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Original Article

ORIGINAL ARTICLE

Increased expression of SCARF genes favoring SARS-CoV-2 infection in key target organs in CKD

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ABSTRACT

Background. Chronic kidney disease (CKD), especially diabetic CKD, is the condition that most increases the risk of lethal coronavirus disease 2019 (COVID-19) caused by severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2). However, the underlying molecular mechanisms are unclear. SARS-CoV-2 and coronavirus-associated receptors and factors (SCARFs) regulate coronavirus cell entry and/or replication. We hypothesized that CKD may alter the expression of SCARF genes.

Methods. A literature search identified 34 SCARF genes of which we selected 21 involved in interactions between SARS-CoV/SARS-CoV-2 and host cells, and assessed their mRNA expression in target tissues of COVID-19 (kidneys, lungs, aorta and heart) in mice with adenine-induced CKD.

Results. Twenty genes were differentially expressed in at least one organ in mice with CKD. For 15 genes, the differential expression would be expected to favor SARS-CoV-2 infection and/or severity. Of these 15 genes, 13 were differentially expressed in the kidney and 8 were validated in human CKD kidney transcriptomics datasets, including those for the most common cause of CKD, diabetic nephropathy. Two genes reported to protect from SARS-CoV-2 were downregulated in at least two non-kidney target organs: Ifitm3 encoding interferon-induced transmembrane protein 3 (IFITM3) in lung and Ly6e encoding lymphocyte antigen 6 family member 6 (LY6E) in aorta.

Conclusion. CKD, including diabetic CKD, is associated with the differential expression of multiple SCARF genes in target organs of COVID-19, some of which may sensitize to SARS-CoV-2 infection. This information may facilitate developing therapeutic strategies aimed at decreasing COVID-19 severity in patients with CKD.

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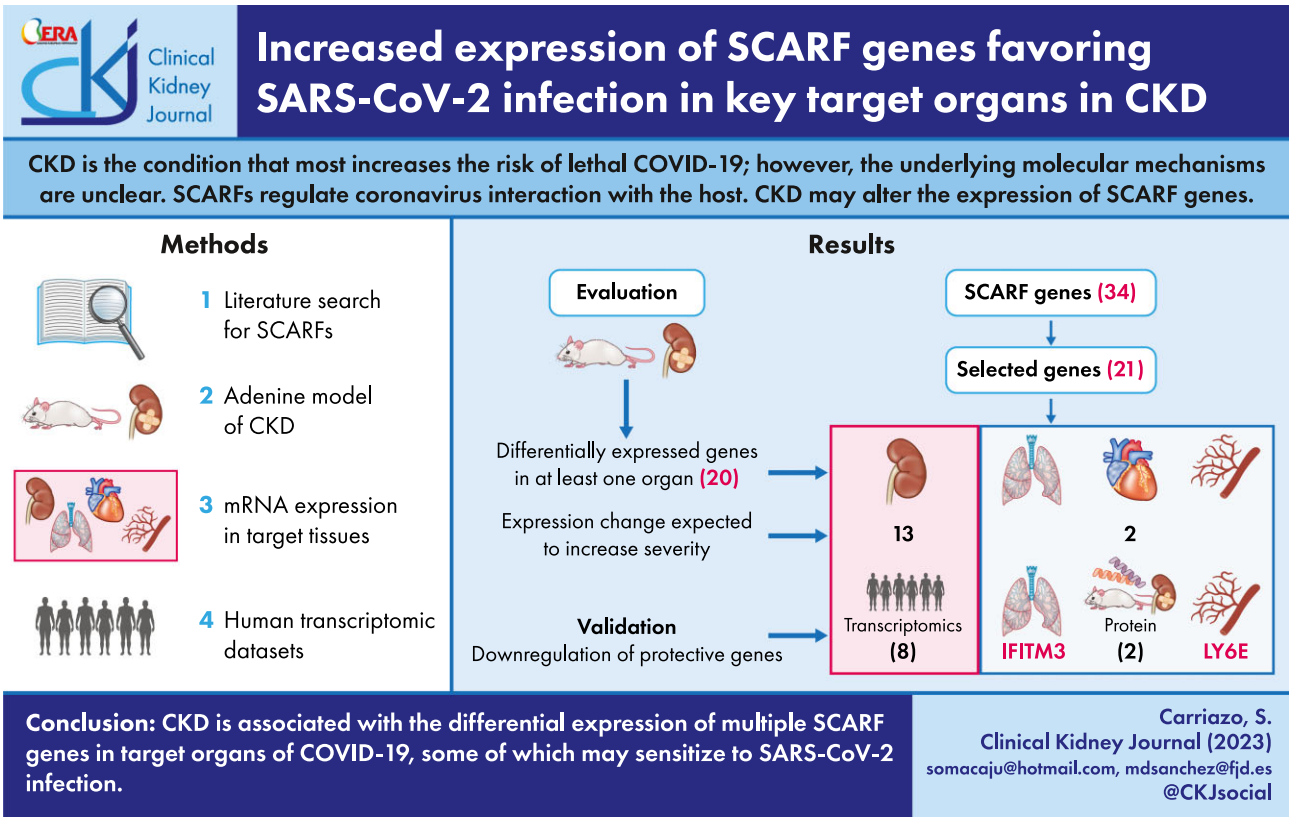
GRAPHICAL ABSTRACT



Keywords: chronic kidney disease, mortality, risk factor, SARS-CoV-2, SCARF

KEY LEARNING POINTS

What was known:

- Chronic kidney disease (CKD) patients represent the largest number of persons at high risk of death from coronavirus disease 2019 (COVID-19). Diabetic nephropathy is the most common cause of CKD. Acute kidney injury (AKI) is frequent in COVID-19 and is also associated with an increased risk of death.
- Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) and coronavirus-associated receptors and factors (SCARFs) regulate viral cell entry and/or replication.
- The cellular and molecular mechanisms underlying this increased risk are unclear.

This study adds:

- CKD is associated with the differential expression of SCARF genes in target organs of COVID-19 which may increase the risk for SARS-CoV-2 infection and/or severity.
- A high percentage of genes reported to increase the risk for SARS-CoV-2 infection are overexpressed in kidneys with CKD, including diabetic CKD.
- Two genes reported to protect from SARS-CoV-2 were downregulated in targets organs in CKD: Ifitm3 in the lung and Ly6e in the aorta.

Potential impact:

- Identifying potential mechanisms that may increase the risk for lethal COVID-19 or AKI in CKD patients may allow development of therapeutic strategies aimed at decreasing the severity of COVID-19 in future coronavirus pandemics in this population.

INTRODUCTION

Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) causes coronavirus disease 2019 (COVID-19). The SARS-CoV-2 pandemic has caused over 6.6 million deaths [1]. SARS-CoV-2 causes primarily pulmonary damage but can injure other organs and systems like the kidneys or the gastrointestinal, cardiovascular or nervous systems [2]. The main risk factor for severe COVID-19 is older age [3]. Additionally, preexistent conditions and genetic factors increase the risk for severe COVID-19 [3, 4]. Chronic kidney disease (CKD) is the preexistent condition that most increases the risk of death from COVID-19, especially advanced CKD that requires kidney replacement therapy, but patients with a glomerular filtration rate <30 mL/min/1.73 m^2 were ranked fifth among conditions at highest risk of COVID-19 death [3, 5]. Diabetic nephropathy is the most common cause of CKD and is associated with a higher risk of COVID-19 death. In the USA, an excess of 7000 to 10 000 deaths in patients with kidney failure occurred during the first months of the pandemic. In the Spanish region of Madrid, COVID-19 was the most frequent cause of death in 2020 among patients on kidney replacement therapy [6]. Indeed, in 2020 the annual mortality rate doubled compared with prior years and for the first time since records began, the prevalence of kidney replacement therapy decreased [6]. Moreover, according to the Global Burden of Disease collaboration, CKD is the most common preexistent condition that increases the risk of severe COVID-19. The increased risk of death associated to CKD is not limited to the initial COVID-19 admission and persists elevated for months [7]. Indeed, patients on dialysis are at the highest risk of COVID-19 death and this is related to injury to non-kidney organs, as the kidney has already failed before SARS-CoV-2 infection [3, 5]. Additionally, acute kidney injury (AKI) is frequent in COVID-19 and is associated with an increased risk of death. Approximately 28% of hospitalized COVID-19 patients presented AKI and 9% needed kidney replacement therapy [8]. Finally, CKD may be a long-term sequela of COVID-19.

The cellular and molecular mechanisms underlying the increased risk of severe COVID-19 in persons with CKD are unclear. CKD and especially, kidney failure, is a risk factor for infection, death from infection and impaired vaccine responses [9]. Thus, the increased risk of severe COVID-19 may result from non-specific impairment of antiviral responses in CKD patients. However, this would not fully explain why CKD is the preexistent condition that is associated with the highest risk of COVID-19 death, given that other conditions (e.g. diabetes) are also associated with an increased risk of multiple types of infection. Uremic toxins that accumulate in CKD, and the resulting disruption of homeostasis, have a multisystemic impact, including the differential expression of multiple genes in several organs [10]. This may include dysregulation of the specific host molecular machinery that modulates SARS-CoV-2 entry and proliferation, although this possibility has not been systematically addressed so far. SARS-CoV-2 and coronavirus-associated receptors and factors (SCARFs) are molecules required for and/or that regulate coronavirus cell entry and/or viral replication [11–14].

We have now studied changes in the expression of genes encoding SCARFs in target organs of COVID-19 in mice with CKD. Interestingly, several SCARF genes were differentially expressed under CKD conditions in organs such as the kidneys, heart, aorta and lungs, in a manner that may be expected to favor viral entry into cells and/or viral proliferation. Kidney findings were validated in human transcriptomics databases, including those of diabetic CKD. These differentially expressed molecules may be novel therapeutic targets for approaches aimed at improving the ability of patients with CKD to effectively fight SARS-CoV-2 and, potentially, future coronavirus pandemics.

MATERIALS AND METHODS

Methods are expanded in the Supplementary data.

SCARF genes

SCARF genes were identified from the literature. Twenty-eight SCARF genes were reported by Singh *et al.* [11]. We focused on 17 factors reported to be directly related to SARS-CoV-2 or SARS-CoV infection. Four additional factors were reported shortly thereafter as relevant to the SARS-CoV-2–host interaction (AT1, AVPR1B, NRP1, NRP2) [12, 13] and were incorporated into the present analysis (Fig. 1). Thus, the expression of a total of 21 genes was evaluated in kidneys, lungs, aorta and heart of mice with CKD (Fig. 2).

Adenine-induced CKD

To explore the systemic impact of CKD, CKD was induced in 12-week-old C57BL/6 wild-type male mice fed a standard pellet chow or an adenine-rich diet (0.2% adenine) for 4 weeks. This model was chosen because it is non-invasive, results in increased serum levels of uremic retention solutes, including urea and creatinine, is fast and reproducible, and its severity may be modulated adjusting the adenine concentration or the duration of treatment.

RNA extraction and RT-PCR

Real-time polymerase chain reaction (RT-PCR) data have been previously described [15].

Histology and immunohistochemistry

Conventional histological assessment included hematoxylin–eosin and periodic acid–Schiff (PAS) is staining. Immunohistochemistry was performed as previously described on paraffin-embedded 3-µm thick tissue sections using the Envision detection kit (Dako) according to the manufacturer's instructions [16]. Kidney, lung, heart and aorta sections were counterstained with Carazzi's hematoxylin. Primary antibodies were goat polyclonal anti-Ly6E (1:100, Abyntek Biopharma) and rabbit polyclonal anti-interferon-induced transmembrane protein 3 (IFITM3) (1:100, Abcam). Negative controls included incubation with a non-specific immunoglobulin of the same isotype as the primary antibody. No specific immunohistochemistry signal was observed in the heart.

Western blot

Western blot was performed as previously described [17] and detailed in Supplementary data, Methods.

Data mining

Data mining to validate kidney findings in murine adenine-induced CKD was performed in in-house murine kidney transcriptomics datasets and in human CKD transcriptomics datasets from Nephroseq v5 (http://v5.nephroseq.org/).

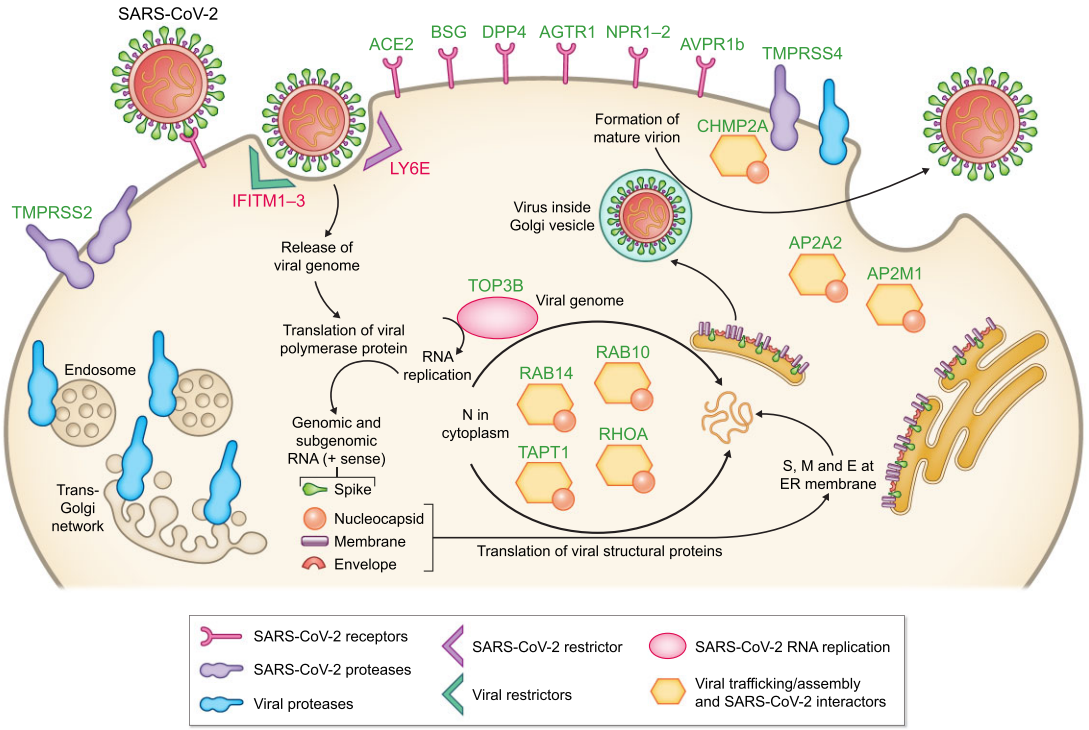


Figure 1: SCARF genes and key functions. Adapted from reference [11], using additional information obtained from references [12, 13].

In-house murine kidney transcriptomics datasets have been described previously [18–21].

Information on gene expression in human CKD transcriptomics datasets was obtained from Nephroseq v5 [22]. A high-sensitivity approach was used, in which statistically significant differences ($P < .05$) in gene expression or correlation with analytical values were selected. Expression of genes of interest by individual cell types in healthy human tissue were identified from ProteinAtlas [23].

Statistics

Statistical analysis was performed using GraphPad Prism Software 8 (La Jolla, CA, USA). Results are expressed as mean ± standard error of the mean (SEM). Significance at the $P < .05$ level was assessed by Student's *t*-test for two groups of data. Statistical results for Nephroseq data are presented as provided in the web page.

Ethics approval and consent to participate

This study was approved by the Ethics Committee of IIS-Fundacion Jimenez Diaz.

RESULTS

SCARF gene expression in mice with CKD

The key target organ in COVID-19 is the lung, while cardiovascular and kidney complications are causes of death in severe COVID-19. Thus, we induced CKD in mice and studied the gene expression of 21 SCARF genes in the kidney, aorta, heart and lung.

Adenine-induced kidney injury resulted in increased serum creatinine and urea levels (Fig. 3A and B), reflecting increased levels of uremic retention solutions, as well as in kidney tubular atrophy and interstitial fibrosis (Fig. 3C). Histological examination of aorta, heart and lungs did not disclose major abnormalities (Fig. 3D–F).

Overall, 20 of 21 SCARF genes showed significant differential expression in at least one organ of mice with CKD as compared with control mice, and for 15/20 (75%) the differential expression would be expected to facilitate SARS-CoV-2 infection and/or severity (Supplementary data, Table S1).

Multiple differentially expressed SCARF genes may contribute to increased severity of kidney injury in CKD

In CKD kidneys, 17 SCARF genes were differentially expressed, all of them upregulated, as assessed by RT-qPCR (Fig. 4, Supplementary data, Fig. S1). For 13 genes (*Dpp4*, *Tmprss2*, *Tmprss4*, *Top3b*, *Ap2m1*, *Ap2a2*, *Chmp2a*, *Rab10*, *Rab14*, *Rhoa*, *Tapt1*, *Nrp1* and *Nrp2*), the increased gene expression would be expected to favor SARS-CoV-2 infection, while for 4 (*Ifitm1*, *Ifitm2*, *Ifitm3* and *Ly6e*), the increased gene expression would be expected to be protective (Fig. 4, Supplementary data, Fig. S1 and Table S1).

To further understand the pattern of kidney SCARF gene expression during kidney injury, the expression of the 21 SCARF genes was assessed in three in-house kidney transcriptomics datasets corresponding to two nephrotoxic models of AKI and one model of early-stage obstructive CKD, as well as in human CKD datasets available at Nephroseq [22] (Supplementary data, Table S2). Same-direction changes in gene expression were observed in murine adenine model and human CKD for 12 of 21 genes (57%), while just 8/21 (38%) genes displayed the same

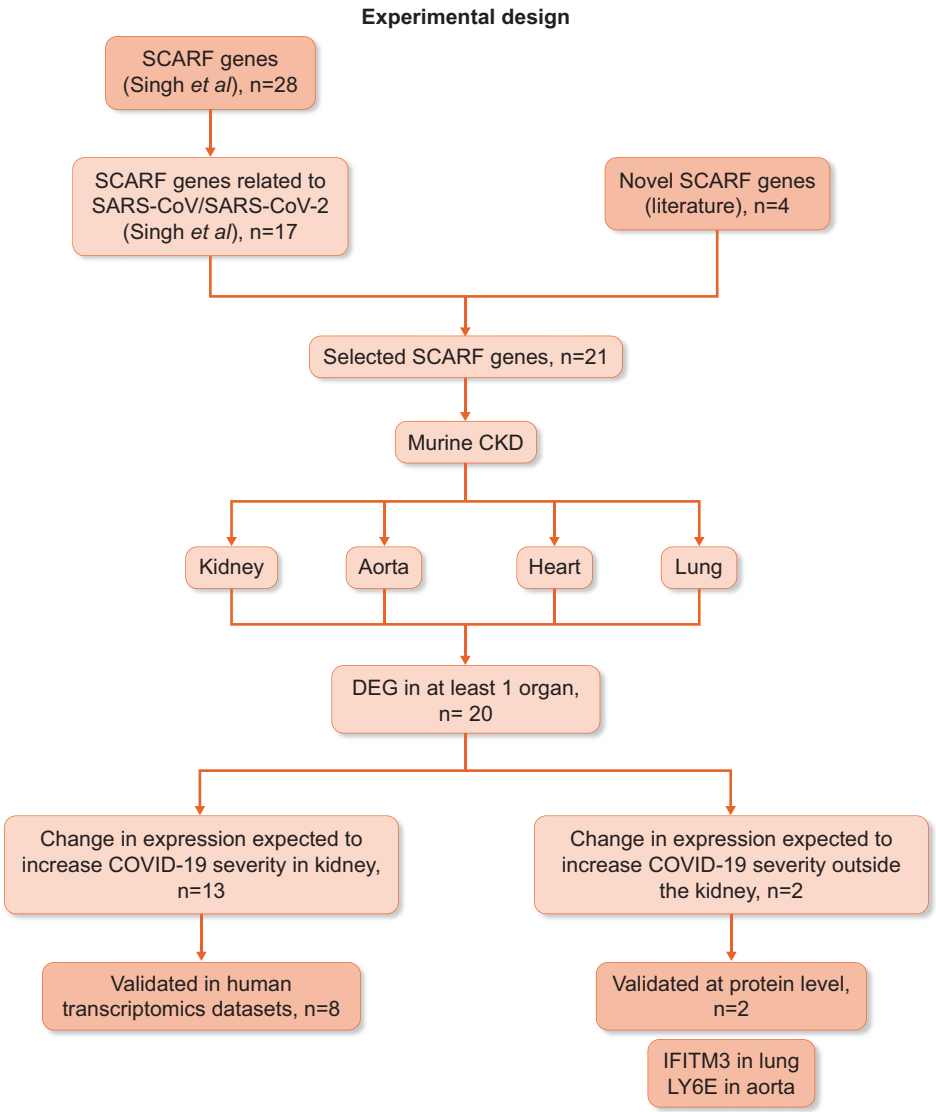


Figure 2: Experimental design. The list of SCARF genes was obtained from references [11–13]. Murine CKD was induced by adding 0.2% adenine to the diet for 4 weeks. For human validation of kidney mRNA expression changes in CKD, the Nephroseq database was searched. In murine tissue, protein validation was achieved by western blot and/or immunohistochemistry.

direction of change in murine adenine CKD and murine models of AKI or early CKD. Thus, changes observed in murine CKD appear representative of changes in human CKD, but the pattern of gene expression appears to change over time as kidney injury shifts from acute to chronic in mice. Interestingly, the most coherent changes in gene expression across species and conditions corresponded to two genes that may protect for SARS-CoV-2 expression (*IFITM2* and *IFITM3*) which were increased in all conditions tested. However, the current manuscript is focused on understanding changes that might explain the clinical observation of more severe COVID-19 in patients with CKD. Thus, we next focused on genes whose increased expression may favor SARS-CoV-2 infection.

Supplementary data, Tables S3 and S4 show detailed human kidney expression data for the 13 genes that are upregulated in murine CKD kidney, and the increased gene expression of which would be expected to favor SARS-CoV-2 infection (*Dpp4*, *Tmprss2*, *Tmprss4*, *Top3b*, *Ap2m1*, *Ap2a2*, *Chmp2a*, *Rab10*, *Rab14*, *Rhoa*, *Tapt1*, *Nrp1* and *Nrp2*). For 8/13 (61%) genes (*Tmprss4*, *Chmp2a*, *Rab10*, *Rab14*, *Rhoa*, *Tapt1*, *Nrp1* and *Nrp2*), the kidney gene expression was also found upregulated in human CKD, and for 4 in diabetic CKD (*Ap2a2*, *Tmprss4*, *Rhoa* and *Nrp1*) (Supplementary data, Table S3). Thus, these eight genes would be prime candidates to explain an increased sensitivity of kidney tissue to SARS-CoV-2 infection in humans whose role may be assessed experimentally in mice, as the change of expression in human and murine CKD was in the same direction and would be expected to result in increased sensitivity to SARS-CoV-2 infection. Specifically, for 4/8 genes (*TMPRSS4*, *RAB10*, *RHOA* and *NRP1*), a correlation was found between lower estimated glomerular filtration rate (eGFR) and higher kidney gene expression in human CKD datasets, all of them including diabetic CKD (Supplementary data, Table S4).

SCARF gene expression in other COVID-19 target organs from mice with CKD

We next explored the impact of CKD on SCARF gene expression in three key targets of COVID-19: the lungs and the

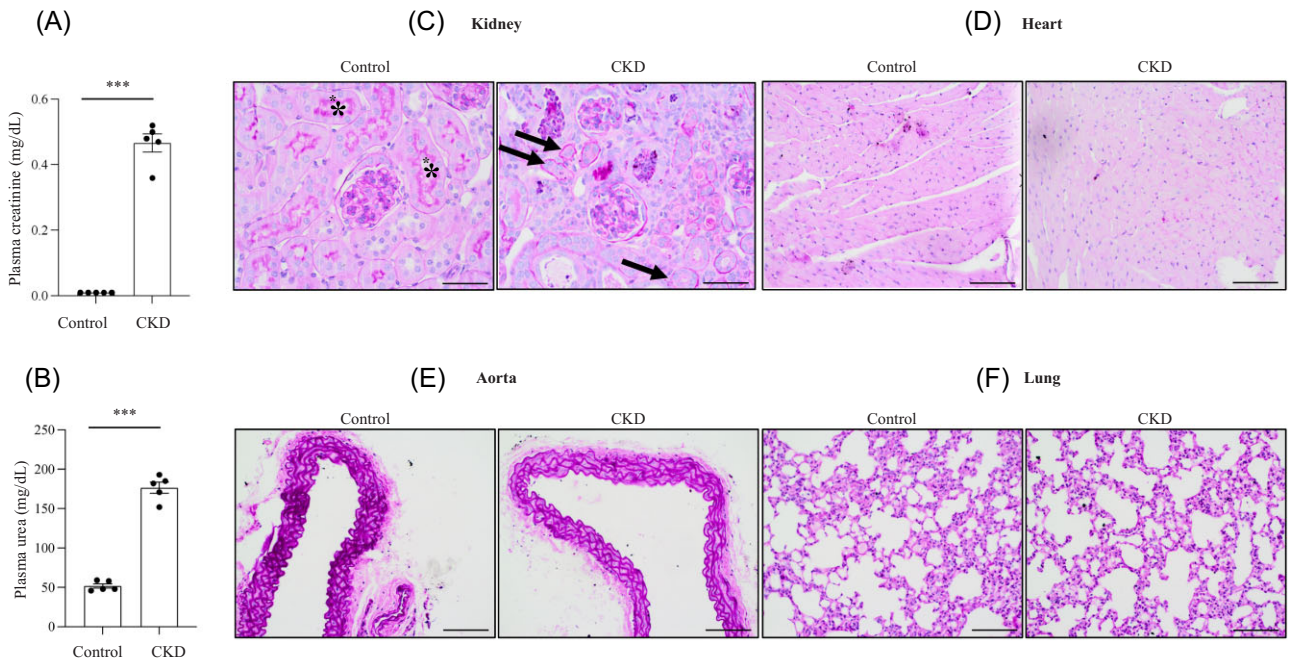


Figure 3: Adenine-induced CKD in mice. Mice were fed with 0.2% adenine or vehicle embedded in standard chow for 4 weeks. (**A**) Plasma creatinine, ***$P < .0001$ vs vehicle. (**B**) Plasma urea, ***$P < .0001$ vs vehicle. Data presented as mean ± SEM. (**C–F**) Histology. PAS staining. (**C**) In kidneys, note evidence for CKD, such as loss of brush border in proximal tubules (asterisk in control kidney) and thickening of tubular basement membranes (arrows in CKD kidney). (**D–F**) No major abnormalities were observed in heart (**D**), aorta (**E**) and lungs (**F**). Original magnification ×40 (kidney) and ×20 (aorta and lung).

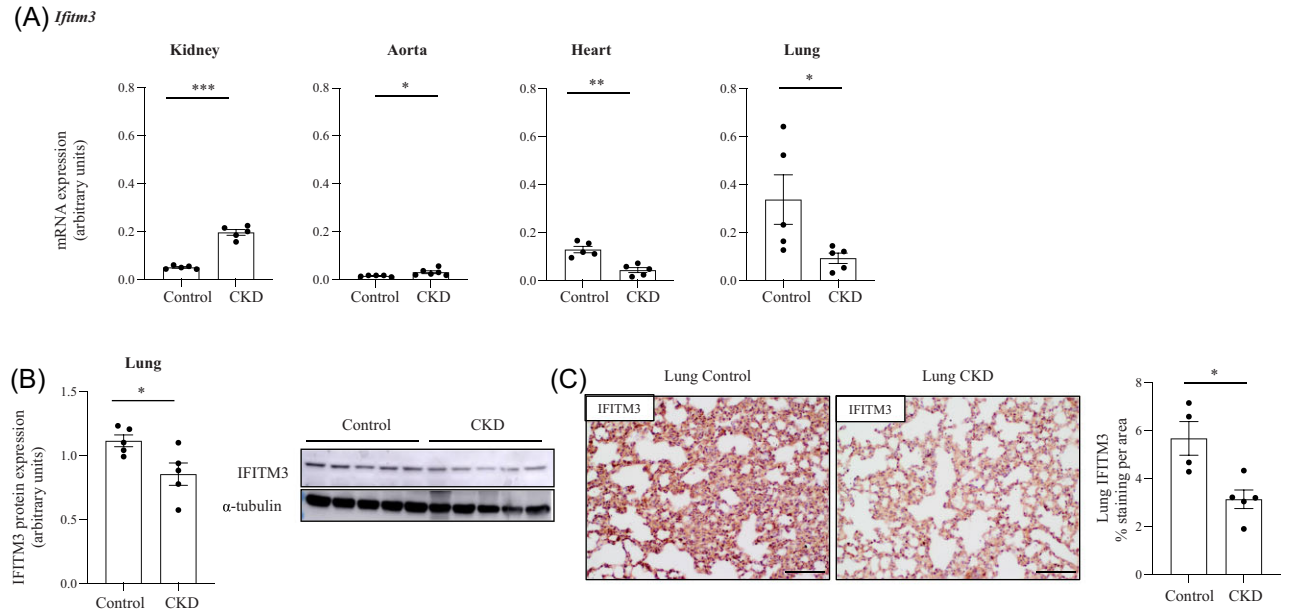


Figure 4: *Ifitm3* mRNA and IFITM3 protein are downregulated in the lung of mice with CKD. Gene expression was assessed by RT-qPCR in tissues from control mice and mice with adenine-induced CKD. (**A**) *Ifitm3* gene expression in kidney, aorta, heart and lung. (**B**) IFITM3 western blot in the lung and quantification. (**C**) IFITM3 immunohistochemistry in the lung and quantification. *$P < .05$ vs control; **$P < .001$ vs control, ***$P < .0001$ vs control, $n = 5$ per group. Data are presented as mean ± SEM. Original magnification ×20.

vasculature, represented by the aorta and the heart (Figs 4 and 5, Supplementary data, Fig. S1 and Table S1).

In the lungs, 10/21 (48%) genes were differentially expressed, all of them downregulated, in CKD lungs (Figs 4 and 5, Supplementary data, Fig. S1 and Table S1). The downregulated expression of one gene (*Ifitm3*) would be compatible with increased sensitivity to SARS-CoV-2 infection.

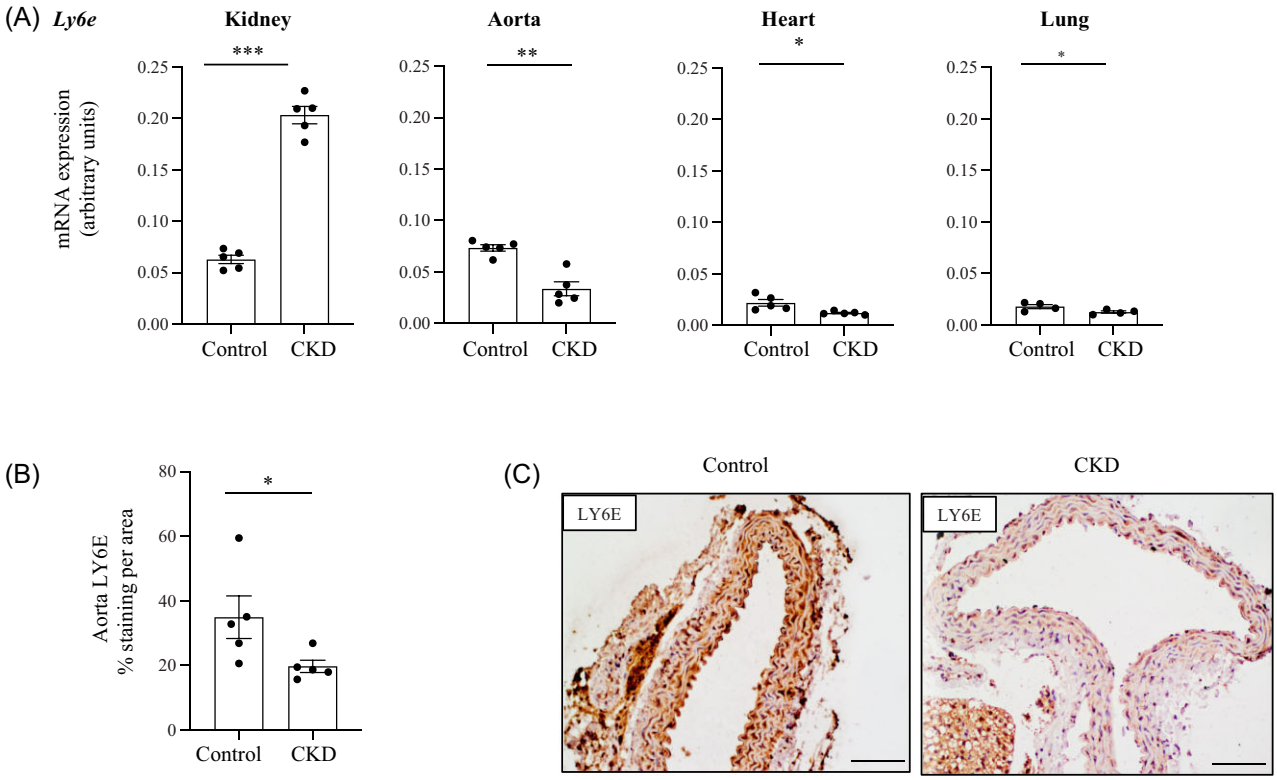


Figure 5: *Ly6e* mRNA and LY6E protein are downregulated in the aorta of mice with CKD. Gene expression was assessed by RT-qPCR in tissue from control mice and mice with adenine-induced CKD. (**A**) *Ly6e* gene expression in kidney, aorta, heart and lung. (**B–C**) LY6E immunohistochemistry in the aorta and quantification. $^{*}P < .05$ vs control; $^{**}P < .001$ vs control, $^{***}P < .0001$ vs control, $n = 5$ per group. Data are presented as mean ± SEM. Original magnification ×20.

In the aorta, 14/21 (67%) SCARF genes were differentially expressed in CKD. One (*Ifitm3*) was upregulated, and 13 (*Ap2a2*, *Ap2m1*, *Avpr1b*, *Bsg*, *Chmp2a*, *Nrp1*, *Nrp2*, *Rab10*, *Rab14*, *Rhoa*, *Tapt1*, *Top3b* and *Ly6e*) downregulated (Figs 4 and 5, Supplementary data, Fig. S1 and Table S1). Only one gene (*Ly6e*) showed a differential expression expected to favor SARS-CoV-2 infection, as it is known to protect from SARS-CoV-2 infection (Fig. 5B).

In the heart, three SCARF genes (*Ace2*, *Tmprss2* and *Tmprss4*) could not be amplified and 5/18 (28%) genes were differentially expressed, all of them downregulated in CKD (Figs 4 and 5, Supplementary data, Fig. S1 and Table S1). The downregulated expression of two genes (*Ifitm3* and *Ly6e*) would be compatible with increased sensitivity to SARS-CoV-2 infection.

Based on these data, *Ifitm3* and *Ly6e* were chosen for validation of the impact of changes in gene expression on protein levels.

Decreased lung IFITM3 expression may predispose persons with CKD to severe COVID-19

The highest *Ifitm3* baseline expression was found in the lung, suggesting that this SCARF may be important to protect from SARS-CoV-2 in the lung, while the baseline expression in other tissues was approximately 50% or more lower than in the lungs (Fig. 4A). The amount and localization of IFITM3 was assessed by western blot and immunohistochemistry. Both techniques confirmed the decreased IFITM3 protein levels in CKD lung as compared with control lung (Fig. 4B and C) and this was concordant with decreased mRNA levels in CKD lung (Fig. 4A). The localization of IFITM3 in murine lungs was consistent with reports in the Human Protein Atlas for *IFITM3* mRNA and protein in human lung [23, 24] (Supplementary data, Fig. S2).

Concordant findings between mRNA and protein levels were also found for the kidney (Fig. 4A, Supplementary data, Fig. S3). In this case, both the mRNA and protein levels of IFITM3 were increased in CKD, as assessed by RT-qPCR (Fig. 4A), western blot and immunohistochemistry (Supplementary data, Fig. S3A and B). By contrast, neither western blot nor immunohistochemistry identified IFITM3 protein expression in the heart (not shown). This may be explained by a combination of low baseline mRNA levels, a further decrease in mRNA expression in CKD (Fig. 4A) and the lack of gene expression in the most abundant cell types, the cardiomyocytes, according to the Human Protein Atlas which localized *IFITM3* expression to human endothelial cells, vascular smooth muscle cells, fibroblasts and the heart [23], which was consistent with human heart immunohistochemistry data in the Atlas [24] (Supplementary data, Fig. S2).

Decreased aorta LY6E expression may predispose persons with CKD to vascular involvement in COVID-19

The highest *Ly6e* baseline expression was found in the aorta followed by the kidney, suggesting that this SCARF may be important to protect from SARS-CoV-2 in these tissues, while the baseline expression in heart and lung was very low (Fig. 5A).

Immunohistochemistry confirmed a decrease in LY6E protein expression in the aortic wall, in both endothelium and the medial layer (Fig. 5B). These data were consistent with the constitutive expression of *LY6E* mRNA by human endothelial and

vascular smooth muscle cells as reported in the Human Protein Atlas [24] (Supplementary data, Fig. S4). The increased CKD kidney *Ly6e* mRNA expression (Fig. 5A) was concordant with increased LY6E protein levels (Supplementary data, Fig. S5A and B). By contrast, CKD lung *Ly6e* mRNA expression was similar to control lung mRNA (Fig. 5A), while *LY6E* protein levels were increased in CKD lung (Supplementary data, Fig. S5C and D).

The Human Protein Atlas localized *LY6E* mainly to human endothelial and vascular smooth muscle cells [24] (Supplementary data, Fig. S4). In this regard, we could not optimize murine heart immunohistochemistry to study LY6E protein and western blot did not show changes in heart LY6E protein (not shown).

DISCUSSION

Diabetes is the most common cause of CKD. To gain insight into potential molecular changes that may predispose patients with CKD to more severe COVID-19, we have assessed the expression of SCARFs in kidney, heart, vascular and lung tissue in a murine model of CKD. The main finding was that CKD is associated with deregulated expression of multiple SCARF genes both in the injured kidney and in the lungs and cardiovascular systems. Kidney findings were validated in human diabetic CKD. Overall, these data indicate that during CKD, the differential expression of genes that modulate SARS-CoV-2 biology is not limited to the kidney tissue and may be observed in aorta, heart and/or lung. In CKD kidneys, a majority of differentially expressed genes (13) would be expected to result in increased sensitivity to SARS-CoV-2 infection and, potentially, to virus-induced kidney injury during COVID-19. Additionally, two genes were found to be differentially expressed in target organs other than kidneys in a manner that might be expected to facilitate SARS-CoV-2 infection or increase its severity: specifically, *Ifitm3* mRNA and IFITM3 protein were significantly downregulated in lungs, while *Ly6e* mRNA and LY6E protein were significantly downregulated in aorta. This suggests that IFITM3 is a key candidate that may contribute to the increased sensitivity of patients with CKD to severe COVID-19, given that the lungs are the usual port of entry of SARS-CoV-2, and that therapeutic approaches that promote IFITM3 expression should be explored as a potential means to decrease the severity of COVID-19 in future coronavirus pandemics in the context of CKD.

CKD patients with COVID-19 have a higher risk of hospitalization, need for mechanical ventilation, and mortality, and patients with kidney failure had an almost 4-fold higher risk for mortality than those without kidney failure [3, 5]. It has been suggested that suboptimal innate and adaptive immune system activation increases the susceptibility to infections in patients with CKD [13]. A Mendelian randomization study to evaluate the role of kidney function in severe COVID-19 in people with European Ancestry found that higher eGFR, but not lower albuminuria, was associated with lower risk of severe COVID-19 (odds ratio 0.90, 95% confidence interval 0.83–0.98) [25]. This further supports the involvement of the kidney function of eliminating uremic toxins in the increased sensitivity to severe COVID-19.

Decreased IFITM3 levels were found in lungs in mice with CKD. IFITMs are expressed in many cell types that behave as innate immune responders to virus infections and regulate the fusion of invading viruses and endocytic vesicles, and direct them to lysosomes [26, 27]. IFITM3 has a key role in adaptative immunity by preventing viruses from traversing the cellular lipid bilayer as they alter membrane rigidity and curvature thus inhibit virus membrane fusion [27–32]. Specifically, IFITM3 had anti-influenza A properties in murine models [33–36]. *IFITM3* gene variants have been associated with the clinical response to influenza and other viruses. The *IFITM3* rs12252 C allele is a risk factor for COVID-19 hospitalization among Chinese, Caucasian Spanish and Arab populations [37–40]. The combined haplotypes of rs12252 and rs34481144 correlated with the reported standardized mortality ratios in ethnic groups in England [41].

As would be expected for an innate immunity protein with antiviral properties, IFITM3 is upregulated in SARS-CoV-2-infected lung epithelial cells [42]. However, it was downregulated in the lungs of mice with CKD, potentially contributing to the increased susceptibility to severe COVID-19 in the uremic milieu. Indeed, IFITM3 knockout (KO) mice had higher lung SARS-CoV-2 viral titers and increased lung inflammatory cytokine levels, CD45-positive immune cell infiltration and histopathological injury than wild-type (WT) control mice [43]. Viral antigen staining was disseminated in lung tissue and pulmonary vasculature in IFITM3 KO mice, while it was observed just in limited regions in WT lungs. Complete virus clearance was delayed in IFITM3 KO mice. Transcriptomics analysis of infected lungs identified upregulation of gene signatures related with interferons, inflammation, angiogenesis and coagulation in IFITM3 KO as compared with WT mice, which would be consistent with more severe inflammatory injury and thromboembolic complications in persons with low IFITM3 expression, such as, potentially, CKD patients. Interestingly, cardiac dissemination of virus was detected in 8/8 IFITM3 KO vs 1/8 control mice, suggesting that IFITM3 limits extrapulmonary dissemination of SARS-CoV-2, concordantly with a previous study showing that IFITM3 protects the heart during influenza virus infection [44]. In this regard, *Ifitm3* was also downregulated in the heart of mice with CKD at the mRNA level, although we were unable to validate this result at the protein level.

The identification of downregulated IFITM3 in COVID-19 target tissues in CKD offers the opportunity for therapeutic intervention aimed at increasing IFITM3 expression. In this regard, valproic acid upregulated *IFITM3* mRNA expression and its antiviral action in a Toxicogenomics Database [42]. Furthermore, the results are in line with the known negative impact of deficiency of an effective interferon response on the severity of COVID-19, as interferon increases IFITM3 [45]. However, clinical trials with diverse type I and III interferons have been inconclusive [45]. It would be worth revisiting the results of these trials from a CKD perspective.

Ly6e mRNA expression was downregulated in the heart and aorta of mice with CKD. However, downregulated LY6E protein was only confirmed in the aorta. *LY6E* encodes a glycosylphosphatidylinositol (GPI)-anchored cell surface protein that modulates T-cell activation and proliferation and has an antiviral immune effect that has been described to control coronavirus infection [46, 47]. LY6E interferes with spike protein–mediated membrane fusion, protecting primary B cells from murine coronavirus infection and severe viral disease [48]. The cellular restriction provided by LY6E extends to multiple coronaviruses, including SARS-CoV, SARS-CoV-2 and Middle East respiratory syndrome coronavirus (MERS-CoV). Thus, mice lacking LY6E in hematopoietic cells were highly susceptible to murine coronavirus infection. In humans, an association between *LY6E* gene variants and SARS-CoV-2 pneumonia was found in an African Ancestry population [47]. As was the case for IFITM3, and consistent with its antiviral role, LY6E was upregulated in

alveolar tissue, large airways and heart tissue samples containing significant levels of SARS-CoV-2, which corresponds to early stages of the infection in COVID-19 patients [48]. However, the role of Ly6E in the vasculature in relation to viral infection is unknown.

Additionally, 13 SCARF genes were upregulated in the kidney of murine CKD and their increased expression would be consistent with increased local susceptibility to SARS-CoV-2 infection. Eight of these SCARF genes were also upregulated in human kidneys with CKD, including four specifically in diabetic CKD datasets. This points to an increased susceptibility of CKD kidneys to SARS-CoV-2-induced kidney injury, which may be mediated by a different set of genes from those potentially mediating the increased sensitivity of CKD patients to SARS-CoV-2-induced lung and cardiovascular injury. In this regard, COVID-19 may have direct and indirect negative impacts on the kidney. Kidney disease in COVID-19 is probably multifactorial, with important contributions of systemic inflammation resulting from severe extrarenal disease leading to kidney epithelial cell injury and maladaptive repair. Additionally, SARS-CoV-2 may infect kidney cells, including podocytes and tubular cells, triggering responses involved in inflammation and fibrosis [49].

Several limitations should be acknowledged. Lung and cardiovascular tissue from patients with CKD were not studied. However, an extensive analysis of kidney transcriptomics databases showed concordance between findings in murine and human CKD at the kidney tissue level and for external tissues, concordance between mRNA and protein levels was characterized. Furthermore, the uremic toxins that may change SCARF gene expression were not identified. Young mice were studied while the risk of severe COVID-19 is increased in aged humans [3, 5]. However, older age results in a higher risk of severe COVID-19 in person without CKD (21-fold higher risk for those over 80 years old) than in persons with CKD (3.5 higher risk) [3, 5]. Definite proof of the involvement of the identified genes in severe COVID-19 would require animal models of CKD, SARS-CoV-2 infection, and genetic manipulation of IFITM3 or LY6E expression in specific organs. In this regard, humanized models of SARS-CoV-2 infection in mice are available [50]. Finally, the changes in the expression of some genes may be expected to be protective against COVID-19. Given the clinical observation of more severe COVID-19 in patients with CKD, we hypothesize that the impact of the observed changes that may favor viral infection outweighs the impact of changes that may protect from viral infection.

In conclusion, CKD, of which the most common cause is diabetes, is associated with the differential expression of multiple SCARF genes in target organs of COVID-19. Specifically, the decreased expression of *Ifitm3* or *Ly6e* may contribute to increase the severity of COVID-19 in target organs such as the lung and the cardiovascular system, respectively, in the context of CKD. Additionally, the differential expression of multiple additional SCARF genes may sensitize CKD kidneys to kidney injury in infected individuals, as validated for human CKD, including diabetic CKD. This information may allow development of therapeutic strategies aimed at decreasing the severity of COVID-19 of future coronavirus pandemics in patients with CKD. In this regard, SARS-CoV-2 is the third novel coronavirus that causes lethal human disease identified in the 21st century, following SARS-CoV (2003) and MERS-CoV (2013) [51]. Given the lung port of entry of SARS-CoV-2, lung IFITM3 expression emerges as a key potential target of therapeutic approaches aimed at increasing the resilience of CKD patients to SARS-CoV-2.

SUPPLEMENTARY DATA

Supplementary data are available at *ckj* online.

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AUTHORS' CONTRIBUTIONS

A.O., M.D.S.-N. and S.C. contributed to the concept, study design and manuscript drafting; M.R. and A.P.-C. performed animal studies; S.C. did the data mining. All authors reviewed and revised the manuscript and approved the final manuscript as submitted.

DATA AVAILABILITY STATEMENT

The datasets used and/or analysed during the current study are available from the corresponding author on reasonable request.

CONFLICT OF INTEREST STATEMENT

A.O. is former Editor-in-Chief of *CKJ* and has received grants from Sanofi and consultancy or speaker fees or travel support from Advicciene, Astellas, AstraZeneca, Amicus, Amgen, Fresenius Medical Care, GSK, Bayer, Sanofi-Genzyme, Menarini, Mundipharma, Kyowa Kirin, Alexion, Freeline, Idorsia, Chiesi, Otsuka, Novo-Nordisk, Sysmex and Vifor Fresenius Medical Care Renal Pharma, and is Director of the Catedra Mundipharma-UAM of diabetic kidney disease and the Catedra Astrazeneca-UAM of chronic kidney disease and electrolytes. S.C. has received honoraria for consultancy from Otsuka and travel support from Menarini.

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