Since January 2020 Elsevier has created a COVID-19 resource centre with free information in English and Mandarin on the novel coronavirus COVID-19. The COVID-19 resource centre is hosted on Elsevier Connect, the company's public news and information website.

Can J Diabetes 45 (2021) 162–166

Contents lists available at ScienceDirect

Canadian Journal of Diabetes

journal homepage: www.canadianjournalofdiabetes.com

Original Research

Overexpression of the Severe Acute Respiratory Syndrome Coronavirus-2 Receptor, Angiotensin-Converting Enzyme 2, in Diabetic Kidney Disease: Implications for Kidney Injury in Novel Coronavirus Disease 2019

Richard E. Gilbert MD, PhD [a,*]; Lauren Caldwell BSc [b]; Paraish S. Misra MD, PhD [c]; Kin Chan MSc [b]; Kevin D. Burns MD [d]; Jeffrey L. Wrana PhD [b]; Darren A. Yuen MD, PhD [a]

[a] Keenan Research Centre for Biomedical Science, Li Ka Shing Knowledge Institute, Unity Health Toronto, St. Michael's Hospital, Toronto, Ontario, Canada
[b] Center for Systems Biology, Lunenfeld-Tanenbaum Research Institute, Mt. Sinai Hospital and Department of Molecular Genetics, University of Toronto, Toronto, Ontario, Canada
[c] Division of Nephrology, Department of Medicine, University of Toronto, Ontario, Ontario, Canada
[d] Division of Nephrology, Department of Medicine, Ottawa Hospital Research Institute, University of Ottawa, Ottawa, Ontario, Canada

Key Messages

- Severe acute respiratory syndrome coronavirus-2 (SARS-CoV-2), the etiologic agent of the coronavirus disease 19 pandemic, enters cells by binding to angiotensin-converting enzyme 2 on cell surfaces.
- Beyond the lungs, the virus can infect the kidney, causing acute injury.
- Angiotensin-converting enzyme 2 expression is increased approximately 2-fold in diabetic kidney disease biopsies.

ARTICLE INFO

Article history:
Received 15 June 2020
Received in revised form
9 July 2020
Accepted 13 July 2020

Keywords:
ACE 2
coronavirus
COVID-19
diabetes
kidney
renin-angiotensin-aldosterone system
RNA Seq
SARS-CoV-2

ABSTRACT

Objectives: Diabetes is associated with adverse outcomes, including death, after coronavirus disease 19 (COVID-19) infection. Beyond the lungs, Severe Acute Respiratory Syndrome Coronavirus-2 (SARS-CoV-2), the etiologic agent of the COVID-19 pandemic, can infect a range of other tissues, including the kidney, potentially contributing to acute kidney injury in those with severe disease. We hypothesized that the renal abundance of angiotensin-converting enzyme (ACE) 2, the cell surface receptor for SARS-CoV-2, may be modulated by diabetes and agents that block the renin-angiotensin-aldosterone system (RAAS).
Methods: The expression of ACE 2 was examined in 49 archival kidney biopsies from patients with diabetic kidney disease and from 12 healthy, potential living allograft donors using next-generation sequencing technology (RNA Seq).
Results: Mean ACE 2 messenger RNA was increased approximately 2-fold in diabetes when compared with healthy control subjects (mean ± SD, 13.2±7.9 vs 7.7±3.6 reads per million reads, respectively; p=0.001). No difference in transcript abundance was noted between recipients and nonrecipients of agents that block the RAAS (12.2±6.7 vs 16.2±10.7 reads per million reads, respectively; p=0.25).
Conclusions: Increased ACE 2 messenger RNA in the diabetic kidney may increase the risk and/or severity of kidney infection with SARS-CoV-2 in the setting of COVID-19 disease. Further studies are needed to ascertain whether this diabetes-related overexpression is generalizable to other tissues, most notably the lungs.

Mots clés:
ECA 2
coronavirus
COVID-19
diabète
reins
système rénine-angiotensine-aldostérone
RNA-Seq
SRAS-CoV-2

RÉSUMÉ

Objectifs : Le diabète est associé à des issues défavorables, dont le décès après l'infection par la maladie à coronavirus 2019 (COVID-19, de l'anglais *COronaVIrus Disease 2019*). Outre les poumons, le SRAS-CoV-2, l'agent étiologique de la pandémie de la COVID-19, peut infecter de nombreux autres tissus, notamment les reins, d'où la possibilité de contribuer à l'insuffisance rénale aiguë chez les patients qui en sont atteints gravement. Nous avons posé l'hypothèse que l'excès de l'enzyme de conversion de l'angiotensine

* Address for correspondence: Richard E. Gilbert MD, PhD, Division of Endocrinology, St. Michael's Hospital, 61 Queen Street East, Toronto, Ontario M5C 2T2, Canada.
E-mail address: richard.gilbert@utoronto.ca

1499-2671/
https://doi.org/10.1016/j.jcjd.2020.07.003

(ECA) 2 dans les reins, le récepteur à la surface des cellules sur lequel se fixe le SRAS-CoV-2, peut être modulé par le diabète et les agents qui bloquent le système rénine-angiotensine-aldostérone (SRAA).
Méthodes : Nous avons examiné l'expression de l'ECA 2 dans 49 biopsies rénales d'archives provenant de patients atteints d'une néphropathie diabétique et de 12 biopsies de tissus prélevés de donneurs vivants potentiels en bonne santé à des fins d'allogreffes à l'aide de la technologie de séquençage de nouvelle génération (RNA-Seq, de l'anglais *RNA sequencing*).
Résultats : L'expression moyenne de l'ARN messager de l'ECA 2 s'est révélée environ 2 fois plus élevée chez les sujets diabétiques que chez les sujets témoins en bonne santé (moyenne ± ÉT, 13,2 ± 7,9 *vs* 7,7 ± 3,6 lectures par million de lectures, respectivement; p = 0,001). Nous n'avons noté aucune différence dans l'excès de transcription entre les receveurs et les non-receveurs d'agents qui bloquent le SRAA (12,2 ± 6,7 *vs* 16,2 ± 10,7 lectures par million de lectures, respectivement; p = 0,25).
Conclusions : L'augmentation de l'ARN messager de l'ECA 2 dans les reins diabétiques peut accroître le risque ou la gravité de l'infection rénale par SRAS-CoV-2, ou les deux, dans le contexte de la COVID-19. D'autres études sont nécessaires pour vérifier si cette surexpression liée au diabète est généralisable à d'autres tissus, plus particulièrement les poumons.

Introduction

Diabetes is associated with an adverse outcome, including death, after coronavirus disease 19 (COVID-19) infection (1); however, whether it also increases susceptibility to infection is unknown. Severe acute respiratory syndrome coronavirus-2 (SARS-CoV-2), the etiologic agent of the pandemic, binds to the cell surface ectoenzyme, angiotensin-converting enzyme (ACE) 2, the abundance of which may be a determinant of virus entry and vulnerability to infection (2). Recent studies have identified the kidney as a site of infection by SARS-CoV-2 (3), and have highlighted acute kidney injury as a common adverse outcome in patients with severe COVID-19 disease (4). Although hemodynamic imbalance and cytokine release are likely contributors to the acute loss of kidney function, the ability of SARS-CoV-2 to infect kidney cells and to induce immunologic injury and microthrombus formation (5) suggests that the virus itself may be directly involved (6).

Substantial advances have been made in the assessment of gene expression in kidney biopsies, focusing in particular on site-specific changes in glomeruli and to a lesser extent tubules (7). ACE 2, however, is highly expressed in macrophages (8) and in the microvasculature (9) that resides in the interstitial space and traditionally not subjected to selective laser capture microscopic microdissection of the kidney (7). Accordingly, we sought to examine the abundance of ACE 2 in whole rather than microdissected biopsy tissue from individuals with diabetic kidney disease.

Methods

After approval from the St. Michael's Hospital research ethics board, our centre compiled a biobank of archived kidney biopsies from which to conduct molecular analyses. Seventy-three patients with a clinical diagnosis of diabetes and a pathologic diagnosis of diabetic nephropathy on biopsy from January 2007 to September 2016 at St. Michael's Hospital were reviewed. All biopsies sampled the renal cortex. Of these, 49 showed only diabetic kidney disease and had adequate documentation of their clinical status and medications at the time of the biopsy with emphasis on their use of agents that block the renin-angiotensin-aldosterone system (RAAS), including ACE inhibitors, angiotensin receptor blockers (ARBs), direct renin inhibitors (DRIs) or mineralocorticoid receptor antagonists (MRAs). Glycated hemoglobin measurements within 3 months of the biopsy were available in 32 participants. Kidney biopsies from healthy control subjects were obtained from 12 potential living allograft donors.

To assess the transcriptome in these biopsies, ten 10-μm-thick sections were cut from formalin fixed paraffin-embedded kidney biopsy blocks or from fresh frozen tissue embedded in Cryomatrix (BD Biosciences, Canada). Total RNA was extracted, ribosomal RNA was removed, and complementary DNA libraries were then prepared and quantitated prior to RNA sequencing (Illumina HiSeq 3000), as previously reported (10). Abundance of ACE 2 messenger RNA was then determined and analyzed according to whether patients were receiving an agent that blocked the RAAS: ACE inhibitors, ARBs, DRIs or MRAs.

Statistical analysis

Data are expressed as mean ± SD, unless otherwise stated. The magnitude of gene expression in control and diabetic kidney disease biopsies was compared using an unpaired Student t test. Within the diabetic kidney disease group, expression levels between those receiving and not receiving treatment with an agent that blocks the RAAS were similarly compared using an unpaired Student t test. $p<0.05$ was considered significant.

Results

At the time of biopsy, approximately three-quarters (38 of 49) of those with diabetic kidney disease were receiving therapy with at least 1 agent that blocks the RAAS: 25 as single agent ACE inhibitor, ARB or MRA treatment with 11 prescribed dual therapy and a further 2 receiving triple ACE, ARB and MRA combination treatment (Table 1). As in most other sites, there were more men than women in the diabetic kidney disease group with the reverse pattern seen in the control, living allograft donors.

The magnitude of ACE 2 gene expression in biopsies obtained from patients with diabetic kidney disease varied widely when compared with that found in biopsies from healthy control subjects. The variability notwithstanding, mean ACE 2 messenger RNA was increased approximately 2-fold in diabetes when compared with healthy control subjects (Figure 1, Supplementary Table 1), whereby mean expression of ACE 2 was 13.2±7.9 reads per million reads (RPMs) in biopsies from subjects with diabetic kidney disease and 7.7±3.6 RPMs in those from control subjects (p=0.001). No difference in transcript abundance was noted between recipients and nonrecipients of agents that block the RAAS (12.2±6.7 vs

Table 1
Clinical data on patients with diabetic kidney disease and healthy control subjects (potential allograft donors)

Clinical parameters	Patients with diabetic kidney disease (n=49)	Healthy control subjects (n=12)
Age, years	56±10	47±9
% female	33	92
Duration of diabetes, years	14±9	N/A
HbA1c, mmol/mol, %	66.1; 8.2%±2.3%	
Baseline estimated glomerular filtration rate, mL/min/1.73 m^2	40±22	89±13
Baseline urine albumin to creatinine ratio, mg/mmol	337±322	0.8±0.9
Medication usage		
RAAS nonusers	11	N/A
RAAS users	38	
DRI only	0	
ACEi only	12	
ARB only	12	
MRA only	1	
DRI + ACEi	3	
DRI + ARB	1	
DRI + MRA	0	
ACEi + ARB	5	
ACEi + MRA	1	
ARB + MRA	1	
ACEi + ARB + MRA	2	

ACEi, angiotensin-converting enzyme inhibitor; *ARB,* angiotensin receptor blocker; *DRI,* direct renin inhibitor; *HbA1C,* glycated hemoglobin; *MRA,* mineralocorticoid receptor antagonist; *N/A,* not applicable; *RAAS,* renin-angiotensin-aldosterone system.
Note: All values are presented as mean ± SD, number of patients or as otherwise indicated.

16.2±10.7 RPMs, respectively; p=0.25) or among those receiving treatment with an ACE inhibitor, ARB, DRI, diuretic or MRA (Figure 2). Similarly, we found no relationship between ACE 2 copy number and either glycated hemoglobin (Spearman rho = 0.05; p=0.8), or between ACE 2 copy number and estimated glomerular filtration rate (ρ=0.10, p=0.5).

Discussion

An increase in ACE 2 expression in the setting of diabetic kidney disease raises the possibility that such individuals may be at higher risk of kidney infection with SARS-CoV-2 in COVID-19 disease, potentially increasing the risk of acute kidney injury and death. Furthermore, the study provides reassurance that any propensity to infection should not be exacerbated by concomitant use of agents that block the RAAS.

The main strengths of this study are the number of biopsies examined, the state-of-the-art technology used to quantify gene expression and the documentation of medications taken at the time of biopsy. We found only a single published study that compared ACE 2 messenger RNA in kidney biopsies from patients with diabetes with that of healthy control subjects using robust methodology (quantitative polymerase chain reaction) (7). In contrast with the current study of 49 biopsies, the number of biopsies in the study by Reich et al (7) was comparatively small, examining only 13 biopsies from patients with diabetes and showing a reduction in expression in diabetes. Moreover, unlike our study, the use of laser capture of proximal tubules and glomeruli in the latter study would not have fully taken into account the abundant ACE 2 expression in infiltrating cells, the postglomerular vasculature and nephron segments beyond the proximal tubule that may display differential expression in diabetes (11). Indeed, macrophages and pericytes have been shown to express high levels of ACE 2, suggesting that these cells may be particularly vulnerable to SARS-CoV-2 infection (8). In addition, the use of next-generation RNA Seq technology

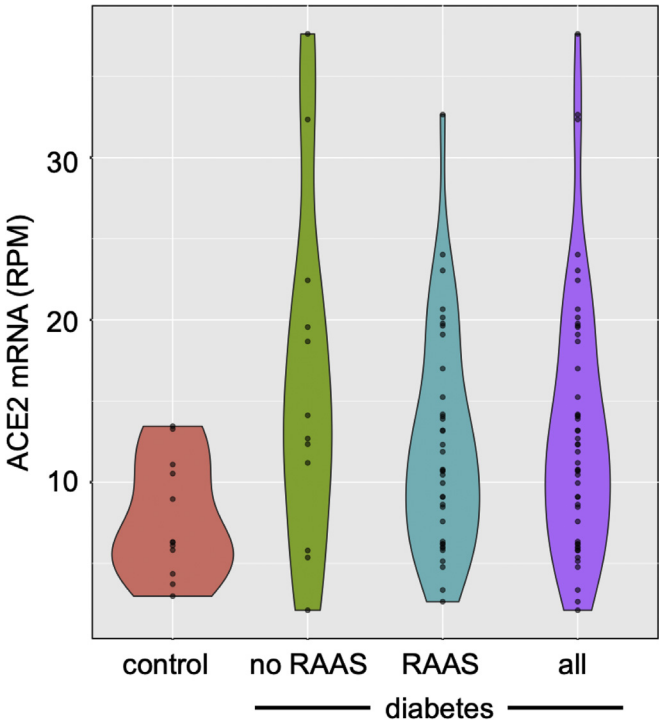

Figure 1. Violin plots showing ACE2 mRNA expression as transcript union RPMs in kidney biopsies from individuals with diabetic kidney disease (purple) and living allograft donors (red) with ACE2 expression in diabetic samples also assessed according to use (blue) or not (green) of agents that block the RAAS: angiotensin-converting enzyme inhibitors, angiotensin receptor blockers, direct renin inhibitors and mineralocorticoid receptor antagonists. *ACE2,* angiotensin-converting enzyme 2; *mRNA,* messenger RNA; *RAAS,* renin-angiotensin-aldosterone system; *RPM,* reads per million read.

enabled a true measurement of ACE 2 transcript levels, as opposed to the relative levels generated by semiquantitative polymerase chain reaction or microarray-based techniques. Finally, archived remnants of formalin-fixed paraffin-embedded core biopsies have not generally been used for RNA Seq because the yield and quality of RNA from such small tissue samples has been insufficient for RNA sequencing. Our technology, developed in bladder tumours (10), overcame these limitations, enabling us to examine ACE 2 expression in small, residual amounts of clinically indicated renal biopsy samples.

In spite of the large number of biopsy specimens studied and reliance on whole rather than microdissected tissue, our study does, however, have several limitations. Most notably, the study was confined to the examination of kidney tissue and not lungs, the principal site of COVID-19 infection. Moreover, having studied only patients with biopsy-proven diabetic kidney disease, we are unable to make any inferences on whether the changes in ACE 2 messenger RNA observed in the current study apply to other organs, most notably the lungs, or if the changes in ACE 2 expression seen in our study group also apply to the kidneys of individuals with diabetes and normal kidney function. In addition, the current study which assessed gene expression in sections cut from kidney biopsies was unable to determine cell-specific patterns of expression that may have differentially affected parenchymal, vascular and inflammatory cells. Finally, although the abundance of a receptor required for SARS-CoV-2 entry was examined, many more components of the cell machinery are required for the virus to infect a cell, initiate virus replication and kill its host; although we assessed gene expression, its translation into protein was not examined.

Diabetes is a well-known risk factor not only for severe bacterial infections, but also for viral infections, such as H1N1 influenza (12). As was the case in previous human coronavirus infections, such as

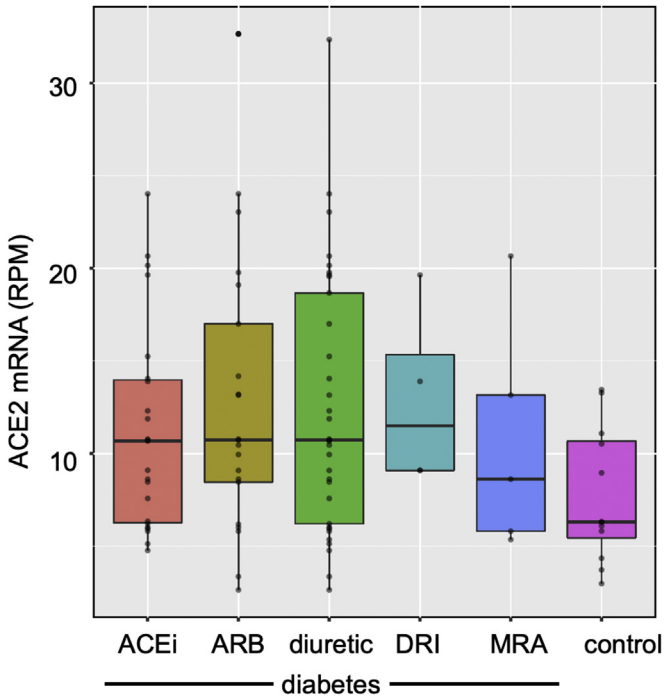

Figure 2. Box plots showing ACE2 mRNA expression as transcript union RPMs in kidney biopsies from individuals with diabetic kidney disease assessed according to use of ACEis, ARBs, DRIs, diuretics and MRAs. *ACE2,* angiotensin-converting enzyme 2; *ACEi,* angiotensin-converting enzyme inhibitor; *ARB,* angiotensin receptor blocker; *DRI,* direct renin inhibitor; *MRA,* mineralocorticoid receptor antagonist; *mRNA,* messenger RNA; *RPM,* reads per million read.

severe acute respiratory syndrome and Middle East respiratory syndrome, individuals with diabetes are also at higher risk of adverse outcomes with COVID-19 (13). Although this may reflect a generalized predisposition to poor outcome with infectious diseases, it may also be a consequence of an increased propensity for cellular entry and invasion by SARS-CoV-2, while noting that the current study assessed ACE 2 gene expression in the kidney and not in the lungs.

Interaction between a virus and its host cells are key determinants of infection severity. For instance, studies of a related murine coronavirus showed a dose-dependent relationship between the number of infective virus particles and the likelihood of death (14). Tissue-specific viral receptor abundance may also influence infectivity (11) and thereby contribute to the extrapulmonary manifestations of COVID-19, such as kidney and vascular disease. Consistent with this clinical observation, single cell RNA sequencing identified cells in the lung, kidney and heart as major sites of ACE 2 expression (2). Accordingly, the augmented kidney ACE 2 expression demonstrated in the present study may signify a greater propensity to renal complications of COVID-19 among individuals with diabetes.

A number of recently published observational studies concluded that the use of agents that block the RAAS increased neither the propensity to infection nor the likelihood of an adverse outcome (15–17). Although these studies did not specifically examine whether this broad conclusion also applied to individuals with diabetes, the current study does not indicate any relationship between the use of agents that block the RAAS and ACE 2 expression in the diabetic setting.

Supplementary Material

To access the supplementary material accompanying this article, visit the online version of the *Canadian Journal of Diabetes* at www.canadianjournalofdiabetes.com.

Acknowledgments

We thank Niki Dacouris, Michelle Nash, Lindita Rapi and Weiqiu Yuan for their assistance with patient data collection. We also thank the contributions of our patient partners in research: Mary Beaucage, Gwen Herrington and Dwight Sparkes. We thank the Network Biology Collaborative Centre (nbcc.lunenfeld.ca) for the RNA-Seq service, a facility supported by the Canada Foundation for Innovation, the Ontarian Government and Genome Canada and Ontario Genomics (OGI-139).

This study was funded by Can-SOLVE CKD, a SPOR grant from the Canadian Institutes of Health Research and a Transformational Grant by the Banting and Best Diabetes Centre in Toronto. R.E.G. is the Canada Research Chair in Diabetes Complications and this work was supported in part by the Canada Research Chairs' Program. D.A.Y. is supported by a CIHR New Investigator Award and the St. Michael's Hospital Foundation.

The study funders were not involved in the design of the study; the collection, analysis and interpretation of data or the writing of the report, nor did they impose any restrictions regarding the publication of the report.

Author Disclosures

R.E.G. reports receiving research grants to his institution from AstraZeneca and Boehringer Ingelheim; serving on advisory panels for AstraZeneca, Boehringer Ingelheim and Janssen and receiving CME speaker honoraria from AstraZeneca, Bayer, Boehringer Ingelheim and Janssen, all unrelated to the current study. He also reports being a shareholder in Certa Therapeutics, OccuRx and Fibrocor Therapeutics and is CSO of Fibrocor Therapeutics. J.L.W. and D.A.Y. are consultants and own shares in Fibrocor Therapeutics. P.S.M. was supported by a Kidney Foundation of Canada KRESCENT postdoctoral fellowship and an Eli Lilly Clinician-Scientist Trainee Fellowship in Diabetes. No other authors have any conflicts of interest to declare.

Author Contributions

R.E.G., J.L.W. and D.A.Y. designed the research. P.S.M., K.C. and L.C. acquired the data. J.L.W. and L.C. contributed to the statistical analyses. R.E.G., J.L.W., K.D.B. and D.A.Y. interpreted the data. R.E.G. drafted the manuscript. All authors reviewed and critically revised the draft for important intellectual content and approved the final manuscript to be published. J.L.W. is the guarantor of this work.

References

1. Onder G, Rezza G, Brusaferro S. Case-fatality rate and characteristics of patients dying in relation to COVID-19 in Italy. JAMA 2020 (In press).
2. Zou X, Chen K, Zou J, Han P, Hao J, Han Z. Single-cell RNA-seq data analysis on the receptor ACE2 expression reveals the potential risk of different human organs vulnerable to 2019-nCoV infection. Front Med 2020 (In press).
3. Farkash EA, Wilson AM, Jentzen JM. Ultrastructural evidence for direct renal infection with SARS-CoV-2. J Am Soc Nephrol 2020 (In press).
4. Cheng Y, Luo R, Wang K, et al. Kidney disease is associated with in-hospital death of patients with COVID-19. Kidney Int 2020 (In press).
5. Sise ME, Baggett MV, Shepard JO, Stevens JS, Rhee EP. Case 17-2020: A 68-year-old man with Covid-19 and acute kidney injury. N Engl J Med 2020;382:2147–56.
6. Puelles VG, Lutgehetmann M, Lindenmeyer MT, et al. Multiorgan and renal tropism of SARS-CoV-2. N Engl J Med 2020 (In press).
7. Reich HN, Oudit GY, Penninger JM, Scholey JW, Herzenberg AM. Decreased glomerular and tubular expression of ACE2 in patients with type 2 diabetes and kidney disease. Kidney Int 2008;74:1610–6.
8. Chen L, Li X, Chen M, Feng Y, Xiong C. The ACE2 expression in human heart indicates new potential mechanism of heart injury among patients infected with SARS-CoV-2. Cardiovasc Res 2020;116:1097–100.
9. Zulli A, Burrell LM, Buxton BF, Hare DL. ACE2 and AT4R are present in diseased human blood vessels. Eur J Histochem 2008;52:39–44.

10. Liu Y, Noon AP, Aguiar Cabeza E, et al. Next-generation RNA sequencing of archival formalin-fixed paraffin-embedded urothelial bladder cancer. Eur Urol 2014;66:982–6.
11. Pan XW, Xu D, Zhang H, Zhou W, Wang LH, Cui XG. Identification of a potential mechanism of acute kidney injury during the COVID-19 outbreak: A study based on single-cell transcriptome analysis. Intensive Care Med 2020 (In press).
12. Allard R, Leclerc P, Tremblay C, Tannenbaum TN. Diabetes and the severity of pandemic influenza A (H1N1) infection. Diabetes Care 2010;33:1491–3.
13. Muniyappa R, Gubbi S. COVID-19 pandemic, coronaviruses, and diabetes mellitus. Am J Physiol Endocrinol Metab 2020;318:E736–41.
14. De Albuquerque N, Baig E, Ma X, et al. Murine hepatitis virus strain 1 produces a clinically relevant model of severe acute respiratory syndrome in A/J mice. J Virol 2006;80:10382–94.
15. Reynolds HR, Adhikari S, Pulgarin C, et al. Renin-angiotensin-aldosterone system inhibitors and risk of Covid-19. N Engl J Med 2020 (In press).
16. Mancia G, Rea F, Ludergnani M, Apolone G, Corrao G. Renin-angiotensin-aldosterone system blockers and the risk of Covid-19. N Engl J Med 2020 (In press).
17. Mehra MR, Desai SS, Kuy S, Henry TD, Patel AN. Cardiovascular disease, drug therapy, and mortality in Covid-19. N Engl J Med 2020 (In press).

Supplementary Table 1
Quality control meta-data for RNA Seq analysis of ACE 2 expression

ID	ACE 2 RPM	ACE 2 count (raw read number)	Raw read count from fastqc	Mapped read numbers	Final unique read numbers	Mapping % (mapped reads/raw reads from fastqc)	Unique reads/mapped reads (%)	Unique reads/raw reads (%)	Condition
S1	11.88	1,166	101,465,082	98,099,054	82,972,027	96.682575	84.5798442	81.77397127	DKD
S10	32.35	3,808	126,566,284	117,684,063	98,667,865	92.98215866	83.8413142	77.95746377	DKD
S11	3.36	642	188,577,850	190,596,410	159,468,358	101.070412	83.6680806	84.56367384	DKD
S12	5.36	520	114,244,968	96,992,737	66,805,465	84.89891388	68.8767706	58.47563019	DKD
S13	6.21	808	175,045,896	129,964,897	97,959,487	74.24618341	75.3738042	55.96217291	DKD
S14	6.29	819	137,316,374	130,057,555	99,596,713	94.71379939	76.5789523	72.53083525	Normal Control
S15	19.77	2,454	125,973,470	124,069,715	106,369,186	98.48876513	85.7334008	84.43776773	DKD
S17	2.98	338	114,739,886	113,279,463	92,896,430	98.72718803	82.0064181	80.96263055	Normal Control
S18	6.32	797	131,967,904	125,958,052	98,037,370	95.4459745	77.8333488	74.28879828	Normal Control
S19	18.67	2,234	125,828,736	119,626,461	92,277,030	95.07085965	77.137641	73.33541839	DKD
S2	32.65	3,358	107,149,218	102,835,831	81,401,804	95.97441112	79.157044	75.97050685	DKD
S20	14.05	1,630	121,341,514	116,008,433	94,539,795	95.6048999	81.4938988	77.91216038	DKD
S21	10.46	1,168	114,541,108	111,565,723	87,783,595	97.4023431	78.6833022	76.63937999	DKD
S23	13.16	1,886	153,494,604	143,258,910	123,152,446	93.33156102	85.964947	80.23242693	DKD
S24	7.58	1,263	176,090,214	166,535,504	132,321,427	94.57396877	79.4553857	75.14411164	DKD
S26	5.13	595	121,143,542	115,801,424	96,940,374	95.59025771	83.7125923	80.02108276	DKD
S28	13.28	1,584	127,927,852	119,193,003	93,410,716	93.1720506	78.3692949	73.01827908	Normal Control
S29	8.96	1,094	124,756,622	122,075,555	98,496,727	97.85096217	80.6850536	78.95110129	Normal Control
S3	19.64	1,911	108,050,092	97,254,218	73,226,930	90.0084546	75.2943487	67.77127964	DKD
S30	4.35	469	116,072,918	107,717,085	72,359,389	92.80122087	67.1754058	62.33959674	Normal Control
S31	5.81	599	126,222,334	102,977,820	76,401,402	81.58446824	74.192095	60.52922615	DKD
S32	37.61	3,643	103,394,492	96,860,281	85,553,782	93.68031036	88.3270017	82.74500928	DKD
S33	9.95	1,149	124,113,112	115,461,620	101,243,718	93.02934891	87.6860363	81.57374863	DKD
S34	6.1	723	128,575,262	118,359,206	93,275,371	92.05441557	78.8070266	72.5453478	Normal Control
S35	13.45	1,796	136,947,722	133,454,499	102,748,768	97.44922884	76.9916105	75.02773065	Normal Control
S36	12.31	1,252	106,491,158	101,699,951	76,515,285	95.50084055	75.2363047	71.85130337	DKD
S38	6.34	660	108,149,060	104,020,509	86,927,172	96.1825364	83.5673396	80.37718682	DKD
S4	9.09	892	105,342,072	98,043,413	83,559,528	93.07146816	85.2270698	79.3220851	DKD
S40	22.44	2,083	102,534,852	92,791,329	80,415,714	90.49735499	86.6629618	78.42768818	DKD
S43	5.82	717	129,310,302	123,119,602	91,843,518	95.21252375	74.5969907	71.02567744	Normal Control
S47	10.53	1,301	125,599,084	123,536,436	97,933,170	98.35775235	79.2747251	77.97283776	Normal Control
S48	11.09	1,358	129,861,432	122,361,656	88,440,726	94.22478569	72.2781375	68.10392018	Normal Control
S49	19.56	2,664	140,716,858	136,163,725	113,595,350	96.76433011	83.4255599	80.72618421	DKD
S5	20.66	2,525	129,277,952	122,208,257	92,915,798	94.53139929	76.0307039	71.87288827	DKD
S50	8.47	1,178	145,194,772	138,993,857	122,500,834	95.72924361	88.1339914	84.37000335	DKD
S51	8.62	1,009	121,066,116	117,037,226	103,152,225	96.67215722	88.1362525	85.20321656	DKD
S52	5.79	664	119,156,404	114,491,282	100,216,841	96.08487514	87.53229	84.10529156	DKD
S53	10.78	1,439	140,139,922	133,387,449	117,250,406	95.18162069	87.9021279	83.66666994	DKD
S54	12.34	1,677	138,605,802	135,790,629	116,695,942	97.96893567	85.9381408	84.19268192	DKD
S55	10.68	1,134	108,659,548	106,132,291	85,310,691	97.67415101	80.3814656	78.51191411	DKD
S56	15.24	1,993	128,886,930	130,769,149	94,009,761	101.4603645	71.8898622	72.93971623	DKD
S57	20.15	3,081	151,341,932	152,896,130	126,284,590	101.0269447	82.5950206	83.44322577	DKD
S58	13.2	1,746	131,157,148	132,245,790	91,054,983	100.8300287	68.8528406	69.42433896	DKD
S59	6.19	1,056	170,223,200	170,469,398	120,482,742	100.1446325	70.6770502	70.77927216	DKD
S60	14.12	1,667	121,045,880	117,986,535	89,261,896	97.47257404	75.6543075	73.74220089	DKD
S61	9.1	1,198	135,099,374	131,562,506	105,719,709	97.38202488	80.3570198	78.25329302	DKD
S62	2.64	366	135,216,050	138,598,101	107,703,422	102.5012201	77.7091614	79.65283855	DKD
S63	5.95	786	133,282,782	132,057,096	107,361,864	99.08038684	81.2995797	80.55193806	DKD
S64	10.73	1,520	146,982,650	141,617,337	121,123,920	96.34969638	85.5290197	82.40695075	DKD
S65	23.04	2,726	121,592,562	118,315,222	97,936,482	97.30465421	82.7758934	80.54479681	DKD
S66	2.11	371	173,153,814	175,225,951	137,535,251	101.1967031	78.4902295	79.42952443	DKD
S67	17	2,612	147,157,566	153,576,126	115,308,540	104.3616921	75.0823341	78.35719436	DKD
S68	19.1	2,359	124,788,568	123,450,100	102,452,239	98.92741136	82.9908109	82.10066086	DKD
S69	11.19	1,781	160,522,214	159,145,496	135,084,192	99.14235048	84.8809394	84.15295842	DKD
S7	3.72	550	146,984,322	147,547,677	120,541,988	100.3832756	81.6969745	82.01009901	Normal Control
S70	12.69	1,689	133,615,280	133,031,354	107,608,627	99.56297962	80.8896728	80.53616847	DKD
S71	14.18	2,045	140,677,196	144,153,124	111,566,950	102.4708539	77.39475	79.30706125	DKD
S72	6.01	1,634	281,300,140	271,680,124	207,399,305	96.58015954	76.3395209	73.72883106	DKD
S73	4.77	483	123,833,718	101,246,379	61,657,133	81.75994441	60.8981117	49.79026229	DKD
S8	24.02	2,450	103,952,142	101,965,133	79,035,117	98.08853482	77.511905	76.0302919	DKD
S9	13.9	1,671	130,740,442	120,131,959	109,764,113	91.88584432	91.3696188	83.95574569	DKD

ACE 2, angiotensin-converting enzyme 2; *DKD*, diabetic kidney disease; *ID*, identification; *RPM*, reads per million read.