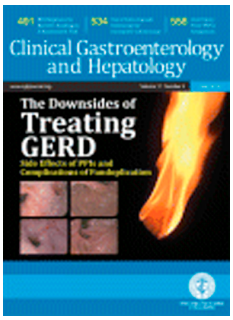
Journal Pre-proof



ACE2 Expression in Pancreas May Cause Pancreatic Damage After SARS-CoV-2 Infection

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PII: S1542-3565(20)30537-1
DOI: https://doi.org/10.1016/j.cgh.2020.04.040
Reference: YJCGH 57158

To appear in: *Clinical Gastroenterology and Hepatology*
Accepted Date: 20 April 2020

Please cite this article as: Liu F, Long X, Zhang B, Zhang W, Chen X, Zhang Z, ACE2 Expression in Pancreas May Cause Pancreatic Damage After SARS-CoV-2 Infection, *Clinical Gastroenterology and Hepatology* (2020), doi: https://doi.org/10.1016/j.cgh.2020.04.040.

Supplementary Material

Supplementary methods

Analysis of Public datasets

We collected bulk RNA-seq data from GTEx (https://gtexportal.org), which contains normal tissue and organ sequencing data from multiple individuals. The data analysis was performed online. In addition, we collected data from single-cell RNA-seq (scRNA-seq) of the pancreas NCBI-GEO (GSE85241 (4 donors with 2,126 pancreatic cells), GSE84133 (4 donors with 8569 pancreatic cells)). The details of the donors are available in the previous report[1, 2]. The scRNA-seq data processing process is as follows: Unique molecular identified (UMI) expression count matrix was obtained from the database, and Seurat object was created. Further quality control was performed, cells with high mitochondrial gene expression > 5% were filtered. The data was normalized and log-transformed with the method “LogNormalize” in NormalizeData function. Before we performed linear dimensional reduction, the data was scaling. And then principal component analysis (PCA) was performed to the data and determined the dimensionality. Finally, after clustering cells based on graph-based clustering approach, the non-linear dimensional reduction based on uniform manifold approximation and projection (UMAP) was performed the data to analyze and visualize the data. All of scRNA-seq data analysis was based on Seurat R package (version: 3.1.4) with the default parameters[3]. The annotation of cell types was

completed based on the featured genes of each cluster and the cell markers of each type pancreas cell from CellMarker database[4] and previously report[1, 2].

More details of clinical data

The criteria for the diagnosis and severity of the patients were followed by the diagnosis and treatment guideline for COVID-19 (trial version 6) issued by National Health Commission of the People's Republic of China. Clinical information included age, gender, amylase and lipase in serum, and the imaging results including bedside ultrasound and abdominal CT.

Mild COVID-19 patients with serum amylase and lipase in the normal range did not undergo the imaging evaluation of the pancreas, and their pancreas was assumed to be normal. Bedside ultrasound of the abdomen was all performed in critically ill patients, and CT was added if there were any abnormalities.

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Supplementary Tables

Supplementary Table 1. The distribution of ACE2 in several type of pancreatic cell in two scRNA-seq datasets.

	GSE85241 (N=55)	GSE84133(N=19)
Exocrine gland		
Duct cell	14 (25.45%)	10 (52.6%)
Acinar cell	31 (56.36%)	1 (5.26%)
Endothelial cell	1 (1.82%)	0
Pancreatic islet		
Alpha cell	4 (7.27%)	0
Beta cell	4 (7.27%)	4 (21.05%)
PP and delta cell	1 (1.82%)	3 (15.79%)

Individual differences between donors from different sources may lead to differences in the results from the two scRNA-seq datasets.

Supplementary Table 2. Summary of the study patients

Characteristics	All patients (n=121)	non-severe (n=54)	Severe (n=67)
Age, years (Range, IQR)	57(Range 18-87, IQR 43-72）	53 (Range 18-83, IQR 39-67）	62 (Range 24-87, IQR 51-73)
Gender, n (%)			
Female	46（38.02%）	21（38.89%）	25（37.31%）
Male	75（61.98%）	33（61.11%）	42（62.69%）
AMS/LPS increased, n (%)	13（10.74%）	1（1.85%）	12（17.91%）
AMS increased	13（10.74%）	1（1.85%）	12（17.91%）
LPS increased	12（9.92%）	1（1.85%）	11（16.41%）
Imaging alteration, N* (%)			
A: Normal	8（3.62%）	1（1.85%）	7（10.44%）
B: Enlargement or dilation	5（4.13%）	0	5（7.46%）
C: Necrosis	0	0	0

Data are median (Range, IQR), n (%), or N* (%), where N is the number of patients with AMS/LPS increased; IQR=interquartile range; AMS=Amylase, LPS=Lipase

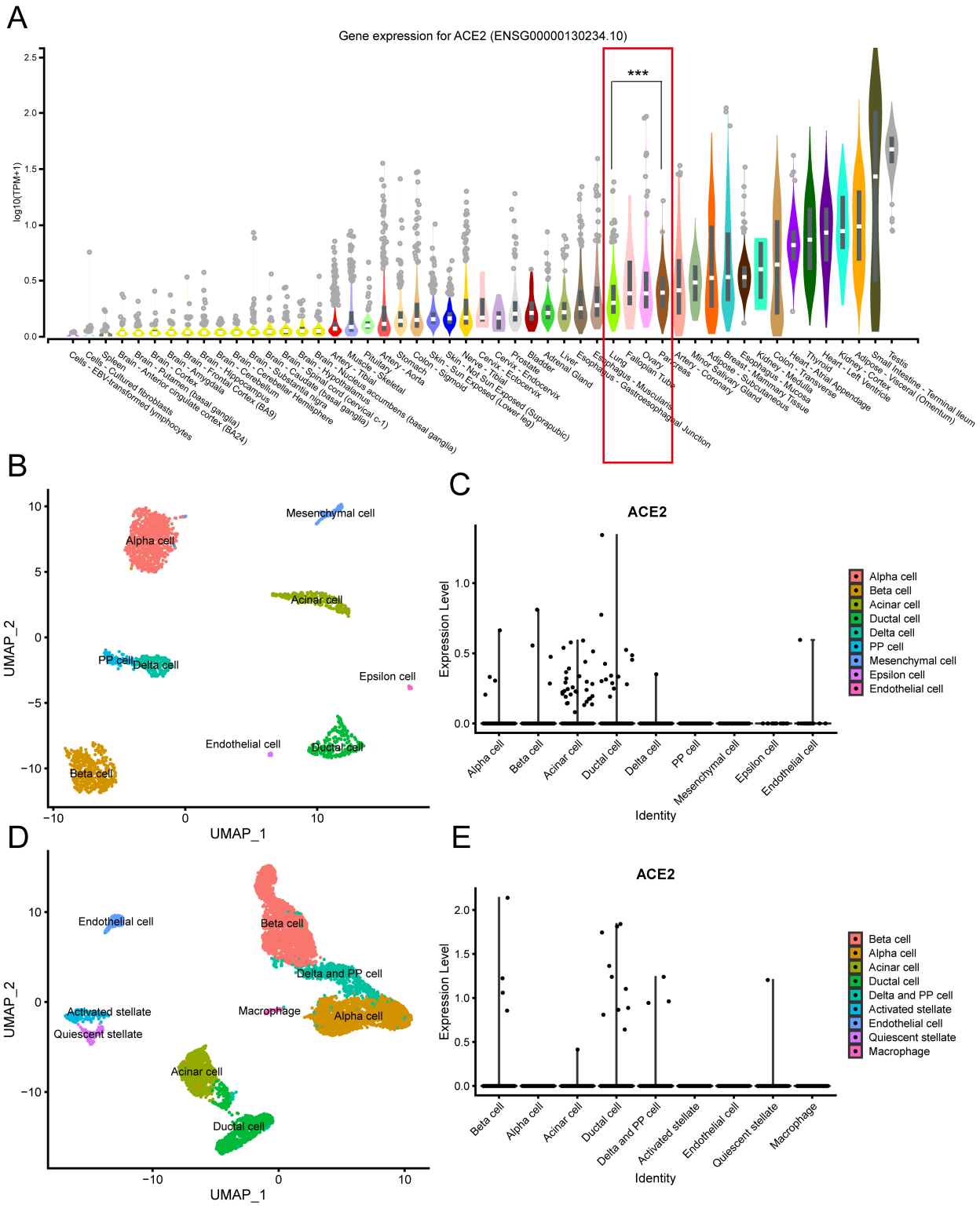
Supplementary Table 3. Clinical Characteristics of COVID-19 patients with pancreatic injury.

Variable, n (%) or median (IQR)	Normal range	non-severe (n=1)	severe (n=12)
Age	-	44	62 (53-69)
Male	-	N	6 (50.0%)
BMI	20-25	28.4	27.2 (24.5-28.7)

Incubation period, days	2-7	4	4 (3-7)
Comorbidities			
Hypertension	-	N	5 (41.7%)
Diabetes	-	Y	5 (41.7%)
CHD	-	N	1 (8.3%)
Presenting symptoms			
Fever	-	Y	7 (58.3%)
Short of breath	-	N	8 (66.7%)
Cough	-	N	4 (33.3%)
Fatigue	-	N	4 (33.3%)
Diarrhea	-	N	2 (16.7%)
Headache	-	N	2 (16.7%)
Complications			
ARDS	-	N	6 (50.0%)
Cardiac injury	-	N	2 (16.7%)
Kidney injury	-	N	4 (33.3%)
Liver injury	-	Y	7 (58.3%)
Shock	-	N	4 (33.3%)
NSAIDs used	-	N	2 (16.7%)
Laboratory findings on admission			
WBC ($\times 10^9$/L）	3.5-9.5	5.62	4.56 (3.80-10.20)
Lymphocyte count, $\times 10^9$/L	1.10-3.20	0.89	0.66 (0.43-0.92)
Neutrophil count , $\times 10^9$/L	1.80-6.30	4.13	5.02 (2.27-7.20)
Platelet count, $\times 10^9$/L	125.0-350.0	227	156 (145-280)
Hemoglobin, g/L	130-175	127	125 (121-150)
ALT, U/L	7.0-40.0	45	31 (20-78)
AST, U/L	13.0-35.0	37	34 (31-51)
T-BIL, μmol/L	0-21.0	10.9	11.4 (9.8-14.4)
Albumin, g/L	40.0-55.0	48.5	36.0 (32.5-40.0)
Creatinine, μmol/L	41.0-73.0	38	56 (42-69)
BUN, mmol/L	2.6-7.5	3.2	6.0 (4.4-7.2)
Amylase, U/L	35-135	76	62 (59-121)
>135 U/L	-	N	3 (25.0%)
Lipase, U/L	8-78	56	31 (24-48)
>78 U/L	-	N	3 (25.0%)
PT, s	11.5-14.5	12.5	12.5 (12.0-14.0)
APTT, s	29.0-42.0	37.5	35.6 (34.5-39.8)
INR	0.8-1.2	1.1	1.0 (0.9-1.0)
D-dimer, mg/L	0-1.5	0.2	0.1 (0.1-1.12)
PCT (ng/mL)	0.02-0.05	0.04	0.32 (0.08-0.49)
CRP (mg/L)	0-5.0	16	27.8 (18.8-86.0)
Ferritin, ng/mL	4.63-204	675	998 (701-1160)
Elevated AMS/LPS after admission			
Amylase, U/L	35-135	175	213 (186-277)

>135 U/L	-	Y	12 (100%)
Lipase, U/L	8-78	102	156 (104-228)
>78 U/L	-	Y	11 (91.7%)
Treatments			
Oxygen support	-	N	12 (100%)
Mechanical ventilation	-	N	7 (58.3%)
Antiviral treatment	-	N	9 (75.0%)
Antimicrobial treatment	-	N	10 (83.3%)
Glucocorticoids	-	N	4 (33.3%)
ICU admission	-	N	6 (50.0%)
Outcome			
Discharge	-	Y	7 (58.3%)
Dead	-	-	5 (41.7%)

N=No, Y=Yes, CHD=Coronary heart disease, ARDS=Acute respiratory distress syndrome, NSAIDs= Nonsteroidal Anti-inflammatory Drugs, WBC=White blood cells, ALT=Alanine aminotransferase, AST=Aspartate aminotransferase, T-BIL=Total bilirubin, BUN=Blood urea nitrogen, AMS=Amylase, LPS=Lipase, PT=Prothrombin time, APTT=Activated partial thromboplastin time, INR=International standard ratio, PCT=Procalcitonin, CRP=C-reactive protein, ICU= Intensive care unit.


A
Gene expression for ACE2 (ENSG00000130234.10)
log10(TPM+1)
***
B
C
ACE2
Expression Level
Identity
UMAP_1
UMAP_2
Alpha cell
Beta cell
Acinar cell
Ductal cell
Delta cell
PP cell
Mesenchymal cell
Epsilon cell
Endothelial cell
D
E
ACE2
Delta and PP cell
Activated stellate
Quiescent stellate
Macrophage

ACE2 Expression in Pancreas May Cause Pancreatic Damage After SARS-CoV-2 Infection

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Conflict of Interest

The authors disclose no conflicts.

Funding

This study was funded by the State Key Project on Infection Disease of China (No. 2018ZX10723204-003).

Introduction

Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), a novel coronavirus that causes coronavirus disease 2019 (COVID-19) in humans, has caused a serious public health issue[1]. There is lacking attention to pancreatic injury, which may impact patients' prognosis. In this study, we explored the expression and distribution of ACE2, the receptor of SARS-CoV-2, in the pancreas. Combined with clinical data, we demonstrated that pancreatic injury can occur in some COVID-19 patients.

Methods

Public database was used to explore the expression and distribution of ACE2 in normal pancreases. We also retrospectively analyzed patients diagnosed with COVID-19 from January 1, 2020 to February 15, 2020 in Wuhan Tongji Hospital and Wuhan Jin Yin-tan Hospital. We collected hospital admissions, laboratory tests, and imaging tests from clinical electronic medical records. Severe COVID-19 was defined when patients had one of the following criteria: (1) Shortness of breath, and respiratory frequency ≥ 30/min; (2)Finger pulse oximeter oxygen saturation at rest ≤ 93%; (3) Oxygenation index ≤ 300mmHg. More details about clinical data and public datasets analysis were described in Supplementary Material.

Results

In the GTEx database, we found the mRNA level of ACE2 was higher in the pancreas than in the lung (Figure 1A, $P < 0.001$, Wilcoxon signed rank test). To investigate the

distribution of ACE2 in the pancreas, we analyzed two single-cell RNA-seq (scRNA-seq) datasets. After identifying different types of pancreatic cells (Figure 1B, D), we found ACE2 was expressed in both the exocrine glands and islets (Figure 1C, E). The details are listed in the Supplementary Table 1.

Further, we analyzed the pancreatic injury after SARS-CoV-2 Infection. Our study cohort included 121 COVID-19 patients (46 women and 75 men), with a median age of 57 years (interquartile range, 43 to 72). In mild cases, 1.85% (1/54) had elevated levels of both amylase and lipase. In patients with severe COVID-19, 17.91% (12/64) and 16.41% (11/64) had elevated amylase and lipase, respectively (Supplementary Table 2). On CT scan, 5 patients with severe COVID-19 (7.46%) showed changes in the pancreas, mainly focal enlargement of the pancreas or dilatation of the pancreatic duct, without acute necrosis. Of the 13 patients with pancreatic injury, 3 severe patients showed elevated amylase and lipase on admission. In addition, 2 patients had a history of nonsteroidal anti-inflammatory drug use, and 4 had been treated with glucocorticoids during hospitalization, which may be associated with drug-induced pancreatitis[2] (Supplementary Table 3). Three patients had suspected symptoms of pancreatitis such as abdominal pain or vomiting. Of note, the clinical symptoms could not be recorded among severe patients requiring mechanical ventilation under sedation. Meanwhile, 5 critically ill patients with pancreatic injury died and 8 were discharged. These clinical data show that pancreatic injury can occur in some COVID-19 patients, mainly in those with severe illness.

Discussion

In this study, we focused on the expression of ACE2 in pancreas and the damage to the pancreas in a proportion of patients with SARS-CoV-2 infection. We found ACE2 was expressed in the pancreas of normal people, and this expression was slightly higher in the pancreas than in the lungs, indicating that SARS-CoV-2 might also bind to ACE2 in the pancreas and cause pancreatic injury. Further, scRNA-seq data indicated that ACE2 was expressed in both exocrine glands and islets of the pancreas. In our study cohort, about 1-2% of non-severe and 17% of severe patients with COVID-19 had pancreatic injury In addition, it should be noted that some critically ill patients have already developed pancreatic injury before admission, and the possibility of drug-induced pancreatitis should be considered because of the history of taking NSAIDs and glucocorticoids in some patients. However, we should pay attention to the possibility of damage caused by SARS-CoV-2, based on the analysis of the expression of ACE2 in the pancreas and the high proportion of COVID-19 patients with pancreatic injury. Although these patients did not show signs of necrotizing pancreatitis, the consequences of pancreatic injury can potentially be serious, such as aggravating systemic inflammation, accelerating the occurrence of acute respiratory distress syndrome[3], and even developing into chronic pancreatitis, which may have a serious impact on the health and quality of life of patients. Yang et al. reported that patients infected with SARS-CoV suffered from hyperglycemia, which might be caused by SARS-CoV damaging the pancreatic islets through ACE2[4, 5]. Our results show that increased attention should be paid to the pancreas in patients

with SARS-CoV-2 infection, especially in severe cases.

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Figure legends

Figure 1. The expression and distribution of ACE2 in pancreas. (A) The mRNA level of ACE2 in multiple organs from GTEx samples. (B), (D) The visualization of pancreatic cell distribution in GSE85241 and GSE84133. (C), (E) The expression of ACE2 in different pancreatic cell in GSE85241 and GSE84133. (***: $P < 0.001$)