, arising an assumption that the higher ACE2 expression contributes to the high-risk of COVID-19 in older individuals.[10, 11, 41] [42, 43] On the other hand, other reports indicated ACE2 expression levels were not changed with ages.[44] It is difficult to compare our results with above reports because most of these bioinformatic studies were mainly focused on adult population RNA-seq datasets without comparison to children's samples parallelly, and no clear information for disease conditions used for studies. We examined ACE2 expressions in 26 children and 24 adults' lung tissues at both transcription and protein levels with negative (IgG) and positive controls (Kidney). The smoking factor was also considered. Our findings indicated that compared to children, the older adults

present generally reduced ACE2 amount and expression levels, and these ACE2 positive cells mainly were detected in the lower pulmonary tract.

It was reported that compared with the adult population, children with COVID-19 generally display milder symptoms and lower mortality. [4, 45] The discrepancy between the abundant expression ACE2 in paediatric patients that generally display milder symptoms and lower mortality as compared to aged adults has not been fully understood. One possibility is that the milder disease in paediatric patients with SARS-CoV-2 infection might be associated with the different patterns of immune responses.[5] Our clinical anlaysis also indicated that high sensitivity C-reactive protein (hs-CRP) level was significantly lower in paediatric patients with COVID-19 than older adult counterparts (Figure 1B). In addition, although *ACE2* is expressed in various human tissues and organs, ACE2-expressing organs do not equally participate in COVID-19 pathophysiology, indicating that other mechanisms are also involved in orchestrating cellular infection resulting in tissue damage.

Third, lung progenitor cells were reduced in the older compared with children. It is essential to maintain sufficient lung progenitor cells for lung development and repair.[46-48] ACE2 marker was in SFTPC^{+} AT II cells, but not in lung progenitor cells including *TP63*, *KRT5*, *SOX2*, *PDPN*, *NKX2.1*, or *SCGB1A1* positive cells (Figure 4). SFTPC presented in AT II cells is also considered as bipotent progenitor cells capable of differentiating into AT I cells during the process of lung injury-repair.[28-30] Although ACE2/SFTPC AT II cells are targeted by SARS-CoV-2 infection during the subsequent inflammatory response,[49] other putative lung progenitor cells without expression of ACE2 should not be attacked by SARS-CoV-2. However, decreased lung progenitor cells in the older limit their capacity for lung repair and regeneration. Our follow-up study also revealed that infected older patients displayed a poorer recovery from pneumonia (Figure 5C-E).

There are several limitations in this study. First, a histological study in normal areas of lung tissue that was adjacent to diseased tissue. Whether diseases such as lung abscess or lung

cancer, affect ACE2 expression in normal areas of lung tissue requires further investigation. Second, the relationship between age and ACE2 expression remains controversial. It could not exclude the possibility that such a difference could be affected by selected tissues from older or younger individuals. Additionally, it is possible that ACE2 expression within single-cell types correlates with age, but such differences are not reflected in protein level analysis. Third, at the current stage, only 12 children and 27 adult patients were in the followed-up study. Long term follow-up of more patients will provide more putative information. Nevertheless, this study is the first time to characterize ACE2 expression and distribution as well as the profile of lung progenitor cells in children and older adults.

In summary, compared to children, older patients are more vulnerable to develop severe pneumonia with poor recovery potential from COVID-19. Older patients present generally reduced ACE2-expressing cells and were mainly distributed in the lower pulmonary tract. The lung progenitor cells were also reduced in older adults. These risk factors may be in part contribute to distinct disease severity between children and older patients following SARS-CoV-2 infection.

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Author Contributions

Qizhou Lian supervised the whole project. Qizhou Lian and Zhao Zhang conceptualized and designed the study, collected data, wrote, and revised the manuscript; Che Zhang, Li Huang and Huimin Xia collected clinical data; Zhao Zhang and Liyan Guo collected and analysed the experimental and clinical data with the help from Che Zhang, Li Huang, Jinqiu Zhang, Jieying Wu, Ziyue Li, Xiaoya Zhou, Chui Yan Ma, Xiaoxian Zhang, Jie liu, and Jinjian Yang; Liyan Guo ran the qPCR; Drs Huimin Xia, Che Zhang, Xiaoxia Lu, Xianfeng Wang, collected clinical data with the help of Huiying Liang, Liang Zeng, Yi Xu, Jianbo Shao, Le Li, Wei Liu, Hui Du, Yanheng Wu ,Guangyun Yu, and Junhui Chen; Ruibang Luo performed bioinformatic data analysis; Ka Yi Kwan, Li Liu, Zhiwei Chen provided SARS-CoV-2 pseudovirus for tissue infections; Drs Hua Jiang, Can Liao, Huanhuan Joyce Chen, and Hung-fat Tse provided resources and critically reviewed and revised the manuscript for important content; and all authors approved the final manuscript as submitted and agree to be accountable for all aspects of the work.

Declaration of Interests

The authors declare no competing interests.

Data availability statement

Data are available upon reasonable request. Deidentified participant data.

References

1. Dong E, Du H, Gardner L. An interactive web-based dashboard to track COVID-19 in real time. Lancet Infect Dis **2020**;20:533-534..
2. Polack FP, Thomas SJ, Kitchin N, et al. Safety and Efficacy of the BNT162b2 mRNA Covid-19 Vaccine. N Engl J Med **2020;** doi: 10.1056/NEJMoa2034577.
3. Palacios R, Patino EG, de Oliveira Piorelli R, et al. Double-Blind, Randomized, Placebo-Controlled Phase III Clinical Trial to Evaluate the Efficacy and Safety of treating Healthcare Professionals with the Adsorbed COVID-19 (Inactivated) Vaccine Manufactured by Sinovac - PROFISCOV: A structured summary of a study protocol for a randomised controlled trial. Trials **2020**; 21: 853.
4. Wu Z, McGoogan JM. Characteristics of and Important Lessons From the Coronavirus Disease 2019 (COVID-19) Outbreak in China: Summary of a Report of 72314 Cases From the Chinese Center for Disease Control and Prevention. JAMA **2020**; 323:1239-1242.
5. Brodin P. Why is COVID-19 so mild in children? Acta Paediatr **2020**; 109:1082-1083.
6. Carsetti R, Quintarelli C, Quinti I, et al. The immune system of children: the key to understanding SARS-CoV-2 susceptibility? The Lancet Child & Adolescent Health **2020**; 4: 414-416.
7. Weiren Luo HY, Jizhou Gou, Xiaoxing Li, Yan Sun, Jinxiu Li, Lei Liu. Clinical pathology of critical patient with novel coronavirus pneumonia (COVID-19). Preprints 2020;2020020407.
8. Basil MC, Katzen J, Engler AE, et al. The Cellular and Physiological Basis for Lung Repair and Regeneration: Past, Present, and Future. Cell Stem Cell **2020**; 26:482-502.
9. Chan JF, Yuan S, Kok KH, et al. A familial cluster of pneumonia associated with the 2019 novel coronavirus indicating person-to-person transmission: a study of a family cluster. Lancet **2020**; 395: 514-23.
10. Cai G, Bosse Y, Xiao F, Kheradmand F, Amos CI. Tobacco Smoking Increases the Lung Gene Expression of ACE2, the Receptor of SARS-CoV-2. Am J Respir Crit Care Med **2020**.
11. Lukassen S, Chua RL, Trefzer T, et al. SARS-CoV-2 receptor ACE2 and TMPRSS2 are primarily expressed in bronchial transient secretory cells. EMBO J **2020**: e105114.
12. AE G-R. Is the ACE2 Overexpression a Risk Factor for COVID-19. Arch Med Res **2020**; pii: S0188-4409(20)30378-7.
13. Organization WH. Clinical management of severe acute respiratory infection when novel coronavirus (2019-nCoV) infection is suspected. https://wwwwhoint/publications-detail/clinical-management-of-severe-acute-respiratory-infection-when-novel-coronavirus-(ncov)-infection-is-suspected Accessed 28 May 2020.
14. Commission NH. National Health Commission of the People's Republic of China. Interim diagnosis and treatment of 2019 novel coronavirus pneumonia. 7th ed. March 3, 2020. Accessed March 4, 2020.
15. Liu L, Wei Q, Lin Q, et al. Anti-spike IgG causes severe acute lung injury by skewing macrophage responses during acute SARS-CoV infection. JCI Insight **2019**; 4.

16. Li X, Zhang Y, Yeung SC, et al. Mitochondrial transfer of induced pluripotent stem cell-derived mesenchymal stem cells to airway epithelial cells attenuates cigarette smoke-induced damage. Am J Respir Cell Mol Biol **2014**; 51: 455-65.
17. Hikmet F, Méar L, Edvinsson Å, Micke P, Uhlén M, Lindskog CJMsb. The protein expression profile of ACE2 in human tissues. **2020**; 16: e9610.
18. Plasschaert LW, Zilionis R, Choo-Wing R, et al. A single-cell atlas of the airway epithelium reveals the CFTR-rich pulmonary ionocyte. Nature **2018**; 560: 377-81.
19. Schiller HB, Montoro DT, Simon LM, et al. The Human Lung Cell Atlas: A High-Resolution Reference Map of the Human Lung in Health and Disease. Am J Respir Cell Mol Biol **2019**; 61(1): 31-41.
20. Raredon MSB, Adams TS, Suhail Y, et al. Single-cell connectomic analysis of adult mammalian lungs. Sci Adv **2019**; 5(12): eaaw3851.
21. ProfileKyle J. Travaglini PNN, Lolita Penland, Rahul Sinha, Astrid Gillich, Rene V. Sit, Stephen Chang, Stephanie D. Conley, Yasuo Mori, Jun Seita, Gerald J. Berry, Joseph B. Shrager, Ross J. Metzger, Christin S. Kuo, Norma Neff, Irving L. Weissman, Stephen R. Quake, Mark A. Krasnow. A molecular cell atlas of the human lung from single cell RNA sequencing. BioRxiv **2019**; https://doi.org/10.1101/742320.
22. Zhou P, Yang XL, Wang XG, et al. A pneumonia outbreak associated with a new coronavirus of probable bat origin. Nature **2020**; 579: 270-3.
23. Lu R, Zhao X, Li J, et al. Genomic characterisation and epidemiology of 2019 novel coronavirus: implications for virus origins and receptor binding. Lancet **2020**; 395: 565-74.
24. Sharma G, Goodwin J. Effect of aging on respiratory system physiology and immunology. Clin Interv Aging **2006**; 1: 253-60.
25. Chen Y, Chan VS, Zheng B, et al. A novel subset of putative stem/progenitor CD34+Oct-4+ cells is the major target for SARS coronavirus in human lung. J Exp Med **2007**; 204: 2529-36.
26. Quantius J, Schmoldt C, Vazquez-Armendariz AI, et al. Influenza Virus Infects Epithelial Stem/Progenitor Cells of the Distal Lung: Impact on Fgfr2b-Driven Epithelial Repair. PLoS Pathog **2016**; 12: e1005544.
27. Song L, Langfelder P, Horvath S. Comparison of co-expression measures: mutual information, correlation, and model based indices. BMC Bioinformatics **2012**; 13: 328.
28. Nabhan AN, Brownfield DG, Harbury PB, Krasnow MA, Desai TJ. Single-cell Wnt signaling niches maintain stemness of alveolar type 2 cells. Science **2018**; 359: 1118-23.
29. Desai TJ, Brownfield DG, Krasnow MA. Alveolar progenitor and stem cells in lung development, renewal and cancer. Nature **2014**; 507: 190-4.
30. Liang J, Zhang Y, Xie T, et al. Hyaluronan and TLR4 promote surfactant-protein-C-positive alveolar progenitor cell renewal and prevent severe pulmonary fibrosis in mice. Nat Med **2016**; 22: 1285-93.
31. Hoffmann M, Kleine-Weber H, Schroeder S, et al. SARS-CoV-2 cell entry depends on ACE2 and TMPRSS2 and is blocked by a clinically proven protease inhibitor. **2020**.
32. Singh M, Bansal V, Feschotte C. A Single-Cell RNA Expression Map of Human Coronavirus Entry Factors. Cell Rep **2020**; 32: 108175.
33. Hou YJ, Okuda K, Edwards CE, et al. SARS-CoV-2 Reverse Genetics Reveals a Variable Infection Gradient in the Respiratory Tract. Cell **2020**; 182: 429-46 e14.

34. Aguiar JA, Tremblay BJ, Mansfield MJ, et al. Gene expression and in situ protein profiling of candidate SARS-CoV-2 receptors in human airway epithelial cells and lung tissue. Eur Respir J **2020**; 56.
35. Rizzo A, Mollica V, Massari F. Re: Hanbing Song, Bobak Seddighzadeh, Matthew R. Cooperberg, Franklin W. Huang. Expression of ACE2 and TMPRSS2, the SARS-CoV-2 Receptor and Co-Receptor, in Prostate Epithelial Cells. Eur Urol. In press DOI: 10.1016/j.eururo.2020.04.065. Eur Urol **2020**; 78: e205-e6.
36. Li X, Xu S, Yu M, et al. Risk factors for severity and mortality in adult COVID-19 inpatients in Wuhan. J Allergy Clin Immunol **2020**.
37. Yang X, Yu Y, Xu J, et al. Clinical course and outcomes of critically ill patients with SARS-CoV-2 pneumonia in Wuhan, China: a single-centered, retrospective, observational study. Lancet Respir Med **2020**; 8: 475-81.
38. Sungnak W, Huang N, Becavin C, et al. SARS-CoV-2 entry factors are highly expressed in nasal epithelial cells together with innate immune genes. Nat Med **2020**; 26: 681-7.
39. Brake SJ, Barnsley K, Lu W, McAlinden KD, Eapen MS, Sohal SS. Smoking Upregulates Angiotensin-Converting Enzyme-2 Receptor: A Potential Adhesion Site for Novel Coronavirus SARS-CoV-2 (Covid-19). J Clin Med **2020**; 9.
40. Leung JM, Yang CX, Tam A, et al. ACE-2 expression in the small airway epithelia of smokers and COPD patients: implications for COVID-19. Eur Respir J **2020**; 55.
41. Kong Q, Xiang Z, Wu Y, Gu Y, Guo J, Geng F. Analysis of the susceptibility of lung cancer patients to SARS-CoV-2 infection. Mol Cancer **2020**; 19: 80.
42. Wark P, Pathinyake P, Kaiko G, et al. ACE2 Expression is elevated in Airway Epithelial Cells from aged and male donors but reduced in asthma. **2020**.
43. Wang D, Yin Y, Hu C, et al. Clinical course and outcome of 107 patients infected with the novel coronavirus, SARS-CoV-2, discharged from two hospitals in Wuhan, China. Crit Care **2020**; 24: 188.
44. Smith JC, Sausville EL, Girish V, et al. Cigarette Smoke Exposure and Inflammatory Signaling Increase the Expression of the SARS-CoV-2 Receptor ACE2 in the Respiratory Tract. Dev Cell **2020**; 53: 514-29 e3.
45. Dong Y, Mo X, Hu Y, et al. Epidemiology of COVID-19 among children in China. **2020**; 145.
46. Herring MJ, Putney LF, Wyatt G, Finkbeiner WE, Hyde DM. Growth of alveoli during postnatal development in humans based on stereological estimation. Am J Physiol Lung Cell Mol Physiol **2014**; 307: L338-44.
47. Emery JL. The post natal development of the human lung and its implications for lung pathology. Respiration **1970**; 27: Suppl:41-50.
48. Nikolic MZ, Sun D, Rawlins EL. Human lung development: recent progress and new challenges. Development **2018**; 145.
49. Xu Z, Shi L, Wang Y, et al. Pathological findings of COVID-19 associated with acute respiratory distress syndrome. Lancet Respir Med **2020**; 8: 420-2.

Figure Legends

Figure 1 Retrospective analysis for patients with COVID-19

(A) Diagram of clinical data collection and analysis process.

(B) Distribution of disease severity and ages in patients with COVID-19. Demographics and baseline characteristics of patients with COVID-19. Patients (n = 299) were divided as 0-16 yrs. (Children), 16-50 yrs. (young adults), and >50 yrs. (older adults). Data were presented with number and percentage (Chi-square or Fisher's exact test was used for *p* value).

Figure 2. Laboratory findings of infected patients with different ages.

(A) The comparison of laboratory parameters in peripheral blood testing between children and older patients. Total white blood cells, lymphocytes, neutrophils, platelet, and ratio of neutrophils/lymphocytes were examined. Kruskal-Wallis test was used.

(B) Biochemical indexes of infected patients. hs-CRP, PT, albumin, alanine transferase, FIB, total bilirubin, creatine, creatine kinase, and procalcitonin were examined between children and older patients. Mann-Whitney test was used. FIB: fibrogen; hs-CRP: high-sensitivity C-reactive protein; PT: prothrombin time. Kruskal-Wallis test was used.

Figure 3. Detection of ACE2 positive cells in lung tissues in children and older adults

(A) Typical staining images of bronchus, alveolus, and ACE2 positive cells in the lung of patients with different ages. i, ii, viii, and ix. HE staining showed typical structures of bronchus and alveolus in children and older adults. iii-v and x-xii. Typical images of ACE2 positive cells presented in the airways and lungs of children and older adults. vi-vii and xiii-xiv. Negative (IgG, lung) and positive controls (ACE2, kidney). Scale bars=100 μm or 50 μm.

(B) Statistical results of ACE2 expressed cells in lung of children and adults i. Quantification of ACE2 positive cells in lung tissue sections with different ages from 0-10Y, 10-50Y, 50-60Y, 60-70Y, and > 70Y (n > 3). One-way ANOVA was used. ii. Quantification of ACE2 positive cells in children (<16yrs., n

= 23) and older adults (>50 yrs., n = 14), Mann-Whitney test was used. iii. Relative mRNA expression of *ACE2* in lung tissues of children and older adults (n = 5). unpaired *t* test was used. Br, Bronchus; PA, Pulmonary Alveolus.

(C) Statistical results for the distribution of ACE2 positive cells in the different regions of lungs. i and ii. Quantification of ACE2 positive cells in the regions of bronchus and alveolus at different age groups. One-way ANOVA was used. iii. Comparison of ACE2 positive cells between children (<16yrs., n = 20) and older adults (>50 yrs., n = 11) in the regions of bronchus and alveolus. Kruskal-Wallis test was used.

Figure 4. Co-expressions of *ACE2* and lung progenitor cell markers in children and older adults

(A) Bioinformatic analysis of the expression of *ACE2* and lung progenitor marker genes using three recent single-cell RNA-seq datasets in human lung. i Pearson analysis was used to examine the correlation of ACE2 and lung progenitor marker genes in lung tissue level. ii the percentage of ACE2 positive cells co-expressing lung progenitor cells marker genes in single cell level.

(B) Immunostaining was used to detect the co-expression of ACE2 and lung progenitor marker genes in lung tissue sections. Scale bar = 15μm.

(C) mRNA expressions level of lung progenitor marker genes in the lung tissues of children and older adults. unpaired *t* test was used, n = 5 respectively.

Figure 5. *Ex vivo* SARS-CoV-2 pseudovirus infection in lung tissues and follow-up of patients with COVID-19

(A) Lung tissues from three children and three older adults were infected with SARS-CoV-2 pseudovirus. i-viii. Immunostaining against ACE2 (red) and GFP to detect nCoV in all lung tissue samples. Scale bar = 50 μm.

(B) The efficiency of infection with SARS-CoV-2 pseudovirus in lungs of children and older adults. Cell number with ACE2 and nCOV-GFP positive signals was counted from 10 images in each sample. Data were presented with number and percentage (%). (Fisher's exact test was used, $p = 0.95$).

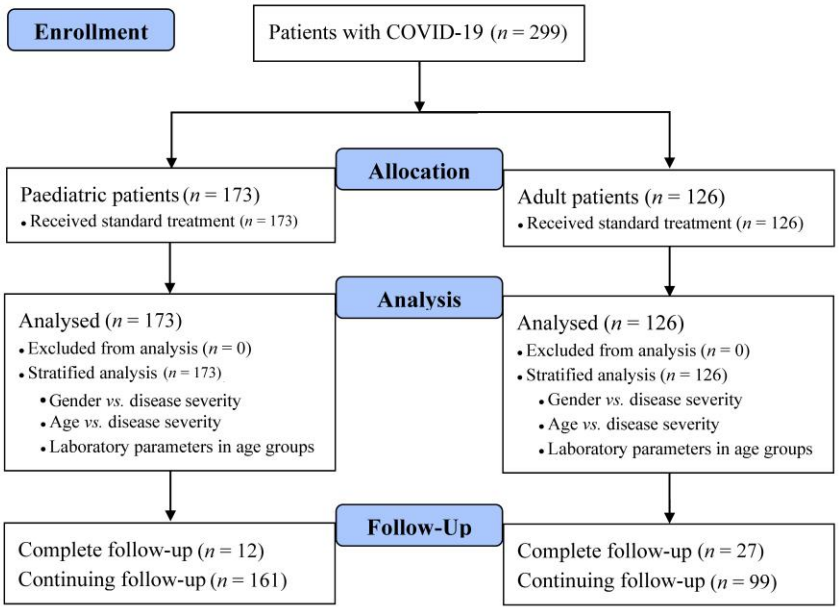
(C). Typical features of computed tomography (CT) images in a 3-year-old pediatric patient and a 64-year-old patient with moderate pneumonia. Lung lesions were detected in the child (i) and the older patient (iv) on admission. Alleviation of lesion was observed at discharge for the child (ii). A new lesion was detected for the older patient (v) who had met the criteria for discharge with improvement in symptoms and negative result of SRAS-CoV-2 test. Based on the radiological assessment in follow-up visit, the child completely recovered without abnormal finding in CT images (iii), while stripe-like high density shadows were detected for the older patient (vi).

(D) The lung recovery of patients and CT examination in follow-up study. Data were presented with number and percent (%); Chi-squared test was used, $p = 0.0025$.

(E) The relationship between the recovery ratios and patients' ages.

Figure 1

A



B

Table 1. Demographics and baseline characteristics of patients with COVID-19

	All patients (*n* = 299)	**0-16 yrs. (*n* = 173)**	**16-50 yrs. (*n* = 89)**	**>50 yrs. (*n* = 37)**	***P* value**
Characteristics					
Gender, *n* (%)					0.995
Males	156 (52.2)	91 (52.6)	46 (48.8)	19 (51.4)	
Females	143 (47.8)	82 (47.4)	43 (51.2)	18 (48.6)	
Disease severity, *n* (%)					0.001
Mild	72 (24.1)	68 (39.3)	4 (4.5)	0 (0.0)	
Moderate	201 (67.2)	104 (60.1)	74 (83.1)	23 (62.2)	
Severe	23 (7.7)	1 (0.6)	9 (10.1)	13 (35.1)	
Critical	3 (1.0)	0 (0.0)	2 (2.3)	1 (2.7)	
Signs and symptoms, *n* (%)					
Fever	148 (49.5)	57 (32.9)	64 (71.9)	27 (73.0)	0.002
Cough	129 (43.1)	57 (32.9)	50 (56.2)	22 (59.5)	0.036
Diarrhea	15 (5.0)	7 (4.0)	7 (7.9)	1 (2.7)	0.366
Sore throat	22 (7.4)	6 (3.5)	12 (13.5)	4 (10.8)	0.019
ICU admission, *n* (%)	24 (8.0)	1 (0.3)	9 (10.1)	14 (37.8)	0.001

Data were presented with patient's number (n) and percent (%). *p* values were calculated using Chi-square test or Fisher's exact test.

Figure 2

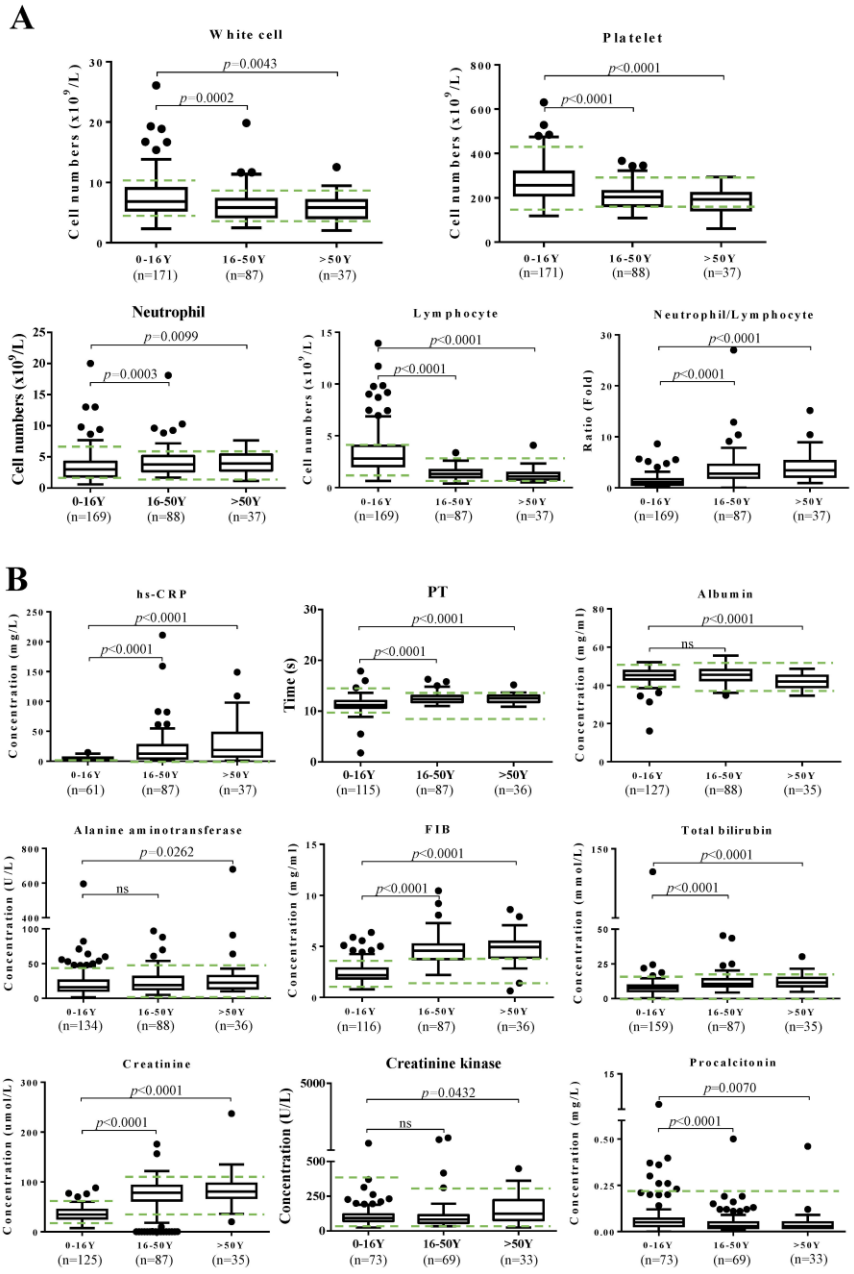

A
White cell
Cell numbers (x10^9/L)
p=0.0043
p=0.0002
0-16Y (n=171)
16-50Y (n=87)
>50Y (n=37)
Platelet
Cell numbers (x10^9/L)
p<0.0001
p<0.0001
0-16Y (n=171)
16-50Y (n=88)
>50Y (n=37)
Neutrophil
Cell numbers (x10^9/L)
p=0.0099
p=0.0003
0-16Y (n=169)
16-50Y (n=88)
>50Y (n=37)
Lymphocyte
Cell numbers (x10^9/L)
p<0.0001
p<0.0001
0-16Y (n=169)
16-50Y (n=87)
>50Y (n=37)
Neutrophil/Lymphocyte
Ratio (Fold)
p<0.0001
p<0.0001
0-16Y (n=169)
16-50Y (n=87)
>50Y (n=37)
B
hs-CRP
Concentration (mg/L)
p<0.0001
p<0.0001
0-16Y (n=61)
16-50Y (n=87)
>50Y (n=37)
PT
Time (s)
p<0.0001
p<0.0001
0-16Y (n=115)
16-50Y (n=87)
>50Y (n=36)
Albumin
Concentration (mg/ml)
p<0.0001
ns
0-16Y (n=127)
16-50Y (n=88)
>50Y (n=35)
Alanine aminotransferase
Concentration (U/L)
p=0.0262
ns
0-16Y (n=134)
16-50Y (n=88)
>50Y (n=36)
FIB
Concentration (mg/ml)
p<0.0001
p<0.0001
0-16Y (n=116)
16-50Y (n=87)
>50Y (n=36)
Total bilirubin
Concentration (mmol/L)
p<0.0001
p<0.0001
0-16Y (n=159)
16-50Y (n=87)
>50Y (n=35)
Creatinine
Concentration (umol/L)
p<0.0001
p<0.0001
0-16Y (n=125)
16-50Y (n=87)
>50Y (n=35)
Creatinine kinase
Concentration (U/L)
p=0.0432
ns
0-16Y (n=73)
16-50Y (n=69)
>50Y (n=33)
Procalcitonin
Concentration (mg/L)
p=0.0070
p<0.0001
0-16Y (n=73)
16-50Y (n=69)
>50Y (n=33)

Figure 3

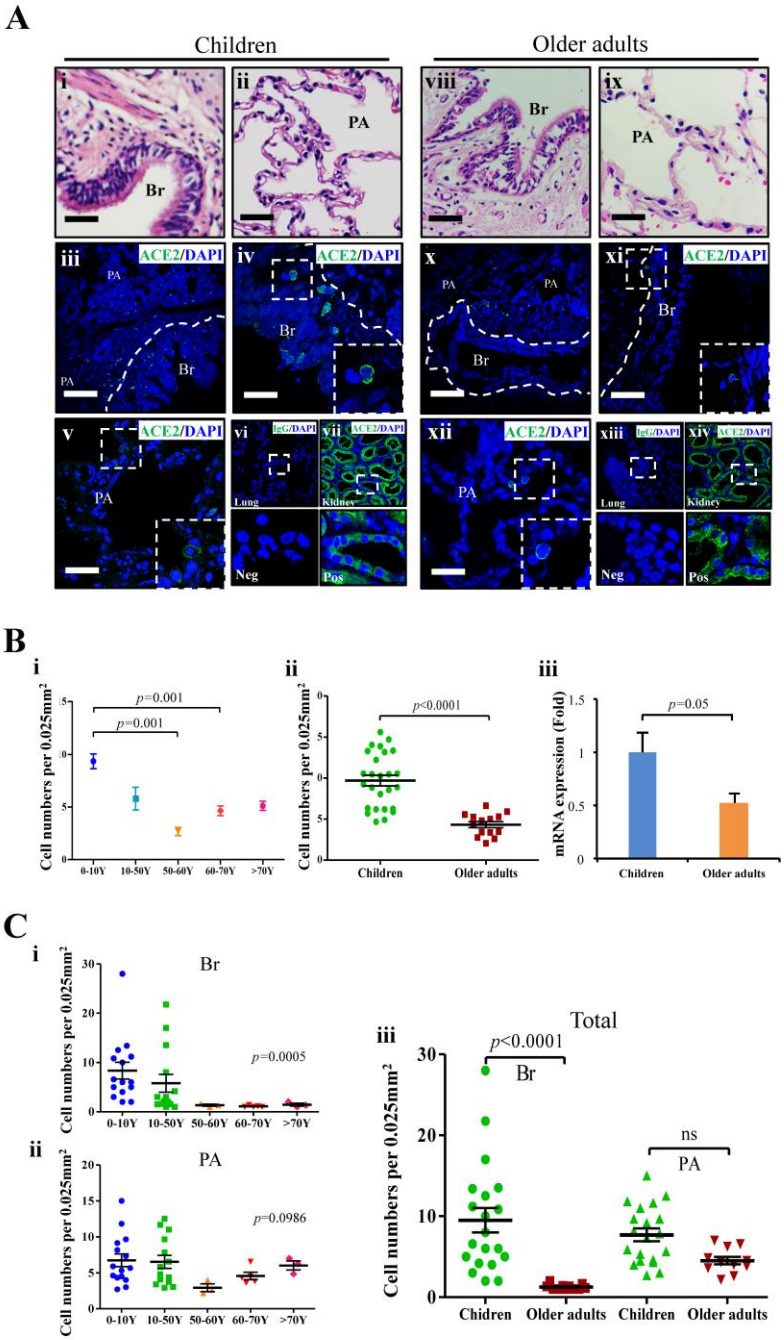

A
Children
Older adults
i
ii
viii
ix
Br
PA
iii
iv
x
xi
ACE2/DAPI
v
vi
vii
xii
xiii
xiv
IgG/DAPI
Lung
Kidney
Neg
Pos
B
i
ii
iii
Cell numbers per 0.025mm²
p=0.001
p=0.001
0-10Y
10-50Y
50-60Y
60-70Y
>70Y
p<0.0001
Children
Older adults
mRNA expression (Fold)
p=0.05
C
Br
PA
p=0.0005
p=0.0986
Total
p<0.0001
ns
Chidren

Figure 4

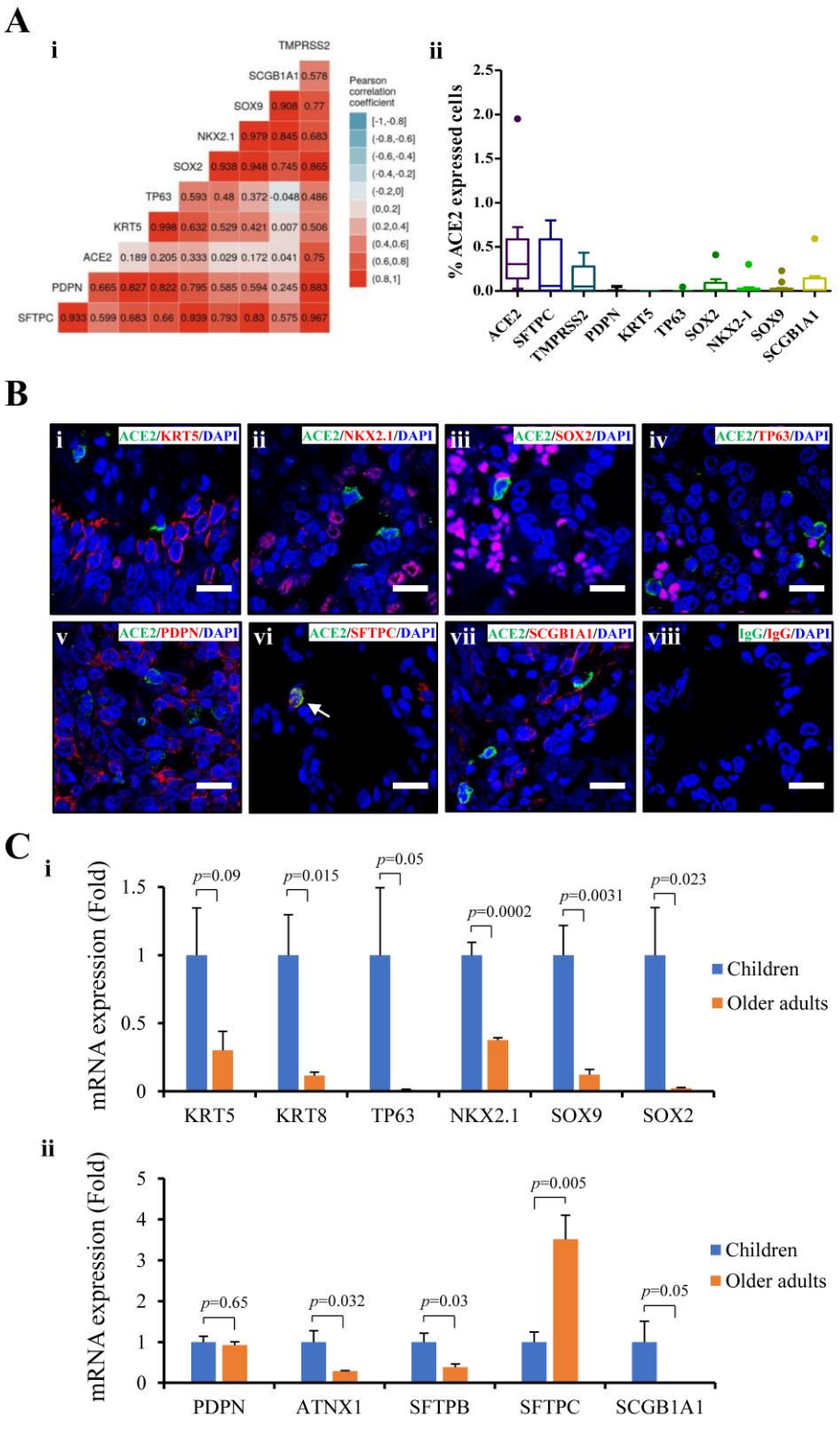
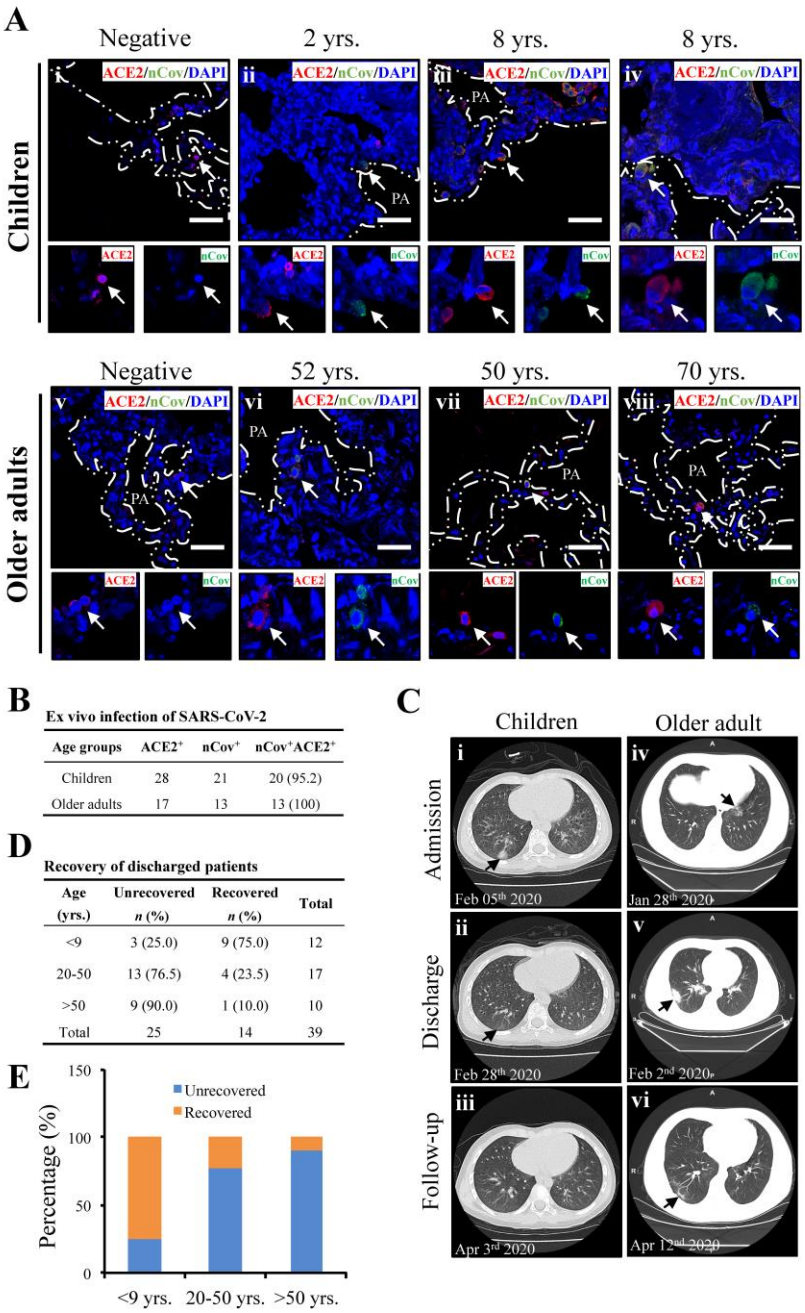

A
i
TMPRSS2
SCGB1A1 0.578
SOX9 0.908 0.77
NKX2.1 0.979 0.845 0.683
SOX2 0.938 0.948 0.745 0.865
TP63 0.593 0.48 0.372 -0.048 0.486
KRT5 0.998 0.632 0.529 0.421 0.007 0.506
ACE2 0.189 0.205 0.333 0.029 0.172 0.041 0.75
PDPN 0.665 0.827 0.822 0.795 0.585 0.594 0.245 0.883
SFTPC 0.933 0.599 0.683 0.66 0.939 0.793 0.83 0.575 0.967
Pearson correlation coefficient
[-1,-0.8]
(-0.8,-0.6]
(-0.6,-0.4]
(-0.4,-0.2]
(-0.2,0]
(0,0.2]
(0.2,0.4]
(0.4,0.6]
(0.6,0.8]
(0.8,1]
ii
% ACE2 expressed cells
2.5
2.0
1.5
1.0
0.5
0.0
ACE2
SFTPC
TMPRSS2
PDPN
KRT5
TP63
SOX2
NKX2-1
SOX9
SCGB1A1
B
i ACE2/KRT5/DAPI
ii ACE2/NKX2.1/DAPI
iii ACE2/SOX2/DAPI
iv ACE2/TP63/DAPI
v ACE2/PDPN/DAPI
vi ACE2/SFTPC/DAPI
vii ACE2/SCGB1A1/DAPI
viii IgG/IgG/DAPI
C
i
mRNA expression (Fold)
1.5
1
0.5
0
p=0.09
p=0.015
p=0.05
p=0.0002
p=0.0031
p=0.023
KRT5
KRT8
TP63
NKX2.1
SOX9
SOX2
Children
Older adults
ii
mRNA expression (Fold)
5
4
3
2
1
0
p=0.65
p=0.032
p=0.03
p=0.005
p=0.05
PDPN
ATNX1
SFTPB
SFTPC
SCGB1A1
Children
Older adults

Figure 5


A
Negative
2 yrs.
8 yrs.
8 yrs.
Children
ACE2/nCov/DAPI
PA
ACE2
nCov
Negative
52 yrs.
50 yrs.
70 yrs.
Older adults
B
Ex vivo infection of SARS-CoV-2
Age groups | ACE2+ | nCov+ | nCov+ACE2+
Children | 28 | 21 | 20 (95.2)
Older adults | 17 | 13 | 13 (100)
D
Recovery of discharged patients
Age (yrs.) | Unrecovered n (%) | Recovered n (%) | Total
<9 | 3 (25.0) | 9 (75.0) | 12
20-50 | 13 (76.5) | 4 (23.5) | 17
>50 | 9 (90.0) | 1 (10.0) | 10
Total | 25 | 14 | 39
E
Unrecovered
Recovered
Percentage (%)
150
100
50
0
<9 yrs.
20-50 yrs.
>50 yrs.
C
Children
Older adult
Admission
Discharge
Follow-up
Feb 05th 2020
Jan 28th 2020
Feb 28th 2020
Feb 2nd 2020
Apr 3rd 2020
Apr 12nd 2020