ACE2 interaction networks in COVID-19: a physiological framework for prediction of outcome in patients with cardiovascular risk factors

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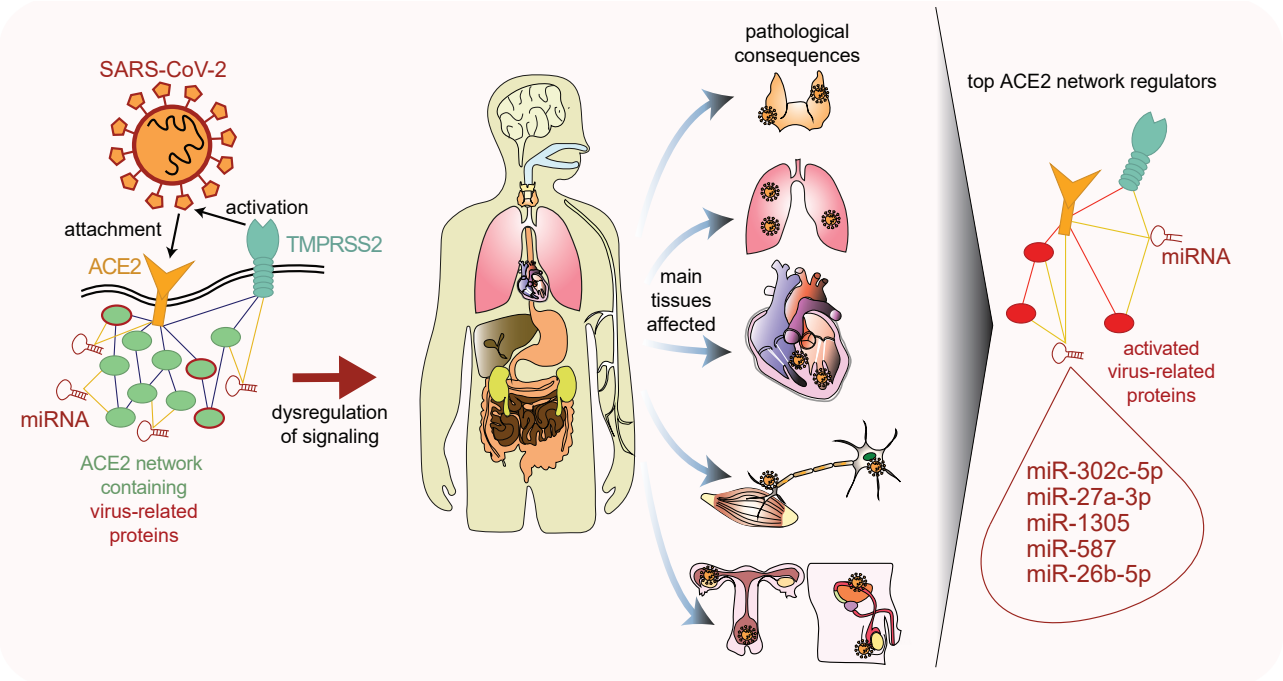

Background: Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) infection (coronavirus disease 2019; COVID-19) is associated with adverse outcome in patients with cardiovascular disease (CVD).

Aim: To characterize the interaction between SARS-CoV-2 and Angiotensin Converting Enzyme 2 (ACE2) functional networks with focus on CVD.

Methods: Using bioinformatic tools, network medicine approaches and publicly available datasets, we investigated ACE2 tissue expression and described ACE2 interaction network which could be affected by SARS-CoV-2 infection. We identified top ACE2 interactors, including miRNAs which are shared regulators between the ACE2, virus-infection related proteins and heart interaction networks, using lung and nervous system networks as a reference. We also identified main SARS-CoV-2 risk groups and performed drug predictions for them.

Results: We found the same range of ACE2 expression confidence in respiratory and cardiovascular systems (averaging 4.48 and 4.64, respectively). Analysing the complete ACE2 interaction network, we identified 11 genes (ACE2, DPP4, ANPEP, CCL2, TFRC, MEP1A, ADAM17, FABP2, NPC1, CLEC4M, TMPRSS2) associated with virus-infection related processes. Previously described genes associated with cardiovascular risk factors DPP4, CCL2 and ANPEP were extensively connected with top regulators of ACE2 network, including ACE, INS and KNG1. Enrichment analysis revealed several disease phenotypes associated with interaction networks of ACE2, heart tissue, and virus-infection related protein, with the strongest associations with the following diseases (in decreasing rank order): obesity, hypertensive disease, non-insulin dependent diabetes mellitus, congestive heart failure, and coronary artery disease. We described for the first time microRNAs-miR (miR-302c-5p, miR-1305, miR-587, miR-26b-5p, and mir-27a-3p), which were common regulators of the three networks: ACE2, heart tissue and virus-infection related proteins.

Conclusion: Our study provides novel information regarding the complexity of signaling pathways affected by SARS-CoV-2 and proposes predictive tools as miR towards personalized diagnosis and therapy in COVID-19. Additionally, our study provides a list of miRNAs with biomarker potential in prediction of adverse outcome in patients with COVID-19 and CVD.

Keywords: angiotensin, COVID-19, SARS-CoV-2, cardiovascular, gene expression, miRNA, microRNA, miR.

Abbreviations

ACE2: Angiotensin Converting Enzyme 2
AGT: Angiotensinogen
ARDS: Acute respiratory distress syndrome
COVID-19: Coronavirus disease 2019
CVD: Cardiovascular disease
DEG: Differentially expressed genes
DM: Diabetes mellitus
eNOS: Endothelial nitric oxide synthase
GO: Gene Ontology
HF: Heart failure
HK: High molecular weight kininogen
INS: Insulin
KKS: Kalikrein-Kinin system
KNG1: Kininogen 1
MERS: Middle-East respiratory syndrome coronavirus
MI: Myocardial infarction
miRNAs, miR: MicroRNAs
NT-proBNP: N-terminal pro-B-type natriuretic peptide
PPI: Protein–protein interaction
RAS: Renin-angiotensin system
REN: Renin
ROS: Reactive oxygen species
SARS-CoV-2: Severe acute respiratory syndrome coronavirus 2
TMPRSS2: Transmembrane protease serine 2

Gene/protein

ACE2: Angiotensin Converting Enzyme 2
ADAM17: ADAM metallopeptidase domain 17
AGT: Angiotensinogen
AGTR1: Angiotensin II Receptor Type 1
ALB: Albumin
ANPEP: Alanyl Aminopeptidase
APN/CD13: Aminopeptidase N/CD13
ATP6AP2: ATPase H+ Transporting Accessory Protein 2
CALM1: Calmodulin 1
CAT: Catalase
CAV1: Caveolin-1
CCL2: C-C Motif Chemokine Ligand 2
CCN2: Cellular Communication Network Factor 2
CDHR2: Cadherin Related Family Member 2
CDK4: Cyclin Dependent Kinase 4
CLEC4M: C-Type Lectin Domain Family 4 Member M
CTSA: Cathepsin A
CTSG: Cathepsin G
DPP4: Dipeptidyl Peptidase 4
ENPEP: Glutamyl Aminopeptidase
EV71: Enterovirus 71
FABP2: Fatty acid-binding protein 2
FoxO2: Forkhead box O-2
INS: Insulin
KNG1: Kininogen 1
KPNA2: Karyopherin Subunit Alpha 2
LNPEP: Leucyl And Cystinyl Aminopeptidase
LTA4H: Leukotriene A4 hydrolase
MAPK: Mitogen-activated protein kinase
MCP-1: Monocyte chemoattractant protein-1
MEP1A: Meprin A subunit alpha
MME: Membrane metalloendopeptidase
MS4A10: Membrane Spanning 4-Domains A10
NFKB1: Nuclear Factor Kappa B Subunit 1
NF-κB: Nuclear factor kappa-light-chain-enhancer of activated B cells
NPC1: Niemann-Pick disease, type C1
PAI-1: Plasminogen activator inhibitor-1
PDGFR-b: Platelet-derived growth factor receptor-beta
PPAR-γ: Peroxisome proliferator-activated receptor gamma
PRCP: Prolylcarboxypeptidase
TFRC: Transferrin Receptor
TGFB1: Transforming Growth Factor Beta 1
THOP1: Thimet Oligopeptidase 1
TMPRSS2: Transmembrane protease, serine 2
VEGFR2: Vascular endothelial growth factor receptor 2

Signaling pathways

AGE-RAGE: Advanced glycation endproducts - Receptor for Advanced glycation end products
ERK1/2/AP-1: Extracellular signal-regulated kinases 1/2/AP-1
PI3K/AKT/Nrf2: Phosphatidylinositol 3'-kinase/AKT/NF-E2-related factor 2
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Introduction

At the end of 2019 in Wuhan (China), a novel coronavirus named severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) has been discovered.[1] The clinical manifestations of SARS-CoV-2 infection, named coronavirus disease 2019 (COVID-19), varies in severity from asymptomatic infection to acute viral pneumonia with fatal outcome. Nearly half of patients who were at risk of acute course of the disease suffered from comorbidities including hypertension, diabetes mellitus (DM) and coronary heart disease.[2,3] Importantly, COVID-19 is associated with increased risk for mortality and adverse cardiovascular events among patients with underlying cardiovascular diseases (CVD).[4] A similar association between the virus and CVD was observed during previous coronavirus outbreaks caused as Middle-East respiratory syndrome coronavirus (MERS) or severe acute respiratory syndrome coronavirus (SARS-CoV).[5,6] Therefore, these data suggest a common factor that is associated with the pathogenesis of COVID-19 and CVD. Most probably, the link between cardiovascular complications and infection may be related to angiotensin-converting enzyme 2 (ACE2), which was found to act as a functional receptor for SARS-CoV-2.[7]

ACE2 is a multi-action cell membrane enzyme that is widely expressed in lungs, heart tissue, intestine, kidneys, central nervous system, testis, and liver.[8] During the 20 years from its discovery, the investigations targeting the complex role of this enzyme established ACE2 as an important regulator in hypertension, heart failure (HF), myocardial infarction (MI), DM, and lung diseases.[9,10] The viral entry to cells is determined by the interaction between SARS-CoV-2 spike (S) protein and N-terminal segment of ACE2 protein, with a subsequent decrease in ACE2 surface expression, which may be enhanced by cofactor transmembrane protease serine 2 (TMPRSS2).[11]

As a consequence of SARS-CoV-2 infection, downregulated ACE2 pathways may lead to myocardial injury, fibrosis, and inflammation which may be responsible for adverse cardiac outcomes.[12] In line with these findings, several reports linked SARS-CoV-2 infection with myocardial damage and HF, accompanied by acute respiratory distress syndrome (ARDS), acute kidney injury, arrhythmias, and coagulopathy. The incidence of myocardial injury ranged from 7 to 28% depending on the severity of COVID-19, accompanied by increased levels of cardiac troponins, creatinine kinase–myocardial band, myohemoglobin and N-terminal pro-B-type natriuretic peptide (NT-proBNP).[13–15]

In the current work, we characterized ACE2 predicted protein-protein interaction (PPI) network in the context of myocardial injury. Our quantitative *in silico* analysis pointed out: (1) the top ACE2 interactors associated with the virus-related processes and playing role in the development of CVD; (2) the most promising microRNAs (miRNAs, miR) for potential diagnostic and prognostic applications; (3) virus-infetion related protein interactions providing information regarding the most potent regulators of ACE2 network; and (4) risk phenotype predictions associated with the alterations in ACE2 network. Our comprehensive analysis investigating ACE2 receptor-related interaction networks, their connection with SARS-CoV-2 interactome, enriched signaling pathways, miRNAs, associated diseases, provide precise targets for developing predictive tools to improve outcome in COVID-19, with potential for reducing health, personal and economic consequences of pandemic.

Methods

Data collection. ACE2-associated genes used for constructing interaction networks were extracted from KEGG database (23 genes from renin-angiotensin system-RAS pathway);[16] stringApp (top 40 ACE2 interactors),[17] Archs4 database https://amp.pharm.mssm.edu/archs4 (top 20 genes with correlated expression), Genecards database https://www.genecards.org (5 interactors and 4 sister terms), literature search.[18–20] In total, we collected 69 genes, which were used further for miRNA prediction analysis and constructing interaction networks.

Tissue Expression analysis. ACE2 and TMPRSS2 tissue expression was evaluated based on a dataset downloaded from the Tissues 2.0 database.[21] This database integrates: transcriptomics datasets from multiple sources and proteomics datasets from humans and other organisms, quantifying gene expression confidence scores across tissues. All tissues were sorted by the decreasing expression confidence of ACE2 and TMPRSS2. Additionally, mean expression confidence for the ACE2 network was calculated for each tissue from the database. Gene expression confidence score were also mapped on the visualisation of the interaction networks.

Interaction networks. To analyze connections between ACE2 and other genes, we constructed a PPI network in Cytoscape 3.7.2, using human interactome data from StringApp 1.5.1 (Search Tool for the Retrieval of Interacting Genes/Proteins) database, including known and predicted protein–protein interactions.[17,22] Interaction networks were composed from a set of genes (nodes) connected by edges which represent functional relationships among these genes. We took into account connections with edge interaction confidence cut-off >0.4, with 1 being the highest possible confidence. We compared the complete tissue-specific ACE2 network, across heart, lungs and nervous system, as well as virus-infection related proteins network. Criteria of selection tissue-specific networks was gene expression confidence score >2 in a given tissue.

Extraction of disease-relevant ontological terms. To improve interpretation of the gene functions, we mined Gene Ontology (GO) database using biomaRt R package for extracting GO terms and further genes associated with following processes: "inflammation" (22 GO terms; 648 genes), "coagulation" (18 GO terms; 223 genes)[23], "angiogenesis" (24 GO terms; 535 genes), "cardiac muscle functions" (176 GO terms; 524 genes), "muscle hypertrophy" (16 GO terms; 85 genes), and "fibrosis" (23 GO terms; 263). Similar methodology was used to extract genes potentially related to "viral infection" (120 GO terms, 1047 genes). Disease relevant gene lists were extracted from the http://t2diacod.igib.res.in/ database.[24] From this database, we used the atherosclerosis, nephropathy, CVD, and neuropathy datasets. Diabetes-related genes were extracted from StringApp disease database, for the term "diabetes type-2". GO term lists used for gene extraction are shown in Supplementary Table 1.

Enrichment analysis. Enrichment analysis is a computational method for increasing the likelihood to identify most significant biological processes related to the study.[25] Enrichment analysis of the networks was done with StringApp, using the Hypergeometric test with Benjamini and Hochberg correction, while the reference was human genome. In all statistical analyses, the significance cutoff was set to corrected p-value ≤0.05. Disease enrichment analysis was performed using http://amp.pharm.mssm.edu/Enrichr/, which implements Benjamini-Hochberg correction.

miRNA predictions. In order to identify miRNAs regulating ACE2 and its 69 interactors, we used R and multimiR package with default settings, similarly to previous publications from our group.[23,26–28] Interaction networks between ACE2-related genes and miRNAs were constructed in R and exported to Cytoscape 3.7.2. Next, the interaction networks were merged with the predicted PPI network for ACE2 constructed using StringApp. Both networks were merged using official gene symbols and ENSG numbers.

Results

ACE2 tissue specific expression. To evaluate the potential susceptibility of the heart for SARS-CoV-2 infection, we ranked all 6668 human tissues from the Tissue 2.0 database, based on provided by the database ACE2 expression confidence (scale 0-5). This analysis revealed that lungs and respiratory systems are in the 14th and 15th place in terms of ACE2 expression confidence, after heart and cardiovascular systems (12th and 13th place, respectively), and before the nervous system (16th place; Figure 1). TMPRSS2 gene expression confidence was lower in lungs and heart than in nervous system (Figure 1). Mean expression of 69 genes from the network was highest in the nervous system in comparison to heart and respiratory system related tissues. Exceptionally high scores for ACE2 expression were assigned to urogenital and reproductive tissues (Figure 1).

ACE2 Interaction networks analysis. Firstly, we analyzed the complete interaction network between ACE2 and associated genes. As expected, ACE2 showed highest number of interactions with other genes (49 interactions), followed by ACE, which is not directly connected with ACE2 (33 interactions), renin (REN, 32 interactions), insulin (INS, 31 interactions), kininogen 1 (KNG1, 30 interactions) and angiotensinogen (AGT, 28 interactions) (Figure 2). Analysis of the ACE2 interaction network revealed 11 genes associated with virus infection related ontological terms (ACE2, DPP4, ANPEP, CCL2, TFRC, MEP1A, ADAM17, FABP2, NPC1, CLEC4M, TMPRSS2). All these genes were connected with ACE2, except for gene FABP2 (6 interactions) and CCL2 (15 interactions). From this group, the highest degree of connectivity with other genes from the network were found for DPP4 (22 interactions), ANPEP (19 interactions) and CCL2 indirectly connected with ACE2 (15 interactions, as presented before) (Figure 2). Two genes CDHR2 and MS4A10 didn't have any known connections with other genes, for edge confidence score >0.4 cutoff. Those results suggest that the above-mentioned genes could be especially affected in SARS-CoV-2 infection, leading to disturbance of the network. All genes from the network are described in Supplementary Table 2.

Integration of ACE2 network with SARS-CoV-2/Human Interactome. To identify how ACE2 network is connected with the SARS-CoV-2/Human Interactome, we included the previously published interactome into our analysis.[30] We found that three proteins from our network, ACE2, CLEC4M, and TMPRSS2, are directly interacting with virus glycoprotein S. In total, 45 proteins from the complete ACE2 network are interacting with 38 from 94 human host proteins for SARS-CoV-2. The strongest connection between networks was associated with INS, which interacted with 12 host proteins from SARS-CoV-2/Human Interactome. Other top interactors were CAT, CCL2, CDK4, CALM1 connected with at least 6 host proteins. Among host proteins directly interacting with virus proteins, the strongest connection with ACE2 network occurs by ALB (31 interactors form ACE2 network) and CAV1 (13 interactors) (Figure 3).

Enrichment analysis of the signaling within ACE2-tissue specific network. By applying enrichment analysis for analyzing signaling pathways associated with analyzed networks we used KEGG database and Reactome databases. According to the KEGG database, only the RAS and protein digestion and absorption pathways were shared between all six compared networks (Figure 4B). Analysis of signaling pathways using the Reactome database showed analogous pathways, for metabolism of angiotensinogen to angiotensin and peptide hormone metabolism. Reactome pathway analysis also showed that intrinsic pathway of fibrin clot formation, neutrophil degranulation, endothelial nitric oxide synthase (eNOS)

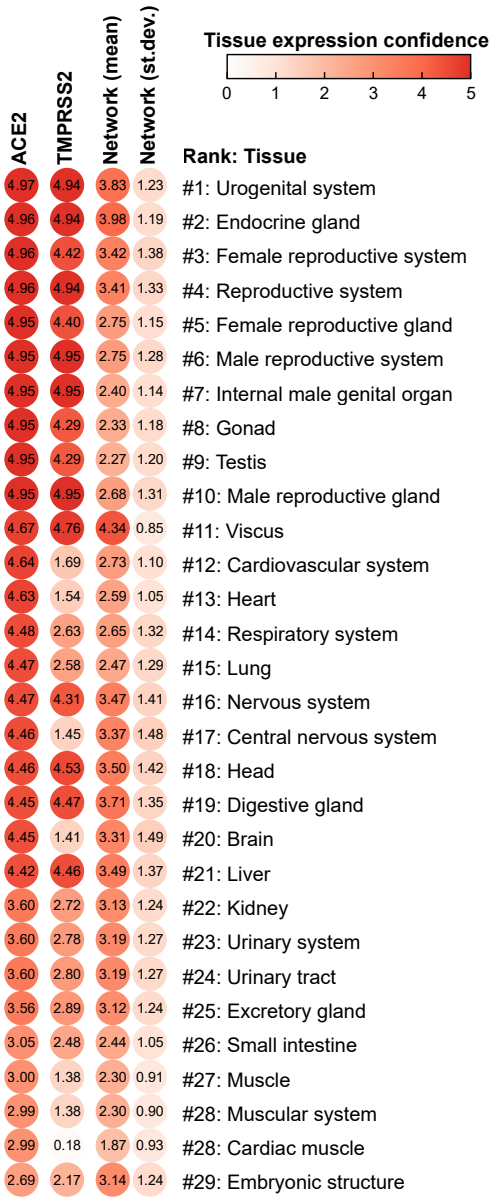

Figure 1. Tissues sorted by potential of being infected by SARS-CoV-2, determined by concentration of membrane receptors ACE2 and TMPRSS2. The virus starts the cell infection by binding to ACE2, a major hub in multiple physiological processes: this binding can block ACE2 network activity. However, the virus will enter the host cell when TMPRSS2 cleavages ACE2. The first column depicts average gene and protein expression confidence for ACE2 receptor; the second column depicts average expression confidence of TMPRSS2. The mean and standard deviation of expression confidence across 69 genes/proteins of ACE2 network are presented in third and fourth columns, respectively. Notice that lungs and respiratory system are ranked as #14-15 in this list, while heart and cardiovascular system #12-13. Nervous and reproductive system are ranked as, #16-17 and #1-10, respectively.

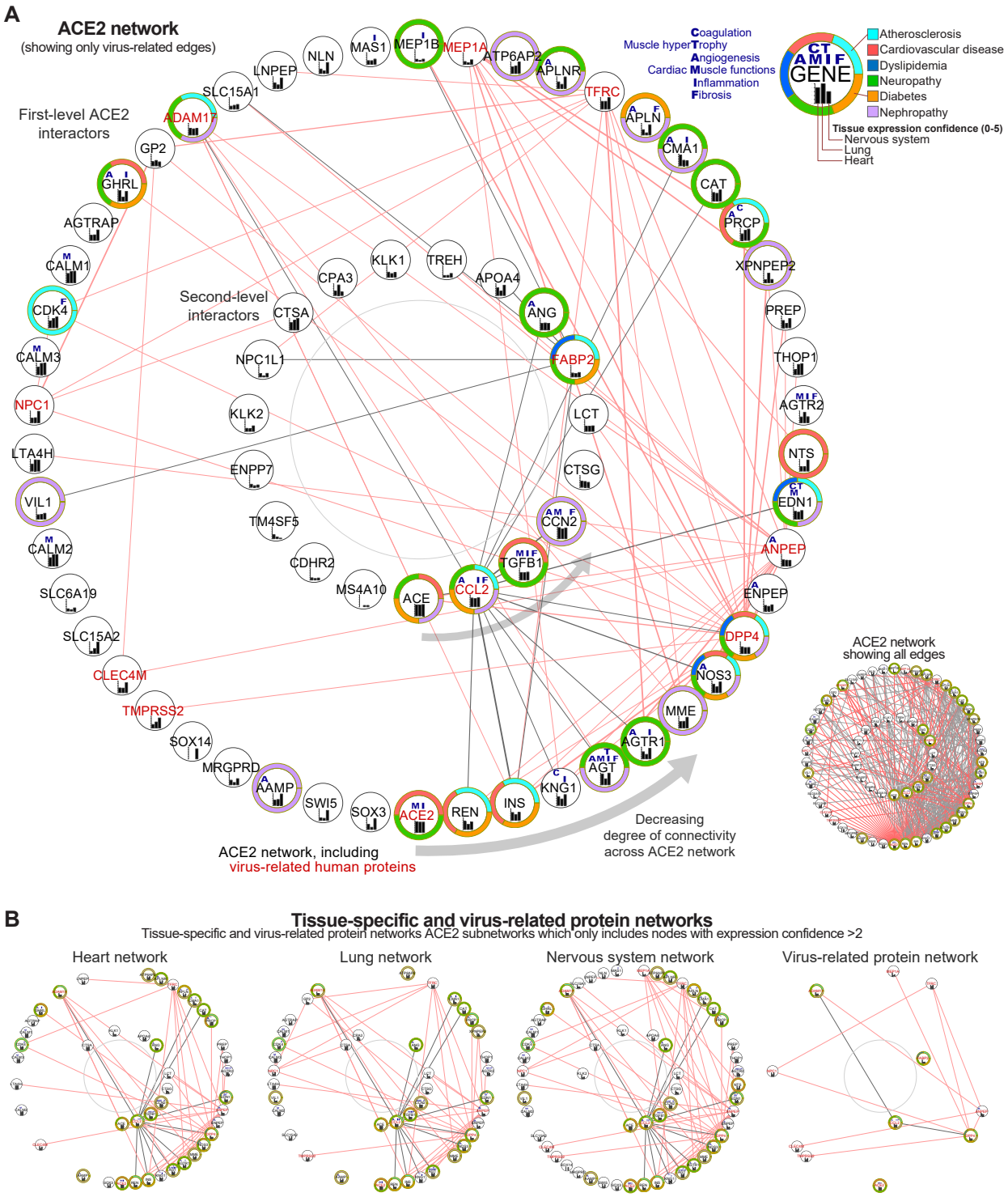

Figure 2. Predicted ACE2 interaction network. (A) Complete ACE2 network, visualized as two circles sorted by the number of connections (degree) with other nodes. The external circle depicts the first level ACE2 interactors; the internal circle depicts the second level of interactors, with genes which do not connect directly with ACE2. For clarity, we showed only the edges associated with virus-related proteins (gene id in red). Edges associated with virus-related proteins are shown in red for first-level (direct) and in grey for the second--level (indirect) ACE2 interactors. Inset in the top right depicts the additional information for each gene/protein, as associated processes (blue letters), associated diseases (color-label ring) and expression confidence across key tissues (black bars). Inset in the bottom right depict the same network including all edges. Notice that the closest ACE2 interactors are REN and INS, which play a central role in the pathophysiology of a number of cardiovascular disorders. The following interactor is KNG1, essential for blood coagulation and assembly of the kallikrein-kinin system. In the network are present 11 virus-infection related proteins forming a dense connection with ACE2 and its top interactors which can affect its functionality. (B) Subsets of ACE2 network containing only highly expressed proteins in the heart, lung, and nervous system; analogous network for virus-related proteins (right).

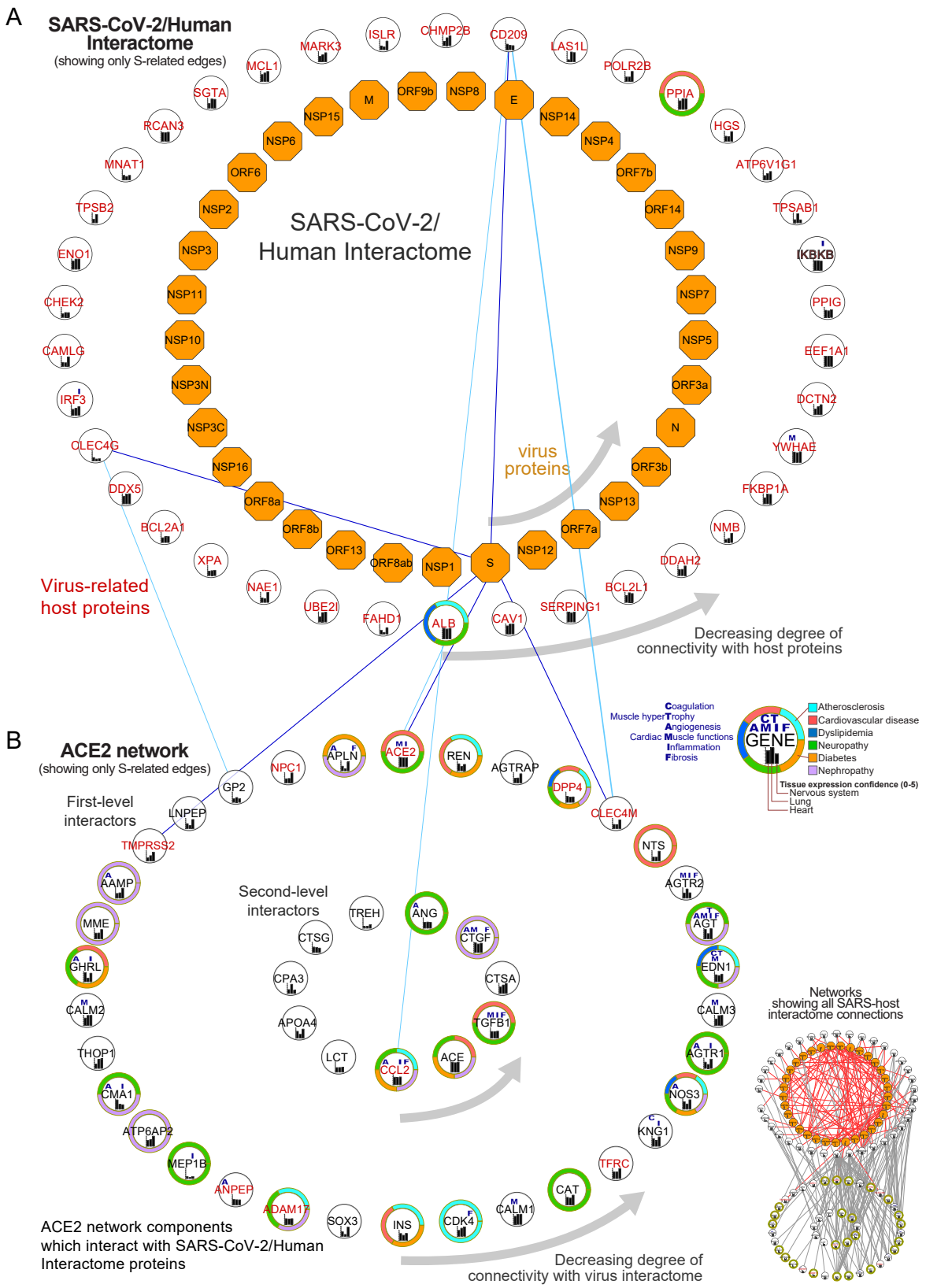

Figure 3. Combined ACE2 network with SARS-CoV-2/Human interactome. (A) depicts ACE2 network components which interact with SARS-CoV-2/Human interactome proteins. (B) depicts the SARS-CoV-2/Human interactome as shown in previously published work 30. Nodes in the network A are sorted by number of connections with virus interactome. Nodes from the network B are sorted by the number of interactions with human proteins. Dark blue edges are showing connections between ACE2 network and virus protein S. Bright blue edges are showing second level interactors of virus glycoprotein S. Virus proteins are shown as orange octagons, while virus-infection related human proteins have red labels. Inset in the bottom right depict the same networks including all SARS-CoV-2/Human interactome edges. Notice that ACE2 interaction network connects directly with virus protein S through ACE2, CLECAM4 and TMPRSS2. Also, SARS-CoV-2 interactome strongly connects with the ACE2 network through INS, CDK4, CCL2 and ALB, all of them associated with atherosclerosis processes.

activation, innate immune system pathway, and protein metabolism related pathways were shared across all networks, except for virus-infection related proteins networks. Platelet activation associated with formation of blood clots was enriched in lung and heart tissue, while digestion of dietary carbohydrate (KEGG) was enriched only in the complete network (Figure 4A).

Enrichment analysis of the disease terms associated with ACE2-tissue specific network. We performed enrichment analysis of DisGenet disease and Rare Diseases AutoRIF database using EnrichR website to evaluate phenotypes associated with ACE2 interaction in different tissues. It enabled us to identify the disease traits which would be helpful in precise identification of the risk groups of patients with COVID-19. This analysis guides the identification of phenotypes which can be triggered by ACE2-network alterations in selected tissues. Moreover, the analysis of rare traits enabled us to precisely characterise the consequences of alterations in the groups of ACE2-network related genes.

The analysis of non-cancerous diseases in the DisGenet database revealed that the highest number of genes from all analyzed networks was associated with following disease phenotypes (in the decreasing order): obesity, hypertensive disease, non-insulin dependent DM, congestive HF, coronary artery disease and atherosclerosis and were observed in all analyzed networks (Figure 5A). Enriched terms not enriched in virus-related network, but containing virus-infection related genes were: Alzheimer's disease, heart failure, diabetes mellitus, asthma and rheumatoid arthritis.

The analysis focused on 11 virus-infection related proteins and diseases shared with other networks, except for diseases mentioned in the last paragraph, revealed that the most significant ones were, SARS, virus diseases, MI and arteriosclerosis (Figure 5A). Additional enrichment analysis of rare disease terms is shown in Figure 5B.

ACE2 network related miRNA predictions. We found 1954 miRNAs regulating components of the ACE2 interaction network. In further analyses, we put special focus on miRNAs which regulated the highest number of the genes from the network as well including ACE2. Analysis of top 10 miRNAs regulating each network (complete network, heart, lung, nervous system tissues and virus-infection related proteins network) revealed overall 16 miRNAs (Figure 6). Five of them were shared between all networks (hsa-miR-302c-5p, hsa-miR-27a-3p, hsa-miR-1305, hsa-miR-587, hsa-miR-26b-5p) (Figure 6C). Signaling pathways associated with 36 genes regulated by those top miRNAs included: RAS pathway, AGE-RAGE signaling pathway in diabetic complications and the Apelin signaling pathway. We also observed enrichment signaling pathways associated with the following diseases: Chagas disease, diseases with heightened arterial wall shear stress, atherosclerosis, Alzheimer's disease, and malaria (Figure 6B).

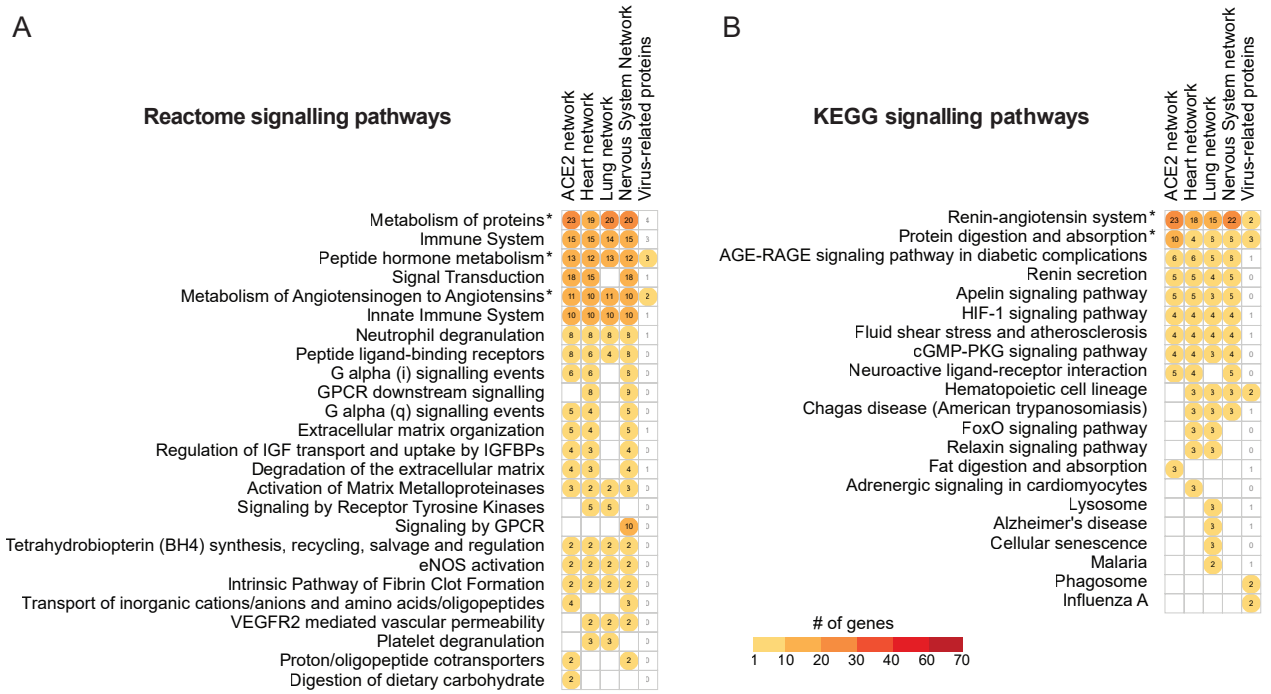

Figure 4. Signaling pathways enriched in ACE2 interaction networks. Using (A) Reactome database, (B) KEGG database, we performed pathway enrichment analysis in the complete ACE2 network (left-most column of symbols) and also in subsets of this network expressed in the heart, lung, and nervous system; we performed this same analysis also for 11 virus-infection related proteins (right-most column of symbols, grey numbers). All circles presented on the graph are associated with significantly enriched pathwyas (FDR corrected p-value <0.05). Pathways marked with asterisks include the ACE2 gene. Notice that all analyzed datasets showed enrichment of pathways related to Renin-angiotensin system and Protein digestion and absorption in both databases. The pathways for "eNOS activation", "Intrinsic Pathway of Fibrin Clot Formation", "Immune System (Reactome)", "AGE-RAGE signaling pathway in diabetic complications", and "Apelin signaling pathway (KEEG)" were enriched in all analyzed tissues. Specific on heart tissue, the "Adrenergic signaling in cardiomyocytes (Reactome)" was enriched.

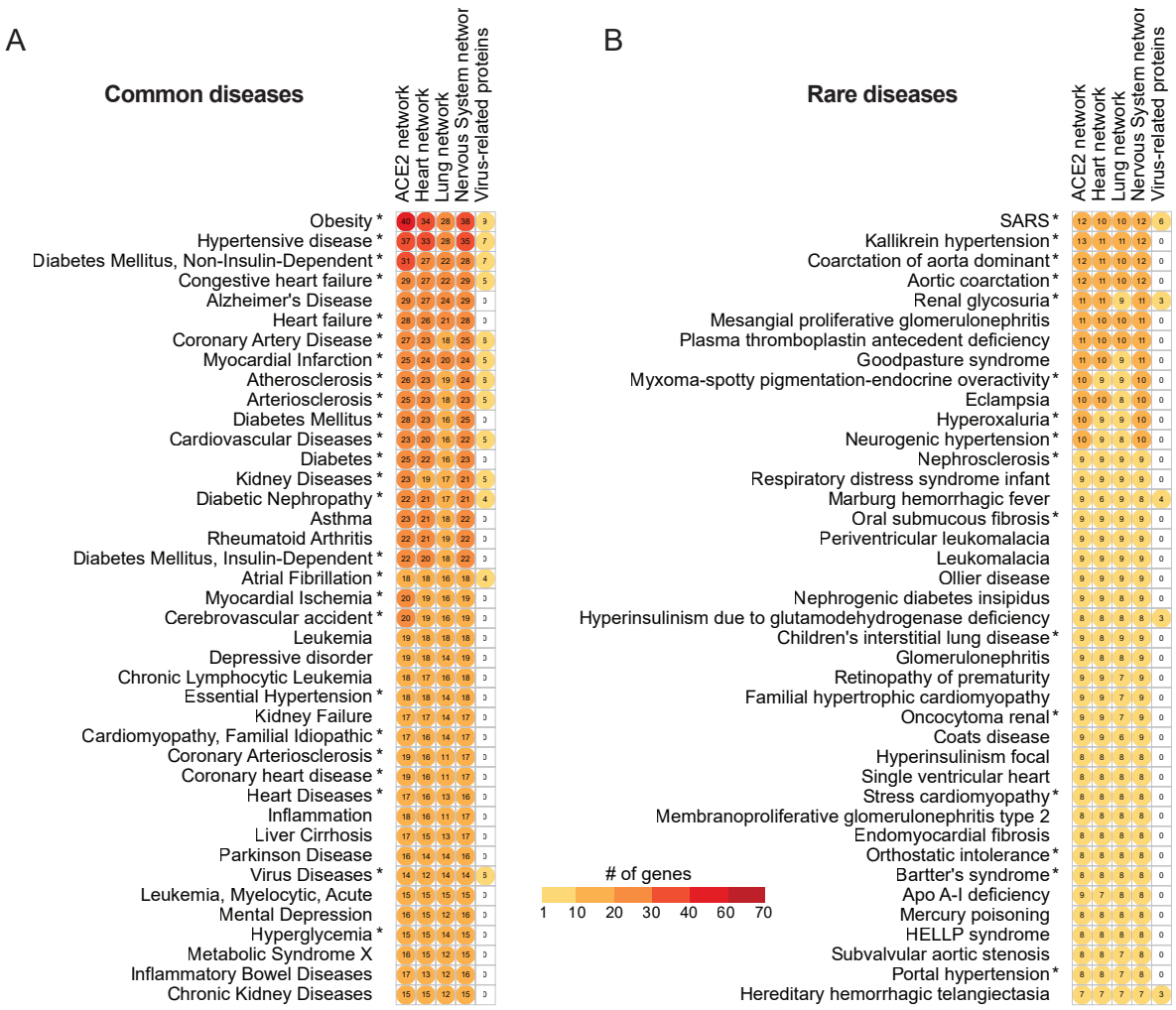

Figure 5. Top 80 potential COVID-19 risk groups, from common and rare diseases significantly associated with ACE2 interaction networks. This list is based on enrichment analysis of DisGeNET, analyzed through EnrichR database. We performed disease enrichment analysis in the complete ACE2 network (left-most column of symbols) and also in subsets of this network expressed in the heart, lung , and nervous system; we performed this same analysis also for 11 virus-infection related proteins (right-most column of symbols). Diseases marked with asterisks include the ACE2 gene. For heart, lung and nervous system tissue, we used the cutoff of expression confidence >2, obtained from Tissue2.0 database. All circles presented on the graph are associated with significantly enriched diseases terms (FDR corrected p-value <0.05). We did not include cancer-related diseases in this analysis. Terms marked with * symbols include ACE2 gene (all except "asthma" include it). The highest number of genes was observed in disease terms shared between all of the networks: SARS*, renal glycosuria*, Marburg hemorrhagic fever, hyperinsulinism due to glutamate dehydrogenase deficiency, hereditary hemorrhagic telangiectasia, Ebola virus disease. Enriched terms not enriched in virus-related network but containing virus-related genes were kallikrein hypertension*, aortic coarctation*, coarctation of aorta dominant*, plasma thromboplastin antecedent deficiency, mesangial proliferative glomerulonephritis, Goodpasture syndrome, eclampsia and hyperoxaluria*. Analysis focused on 11 virus-related proteins and diseases shared with other networks showed that the most significant were, except for mentioned ones also Krabbe leukodystrophy.

Discussion

In the current study, we characterized the interaction between SARS-CoV-2 infection and ACE2 functional networks with a focus on CVD. Using data mining and bioinformatic tools, we described the ACE2 interaction network and evaluated its expression. The main findings of this analysis are the following:

(1) Expression of ACE2 is similar in lungs and heart, which provides a rationale why the cardiovascular system is also a target of SARS-CoV-2 infection;

(2) Change in the heart-relevant ACE2 interaction network by SARS-CoV-2 binding to the receptor likely leads to disturbances in signaling pathways linked to cardiac adverse outcomes;

(3) Genes, which are altered in patients with cardiovascular risk factors (as DPP4, CCL2 and ANPEP) are extensively connected with top regulators of the ACE2 network;

(4) Specific miRNAs (miR-302c-5p, miR-1305, miR-587, miR-26b-5p, and miR-27a-3p) are shared as top regulators between the heart-specific network, complete ACE2 network, virus-infection related proteins network, lung, and nervous system networks;

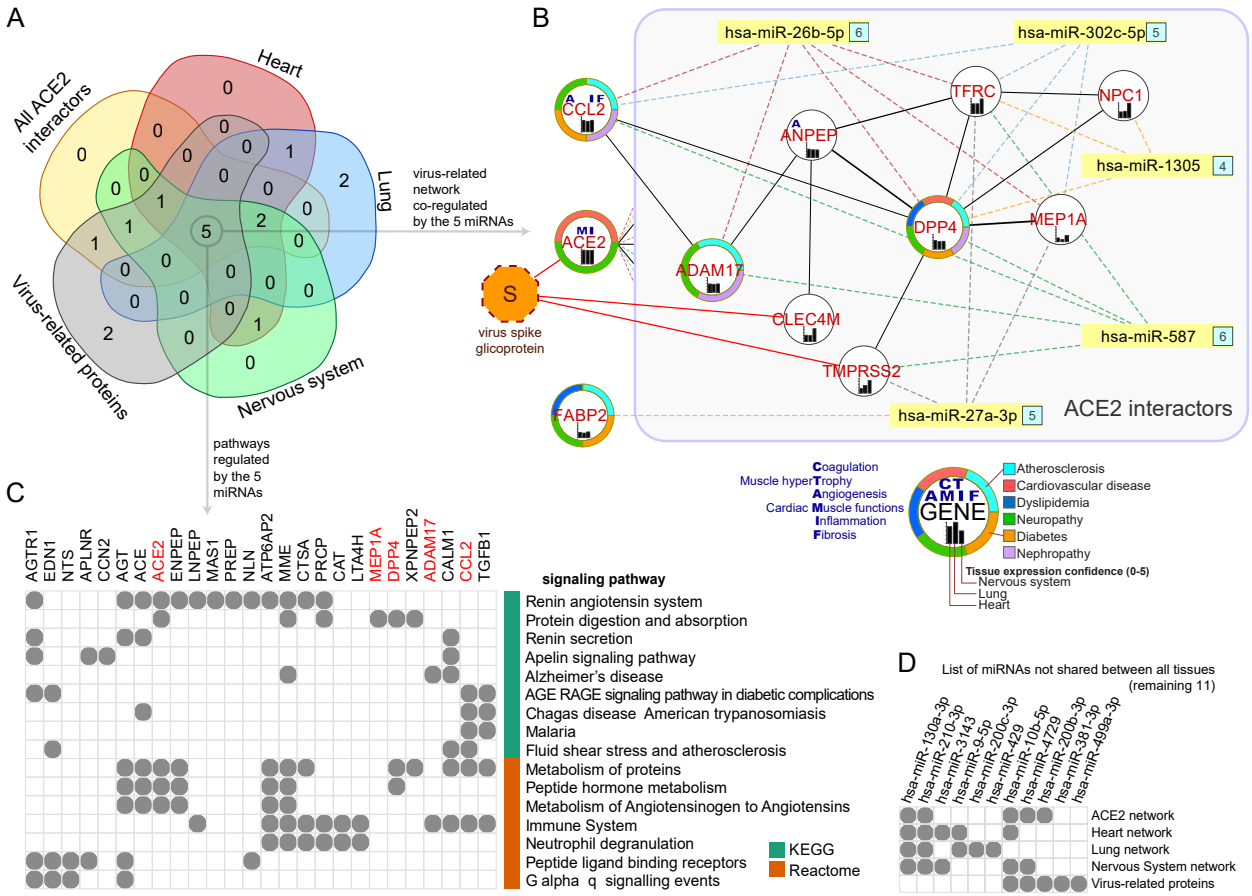

Figure 6. Potencial miRNA modulators of ACE2 network in COVID-19. (**A**) Venn diagram of the top overlapping 10 miRNAs of each network (i.e., complete ACE2 network, and its subnetworks expressed in heart, lung, nervous system, and virus-infection related proteins) regulating the highest number genes. Numbers in diagram depict the the number of shared miRNAs. (**B**) Interaction network between virus-infection related proteins (red labels) and 5 top miRNAs shared between analyzed networks. Numbers on the right side of the miRNAs depict the number of targeted genes within the network. CCL2 and FABP2 genes are not a direct interactors of the ACE2, so they are presented outside of the ACE2-interactors box. "S" refers to SARS-CoV-2 S spike glycoprotein. (**C**) Signaling pathways enriched among 36 targets regulated by the 5 miRNAs shared between all analyzed networks. Genes marked with red are associated with virus-infection related processes. (**D**) Presence of other top miRNAs in the analysed networks.

The high expression of ACE2 in cardiovascular system explains why SARS-CoV-2 infection may target the heart. Recently published studies suggested that the highest expression of ACE2 was found in the digestive tract, followed by testis and kidney.[31,32] Our analysis shows the highest expression of ACE2 in the genitourinary tract and related tissues. Moreover, we found in the genitourinary tract the highest co-expression of TMPRSS2 gene that is known to positively regulate viral entry into the host cell *via* proteolytic cleavage of ACE2. Our analysis also supports the findings of a previous study using integrated analyses of single-cell atlases showing a high expression of ACE2 in ventricular cardiomyocytes, heart macrophages, and pericytes of the heart, lung, and kidney.[33] Therefore, the disturbances in the ACE2 interaction network, by SARS-CoV-2 binding in the ACE2 receptor on cardiomyocytes, might explain cardiac abnormalities presented in COVID-19 patients, including elevated troponin, myocarditis, and sudden cardiac death.[34] Moreover, as myocardial complex interaction network of ACE and its own expression is increased in patients with coexisting CVD, SARS-CoV-2 infection may result in greater damage to cardiomyocytes, and account for greater disease acuity and poorer survival in these patients.

Cardiovascular risk factors related to signaling pathways affected by SARS-CoV-2 binding to ACE2 receptor in heart tissue. Our analysis of ACE2 interaction network, as well as analysis only focused on heart-tissue specific genes, showed that change in ACE2 receptor activity can lead to significant disturbances in signaling pathways linked to well-known complications in COVID-19 disease. Those pathways included RAS, AGE-RAGE signaling pathway in diabetic complications, neuroactive ligand-receptor interaction, innate immune system, neutrophil degranulation and intrinsic pathway of clot formation. Moreover, signaling pathways identified as specific for the heart-relevant ACE2 interaction network were associated with adrenergic signaling in cardiomyocytes, FOXO2 signaling pathway, relaxin signaling pathway, platelet degranulation and VEGFR2 mediated vascular permeability. In these networks, the closest interactors of ACE2 were REN, INS, KNG1, and AGT. Those results were supported by our subsequent analysis showing that genes from the ACE2 network have strongest connection with rare diseases related to high blood pressure, respiratory diseases (i.e. SARS and Goodpasture syndrome), renal diseases, coagulation disorders and several phenotypes like obesity, hypertension, DM that are known to be a risk factors for severe course of COVID-19.

RAS/ACE2, AGE-RAGE, and Apelin signaling as fundamental mediators of the blood pressure dysregulation mediated through ACE2 in COVID-19. Moreover, in our *in silico* analysis using ACE2 functional networks, we found that RAS/ACE2, AGE-RAGE, and Apelin signaling pathways play an important role in SARS-CoV-2 infection. These pathways have a crucial role in pathogenesis of DM, CVD and blood pressure regulations. Therefore, binding of the SARS-CoV-2 to the ACE2 receptor leading to disturbances in the pathways of these key regulators might explain the adverse outcome in COVID-19 patients with coexistence of CVD. Abnormalities of ACE2/RAS pathway signaling and deregulation of angiotensin II as a fundamental mediator of this axis are closely related to pathophysiology of hypertension and progression of cardiovascular remodeling.[35] Furthermore, increased ACE2 expression attenuates hypertension-linked pathophysiological changes and protects against elevated blood pressure whereas loss of ACE2 function exacerbates hypertension.[36] Apelin signaling is involved in many physiological processes such as energy metabolism, blood pressure regulation, and cardiac contractility and plays an important role in organ and tissue pathologies including, DM, obesity, HF as well as HIV-1 infection.[37] *In vitro* analysis showed that apelin is a second catalytic substrate for ACE2 and it can effect as an inotropic and cardioprotective peptide.[38,39]

Thromboembolic complications in COVID-19: the role of KNG1, one of the top ACE2 interactors. In this study we found a strong connection between ACE2 and KNG1 which as a part of Kalikrein-Kinin system (KKS) encoding precursors of kinins.[40] KKS mediators are involved in vessel wall remodeling, intimal hyperplasia[41] and affect nitric oxide and prostacyclin production.[42] Moreover, high molecular weight kininogen (HK) is essential in surface-binding and activity of factor XI, a component of intrinsic pathway of blood coagulation.[43] Plasma kininogen may induce the development of arterial thrombosis, especially under pathologic conditions.[44] 2-chain HKa was also found to inhibit PAI-1 function, interfere with platelet activation and mediate platelet-leukocyte interaction [45,46] and therefore, deregulation of KKS may result in thromboembolic complications and lead to sepsis exacerbation in infections.[47–49]

Pro-inflammatory cytokines modulated by ACE2, a putative mechanism for generalized inflammatory response. Several experimental studies indicated that ACE2 is able to modify acute and chronic inflammatory response.[50,51] In line, we found in our analysis a strong interaction of ACE2 with several genes related with inflammatory response including CCL2 and TGFB1. Loss of function of ACE2 in mutant mice resulted in enhanced production of inflammatory cytokine and collagenase levels, increased ROS, neutrophilic infiltration and MAPK activation.[52] Diminished ACE2 function may deregulate RAS and induce inflammation and vessel permeability and lung edema.[53] It is worth to mention that among SARS-CoV-infected patients the level of pro-inflammatory cytokines, especially CCL2 and TGF-β1 were increased in cells expressing ACE2, while this could not be seen in tissue with undetectable ACE2 expression [54] A comparable pattern of inflammatory cytokines was found in SARS-CoV-2 infection as well.[55]

The role of virus-infection related proteins from ACE2 network in COVID-19 adverse outcomes. Additional analysis of ACE2 interaction network identified 11 virus-infection related proteins involved in significantly enriched pathways. Three of them (ACE2, CLEC4M and TMPRSS2) directly interact with virus glycoprotein S. We identified 10 virus-infection related proteins in the heart-specific interaction network, (except for TMPRSS2) suggesting that the heart tissue could be capable of being as strongly affected as the respiratory system. Three of those genes: DPP4, CCL2 and ANPEP showed especially extensive connections with top regulators of the network. Interestingly, higher plasma DPP4 can be found among patients with obesity, metabolic syndrome and DM, who are at risk of a severe course of COVID-19.[56] Moreover, in experimental models of ARDS, DPP4 inhibitors suppressed pro-inflammatory cytokine production and attenuated LPS-induced lung injury.[57] DPP4 knockin mice were found more susceptible to MERS-CoV infections which resulted in severe inflammatory response and lethal lung disease.[58,59] Therefore, it should be further investigated whether DPP4 inhibitors, widely used for the treatment of DM, may act as therapeutic drugs for ARDS caused by SARS-CoV-2 infection.[60] Interestingly, DPP4 inhibitors are able to at least partially reverse the olfactory dysfunction observed in DM, suggesting that DPP4 pathway may be involved in anosmia which occurs commonly among COVID-19 patients.[61,62] This evidence show that DPP4 could be a crucial factor in regulation of multiple pathological processes associated with COVID-19.

Second crucial cytokine in the network is the chemokine CCL2 also referred to as MCP-1. It has been shown that the CCL2 expression was increased among patients infected with SARS-CoV.[63,64] Possibly, ACE2 mediates viral entry and leads to activation of ERK1/2/AP-1 pathway and upregulation of CCL2. CCL2 protein has been implicated in lung inflammatory disorders and contributes to development of pulmonary fibrosis.[19,65] Besides, in the late stage of disease CCL2 expression levels are constantly elevated among severe SARS patients in comparison to non-severe controls and associated with diffuse pulmonary infiltrates, higher body temperatures and longer hospitalization.[66] It is worth to mention that among SARS-CoV-infected patients the level of pro-inflammatory cytokines, especially CCL2 and TGF-β1 were increased in cells expressing ACE2, while this could not be seen in tissue with undetectable ACE2 expression.[54] A comparable pattern of inflammatory cytokines was found in SARS-CoV-2 infection as well.[55] Significant association of the CCL2 with malaria disease pathway in our study, target of chloroquine, a potent anti inflammatory agent (regulating also ACE2 and INS) make it a promising link between ACE2 and cytokine storm associated with severe COVID-19 disease.

The third gene with extensive ACE2 network connection, ANPEP encodes aminopeptidase N (APN, CD13), which similarly to ACE2 and DDP2 is also recognized as a target for coronavirus named HCoV-22944.[67] APN plays a role in inflammatory response by regulating the activity of various hormones, chemokines and cytokines and was found to be increased in some inflammatory diseases.[68] APN may influence on controlling blood pressure by regulating the metabolism of Ang III.[69]

miRNAs as promising antiviral modulators of the ACE2 network and potential biomarker of HF associated with COVID-19. In the next step of our study, we searched for miRNAs that may regulate expression of ACE2 networks and related processes. Our prediction model revealed that miR-302c-5p, miR-1305, miR-587, miR-26b-5p, and miR-27a-3p are top regulators of complete ACE2 network, heart-specific network as well as virus-infection related network. To our best knowledge, we present here novel results on a potential role of miRNAs as a diagnostic and prognostic tool in heart muscle injury in the course of SARS-CoV-2 infection.

miR-1305 and miR-587: TGF-β signaling pathway regulators in HF progression. MiR-1305 and miR-587 were found to regulate the expression of TGF-β pathway members, SMAD3 and SMAD4.[70,71] SMAD3 negatively regulates the inflammatory response and modulates T-cell activation.[72] The TGF-β/SMAD signaling pathway has been directly related to viral infections.[73] Moreover, this pathway deregulation has been also implicated to ventricular remodeling, myocardial fibrosis and hypertrophy and, as a result, HF progression.[74] The highest expression of miR-587 was found in platelets of patients with acute coronary syndrome and was closely related to the severity of coronary artery stenosis.[75]

miR-26b-5p: anti-fibrotic agent and AGTR1-dependent hypertension modulator. In our study we found that miR-26b-5p may play an important role in pathogenesis of HF in COVID-19 patients as showed by an interaction of ACE2 with ENPEP, AGT, CCN2, CALM1, CCL2, and AGTR1. Noteworthy, previous study showed that in the miRNA-differentially expressed genes (DEG) regulatory network, hsa-miR-26b-5p was one of the most important miRNA and AGTR1 was the most outstanding up-regulated DEGs in the PPI network.[76] It has been suggested that AGTR1 can modulate hypertension, via the regulation of miR-26b-5p in arachidonic acid metabolism. The anti-fibrotic effect of miR-26b-5p was shown in the liver, in the diabetic mouse myocardium and in Ang-II-induced mouse cardiac fibroblasts .[77]

miR-302c-5p: potential antiviral therapeutic and biomarker of HF. Another regulating miRNA from our network was miR-302c-5p playing an important role in many viral infections.[78–80] A study reported an association between the miR-302, KPNA2 axis and EV71-related cytokine storm and showed the potential of miR-302 as an antiviral therapeutic.[80] Our bioinformatic analysis for the first time showed the importance of miR-302c-5p in SARS-CoV-2 infection. Apart from the crucial function of miR-302 in viral infections, it may be also associated with CVD,[81,82] as circulating miR-302 was positively correlated with NT-proBNP levels in acute HF patients and showed strong potential as a novel biomarker for the diagnosis and the differentiation of disease severity of acute HF.[81]

miR-27a-3p: a potential biomarker of acute HF and NF-κB signaling regulator. Also miR-27a-3p was found to be involved in inflammatory response and oxidative stress through several pathways including PPAR-γ, NF-κB and PI3K/AKT/Nrf2 signaling.[83–85] In animal model of acute lung injury, expression of miR-27a-3p in alveolar macrophages was significantly decreased, while overexpression of miR-27a-3p suppressed NF-κB activation and alleviated acute lung injury by binding to its target NFKB1.[83] Moreover, it was also found that miR-27a-3p may target pathways related to atherosclerosis, and may act as a potential biomarker of acute HF.[86,87]

Conclusion

This comprehensive analysis provides novel information regarding the complexity of signaling pathways of SARS-CoV-2 infection affecting the cardiovascular system and forms a basis for a creation of predictive tools and introduction of therapy to improve outcome in COVID-19, and therefore has a potential to reduce economic consequences of the global pandemic. We believe that the results of our analysis could be further validated in laboratory and clinical settings and help to create a paradigm for future studies in this field. MiRNAs identified for the first time in this study can serve as potential biomarkers helping with identification of the pathological changes in COVID-19 or serve as therapeutic targets due to their stability in the serum, forming a basis for personalized therapy in patients with or at risk for CVD as those with obesity, DM or hypertension suffering from COVID-19.

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References

1. Zhu N, Zhang D, Wang W, Li X, Yang B, Song J, Zhao X, Huang B, Shi W, Lu R, Niu P, Zhan F, Ma X, Wang D, Xu W, Wu G, Gao GF, Tan W. A Novel Coronavirus from Patients with Pneumonia in China, 2019. New England Journal of Medicine. 2020. p. 727–733.
2. Wu C, Chen X, Cai Y, Xia J 'an, Zhou X, Xu S, Huang H, Zhang L, Zhou X, Du C, Zhang Y, Song J, Wang S, Chao Y, Yang Z, Xu J, Zhou X, Chen D, Xiong W, Xu L, Zhou F, Jiang J, Bai C, Zheng J, Song Y. Risk Factors Associated With Acute Respiratory Distress Syndrome and Death in Patients With Coronavirus Disease 2019 Pneumonia in Wuhan, China. *JAMA Intern Med* 2020;
3. Yang J, Zheng Y, Gou X, Pu K, Chen Z, Guo Q, Ji R, Wang H, Wang Y, Zhou Y. Prevalence of comorbidities and its effects in coronavirus disease 2019 patients: A systematic review and meta-analysis. International Journal of Infectious Diseases. 2020. p. 91–95.
4. Li B, Yang J, Zhao F, Zhi L, Wang X, Liu L, Bi Z, Zhao Y. Prevalence and impact of cardiovascular metabolic diseases on COVID-19 in China. Clinical Research in Cardiology. 2020. p. 531–538.

5. Leung GM, Hedley AJ, Ho L-M, Chau P, Wong IOL, Thach TQ, Ghani AC, Donnelly CA, Fraser C, Riley S, Ferguson NM, Anderson RM, Tsang T, Leung P-Y, Wong V, Chan JCK, Tsui E, Lo S-V, Lam T-H. The Epidemiology of Severe Acute Respiratory Syndrome in the 2003 Hong Kong Epidemic: An Analysis of All 1755 Patients. Annals of Internal Medicine. 2004. p. 662.
6. Assiri A, Al-Tawfiq JA, Al-Rabeeah AA, Al-Rabiah FA, Al-Hajjar S, Al-Barrak A, Flemban H, Al-Nassir WN, Balkhy HH, Al-Hakeem RF, Makhdoom HQ, Zumla AI, Memish ZA. Epidemiological, demographic, and clinical characteristics of 47 cases of Middle East respiratory syndrome coronavirus disease from Saudi Arabia: a descriptive study. *Lancet Infect Dis* 2013;**13**:752–761.
7. Wang K, Gheblawi M, Oudit GY. Angiotensin Converting Enzyme 2: A Double-Edged Sword. Circulation. 2020.
8. Gheblawi M, Wang K, Viveiros A, Nguyen Q, Zhong J-C, Turner AJ, Raizada MK, Grant MB, Oudit GY. Angiotensin-Converting Enzyme 2: SARS-CoV-2 Receptor and Regulator of the Renin-Angiotensin System. Circulation Research. 2020. p. 1456–1474.
9. Gheblawi M, Wang K, Viveiros A, Nguyen Q, Zhong J-C, Turner AJ, Raizada MK, Grant MB, Oudit GY. Angiotensin-Converting Enzyme 2: SARS-CoV-2 Receptor and Regulator of the Renin-Angiotensin System. Circulation Research. 2020. p. 1456–1474.
10. Patel VB, Zhong J-C, Grant MB, Oudit GY. Role of the ACE2/Angiotensin 1–7 Axis of the Renin–Angiotensin System in Heart Failure. Circulation Research. 2016. p. 1313–1326.
11. Hoffmann M, Kleine-Weber H, Krüger N, Müller M, Drosten C, Pöhlmann S. The novel coronavirus 2019 (2019-nCoV) uses the SARS-coronavirus receptor ACE2 and the cellular protease TMPRSS2 for entry into target cells.
12. Oudit GY, Kassiri Z, Jiang C, Liu PP, Poutanen SM, Penninger JM, Butany J. SARS-coronavirus modulation of myocardial ACE2 expression and inflammation in patients with SARS. *Eur J Clin Invest* 2009;**39**:618–625.
13. Shi S, Qin M, Shen B, Cai Y, Liu T, Yang F, Gong W, Liu X, Liang J, Zhao Q, Huang H, Yang B, Huang C. Association of Cardiac Injury With Mortality in Hospitalized Patients With COVID-19 in Wuhan, China. *JAMA Cardiol* 2020;
14. Clerkin KJ, Fried JA, Raikhelkar J, Sayer G, Griffin JM, Masoumi A, Jain SS, Burkhoff D, Kumaraiah D, Rabbani L, Schwartz A, Uriel N. Coronavirus Disease 2019 (COVID-19) and Cardiovascular Disease. *Circulation* 2020;
15. Guo T, Fan Y, Chen M, Wu X, Zhang L, He T, Wang H, Wan J, Wang X, Lu Z. Cardiovascular Implications of Fatal Outcomes of Patients With Coronavirus Disease 2019 (COVID-19). *JAMA Cardiol* 2020;
16. Kanehisa M. The KEGG Database. 'In Silico' Simulation of Biological Processes. p. 91–103.
17. Doncheva NT, Morris JH, Gorodkin J, Jensen LJ. Cytoscape StringApp: Network Analysis and Visualization of Proteomics Data. *J Proteome Res* 2019;**18**:623–632.
18. Zhang H, Penninger JM, Li Y, Zhong N, Slutsky AS. Angiotensin-converting enzyme 2 (ACE2) as a SARS-CoV-2 receptor: molecular mechanisms and potential therapeutic target. Intensive Care Medicine. 2020. p. 586–590.
19. Chen I-Y, Chang SC, Wu H-Y, Yu T-C, Wei W-C, Lin S, Chien C-L, Chang M-F. Upregulation of the Chemokine (C-C Motif) Ligand 2 via a Severe Acute Respiratory Syndrome Coronavirus Spike-ACE2 Signaling Pathway. *J Virol* American Society for Microbiology Journals; 2010;**84**:7703–7712.
20. pubmeddev, Kuan TC E al. Identifying the regulatory element for human angiotensin-converting enzyme 2 (ACE2) expression in human cardiofibroblasts. - PubMed - NCBI. https://www.ncbi.nlm.nih.gov/pubmed/21864606 (22 April 2020)
21. Palasca O, Santos A, Stolte C, Gorodkin J, Jensen LJ. TISSUES 2.0: an integrative web resource on mammalian tissue expression. *Database* Oxford University Press; 2018;**2018**.
22. Shannon P, Markiel A, Ozier O, Baliga NS, Wang JT, Ramage D, Amin N, Schwikowski B, Ideker T. Cytoscape: a software environment for integrated models of biomolecular interaction networks. *Genome Res* 2003;**13**:2498–2504.
23. Eyileten C, Wicik Z, De Rosa S, Mirowska-Guzel D, Soplinska A, Indolfi C, Jastrzebska-Kurkowska I, Czlonkowska A, Postula M. MicroRNAs as Diagnostic and Prognostic Biomarkers in Ischemic Stroke—A Comprehensive Review and Bioinformatic Analysis. Cells. 2018. p. 249.
24. Rani J, Mittal I, Pramanik A, Singh N, Dube N, Sharma S, Puniya BL, Raghunandanan MV, Mobeen A, Ramachandran S. T2DiACoD: A Gene Atlas of Type 2 Diabetes Mellitus Associated Complex Disorders. *Sci Rep* Nature Publishing Group; 2017;**7**.
25. Huang DW, Sherman BT, Lempicki RA. Bioinformatics enrichment tools: paths toward the comprehensive functional analysis of large gene lists. *Nucleic Acids Res* 2009;**37**:1–13.
26. Ru Y, Kechris KJ, Tabakoff B, Hoffman P, Radcliffe RA, Bowler R, Mahaffey S, Rossi S, Calin GA, Bemis L, Theodorescu D. The multiMiR R package and database: integration of microRNA–target interactions along with their disease and drug associations. Nucleic Acids Research. 2014. p. e133–e133.
27. Pordzik J, Jakubik D, Jarosz-Popek J, Wicik Z, Eyileten C, De Rosa S, Indolfi C, Siller-Matula JM, Czajka P, Postula M. Significance of circulating microRNAs in diabetes mellitus type 2 and platelet reactivity: bioinformatic analysis and review. Cardiovascular Diabetology. 2019.
28. Sabatino J, Wicik Z, De Rosa S, Eyileten C, Jakubik D, Spaccarotella C, Mongiardo A, Postula M, Indolfi C. MicroRNAs fingerprint of bicuspid aortic valve. *J Mol Cell Cardiol* 2019;**134**:98–106.
29. Yoo M, Shin J, Kim J, Ryall KA, Lee K, Lee S, Jeon M, Kang J, Tan AC. DSigDB: drug signatures database for gene set analysis. *Bioinformatics* 2015;**31**:3069–3071.
30. Guzzi PH, Mercatelli D, Ceraolo C, Giorgi FM. Master Regulator Analysis of the SARS-CoV-2/Human Interactome. *J Clin Med Res* 2020;**9**.
31. 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. Cardiovascular Research. 2020. p. 1097–1100.
32. Sungnak W, HCA Lung Biological Network, Huang N, Bécavin C, Berg M, Queen R, Litvinukova M, Talavera-López C, Maatz H, Reichart D, Sampaziotis F, Worlock KB, Yoshida M, Barnes JL. SARS-CoV-2 entry factors are highly expressed in nasal epithelial cells together with innate immune genes. Nature Medicine. 2020.
33. Muus C, Luecken MD, Eraslan G, Waghray A, Heimberg G, Sikkema L, Kobayashi Y, Vaishnav ED, Subramanian A, Smilie C, Jagadeesh K, Duong ET, Fiskin E, Triglia ET, Ansari M, Cai P, Lin B, Buchanan J, Chen S, Shu J, Haber AL, Chung H, Montoro DT, Adams T, Aliee H, Samuel J, Andrusivova AZ, Angelidis I, Ashenberg O, Bassler K, et al. Integrated analyses of single-cell atlases reveal age, gender, and smoking status associations with cell type-specific expression of mediators of SARS-CoV-2 viral entry and highlights inflammatory programs in putative target cells.
34. Zheng Y-Y, Ma Y-T, Zhang J-Y, Xie X. COVID-19 and the cardiovascular system. *Nat Rev Cardiol* 2020;**17**:259–260.
35. Montezano AC, Cat AND, Rios FJ, Touyz RM. Angiotensin II and Vascular Injury. Current Hypertension Reports. 2014.
36. Crackower MA, Sarao R, Oudit GY, Yagil C, Kozieradzki I, Scanga SE, Oliveira-dos-Santos AJ, Costa J da, Zhang L, Pei Y, Scholey J, Ferrario CM, Manoukian AS, Chappell MC, Backx PH, Yagil Y, Penninger JM. Angiotensin-converting enzyme 2 is an essential regulator of heart function. *Nature* 2002;**417**:822–828.

37. Zou M-X, Liu H-Y, Haraguchi Y, Soda Y, Tatemoto K, Hoshino H. Apelin peptides block the entry of human immunodeficiency virus (HIV). FEBS Letters. 2000. p. 15–18.
38. Sato T, Suzuki T, Watanabe H, Kadowaki A, Fukamizu A, Liu PP, Kimura A, Ito H, Penninger JM, Imai Y, Kuba K. Apelin is a positive regulator of ACE2 in failing hearts. Journal of Clinical Investigation. 2013. p. 5203–5211.
39. Wang, Wang W, McKinnie SMK, Farhan M, Paul M, McDonald T, McLean B, Llorens-Cortes C, Hazra S, Murray AG, Vederas JC, Oudit GY. Angiotensin-Converting Enzyme 2 Metabolizes and Partially Inactivates Pyr-Apelin-13 and Apelin-17. Hypertension. 2016. p. 365–377.
40. Moreau ME, Garbacki N, Molinaro G, Brown NJ, Marceau F, Adam A. The kallikrein-kinin system: current and future pharmacological targets. *J Pharmacol Sci* 2005;**99**:6–38.
41. Colman R. Regulation of Angiogenesis by the Kallikrein-Kinin System. Current Pharmaceutical Design. 2006. p. 2599–2607.
42. Bhoola KD, Figueroa CD, Worthy K. Bioregulation of kinins: kallikreins, kininogens, and kininases. *Pharmacol Rev* 1992;**44**:1–80.
43. Scott CF, Schapira M, James HL, Cohen AB, Colman RW. Inactivation of factor XIa by plasma protease inhibitors: predominant role of alpha 1-protease inhibitor and protective effect of high molecular weight kininogen. *J Clin Invest* 1982;**69**:844–852.
44. Merkulov S, Zhang W-M, Komar AA, Schmaier AH, Barnes E, Zhou Y, Lu X, Iwaki T, Castellino FJ, Luo G, McCrae KR. Deletion of murine kininogen gene 1 (mKng1) causes loss of plasma kininogen and delays thrombosis. *Blood* 2008;**111**:1274–1281.
45. Chavakis T, Santoso S, Clemetson KJ, Sachs UJH, Isordia-Salas I, Pixley RA, Nawroth PP, Colman RW, Preissner KT. High Molecular Weight Kininogen Regulates Platelet-Leukocyte Interactions by Bridging Mac-1 and Glycoprotein Ib. Journal of Biological Chemistry. 2003. p. 45375–45381.
46. Chavakis T, Boeckel N, Santoso S, Voss R, Isordia-Salas I, Pixley RA, Morgenstern E, Colman RW, Preissner KT. Inhibition of Platelet Adhesion and Aggregation by a Defined Region (Gly-486–Lys-502) of High Molecular Weight Kininogen. Journal of Biological Chemistry. 2002. p. 23157–23164.
47. Rhaleb N-E, Yang X-P, Carretero OA. The kallikrein-kinin system as a regulator of cardiovascular and renal function. *Compr Physiol* 2011;**1**:971–993.
48. Isordia-Salas I, Pixley RA, Sáinz IM, Martínez-Murillo C, Colman RW. The role of plasma high molecular weight kininogen in experimental intestinal and systemic inflammation. *Arch Med Res* 2004;**35**:369–377.
49. Mattsson E, Herwald H, Cramer H, Persson K, Sjöbring U, Björck L. Staphylococcus aureus induces release of bradykinin in human plasma. *Infect Immun* 2001;**69**:3877–3882.
50. Silva ACS e., Silveira KD, Ferreira AJ, Teixeira MM. ACE2, angiotensin-(1-7) and Mas receptor axis in inflammation and fibrosis. British Journal of Pharmacology. 2013. p. 477–492.
51. Patel VB, Mori J, McLean BA, Basu R, Das SK, Ramprasath T, Parajuli N, Penninger JM, Grant MB, Lopaschuk GD, Oudit GY. ACE2 Deficiency Worsens Epicardial Adipose Tissue Inflammation and Cardiac Dysfunction in Response to Diet-Induced Obesity. *Diabetes* 2016;**65**:85–95.
52. Oudit GY, Kassiri Z, Patel MP, Chappell M, Butany J, Backx PH, Tsushima RG, Scholey JW, Khokha R, Penninger JM. Angiotensin II-mediated oxidative stress and inflammation mediate the age-dependent cardiomyopathy in ACE2 null mice. *Cardiovasc Res* 2007;**75**:29–39.
53. Imai Y, Kuba K, Rao S, Huan Y, Guo F, Guan B, Yang P, Sarao R, Wada T, Leong-Poi H, Crackower MA, Fukamizu A, Hui C-C, Hein L, Uhlig S, Slutsky AS, Jiang C, Penninger JM. Angiotensin-converting enzyme 2 protects from severe acute lung failure. Nature. 2005. p. 112–116.
54. He L, Ding Y, Zhang Q, Che X, He Y, Shen H, Wang H, Li Z, Zhao L, Geng J, Deng Y, Yang L, Li J, Cai J, Qiu L, Wen K, Xu X, Jiang S. Expression of elevated levels of pro-inflammatory cytokines in SARS-CoV-infected ACE2+ cells in SARS patients: relation to the acute lung injury and pathogenesis of SARS. *J Pathol* 2006;**210**:288–297.
55. Huang C, Wang Y, Li X, Ren L, Zhao J, Hu Y, Zhang L, Fan G, Xu J, Gu X, Cheng Z, Yu T, Xia J, Wei Y, Wu W, Xie X, Yin W, Li H, Liu M, Xiao Y, Gao H, Guo L, Xie J, Wang G, Jiang R, Gao Z, Jin Q, Wang J, Cao B. Clinical features of patients infected with 2019 novel coronavirus in Wuhan, China. The Lancet. 2020. p. 497–506.
56. Barchetta I, Cavallo MG, Baroni MG. COVID-19 and diabetes: Is this association driven by the DPP4 receptor? Potential clinical and therapeutic implications. Diabetes Research and Clinical Practice. 2020. p. 108165.
57. Kawasaki T, Chen W, Htwe YM, Tatsumi K, Dudek SM. DPP4 inhibition by sitagliptin attenuates LPS-induced lung injury in mice. *Am J Physiol Lung Cell Mol Physiol* 2018;**315**:L834–L845.
58. Li K, Wohlford-Lenane CL, Channappanavar R, Park J-E, Earnest JT, Bair TB, Bates AM, Brogden KA, Flaherty HA, Gallagher T, Meyerholz DK, Perlman S, McCray PB Jr. Mouse-adapted MERS coronavirus causes lethal lung disease in human DPP4 knockin mice. *Proc Natl Acad Sci U S A* 2017;**114**:E3119–E3128.
59. Fan C, Wu X, Liu Q, Li Q, Liu S, Lu J, Yang Y, Cao Y, Huang W, Liang C, Ying T, Jiang S, Wang Y. A Human DPP4-Knockin Mouse's Susceptibility to Infection by Authentic and Pseudotyped MERS-CoV. Viruses. 2018. p. 448.
60. Iacobellis G. COVID-19 and diabetes: Can DPP4 inhibition play a role? *Diabetes Res Clin Pract* 2020;**162**:108125.
61. Vaira LA, Salzano G, Deiana G, De Riu G. Anosmia and Ageusia: Common Findings in COVID-19 Patients. *Laryngoscope* 2020;
62. Lietzau G, Davidsson W, Östenson C-G, Chiazza F, Nathanson D, Pintana H, Skogsberg J, Klein T, Nyström T, Darsalia V, Patrone C. Type 2 diabetes impairs odour detection, olfactory memory and olfactory neuroplasticity; effects partly reversed by the DPP-4 inhibitor Linagliptin. *Acta Neuropathol Commun* 2018;**6**:14.
63. Wong CK, Lam CWK, Wu AKL, Ip WK, Lee NLS, Chan IHS, Lit LCW, Hui DSC, Chan MHM, Chung SSC, Sung JJY. Plasma inflammatory cytokines and chemokines in severe acute respiratory syndrome. Clinical & Experimental Immunology. 2004. p. 95–103.
64. Huang K-J, Su I-J, Theron M, Wu Y-C, Lai S-K, Liu C-C, Lei H-Y. An interferon-?-related cytokine storm in SARS patients. Journal of Medical Virology. 2005. p. 185–194.
65. Rose CE, Edward Rose C, Sung S-SJ, Fu SM. Significant Involvement of CCL2 (MCP-1) in Inflammatory Disorders of the Lung. Microcirculation. 2003. p. 273–288.
66. Cameron MJ, Bermejo-Martin JF, Danesh A, Muller MP, Kelvin DJ. Human immunopathogenesis of severe acute respiratory syndrome (SARS). *Virus Res* 2008;**133**:13–19.
67. Feng Z, Wang Y, Qi W. The Small Intestine, an Underestimated Site of SARS-CoV-2 Infection: From Red Queen Effect to Probiotics. *Preprints* 2020;
68. Lu C, Amin MA, Fox DA. CD13/Aminopeptidase N Is a Potential Therapeutic Target for Inflammatory Disorders. The Journal of Immunology. 2020. p. 3–11.

69. Danziger RS. Aminopeptidase N in arterial hypertension. *Heart Fail Rev* 2008;**13**:293–298.
70. Zhang L, Sun X, Chen S, Yang C, Shi B, Zhou L, Zhao J. Long noncoding RNA DANCR regulates -Smad 4 axis to promote chondrogenic differentiation of human synovium-derived mesenchymal stem cells. *Biosci Rep* 2017;**37**.
71. Su Y, Feng W, Shi J, Chen L, Huang J, Lin T. circRIP2 accelerates bladder cancer progression via miR-1305/Tgf-β2/smad3 pathway. Molecular Cancer. 2020.
72. Yang X, Letterio JJ, Lechleider RJ, Chen L, Hayman R, Gu H, Roberts AB, Deng C. Targeted disruption of SMAD3 results in impaired mucosal immunity and diminished T cell responsiveness to TGF-beta. *EMBO J* 1999;**18**:1280–1291.
73. Mirzaei H, Faghihloo E. Viruses as key modulators of the TGF-β pathway; a double-edged sword involved in cancer. Reviews in Medical Virology. 2018. p. e1967.
74. Eulertaimor G, Heger J. The complex pattern of SMAD signaling in the cardiovascular system☆. Cardiovascular Research. 2006. p. 15–25.
75. Qiu H, Zhang Y, Zhao Q, Jiang H, Yan J, Liu Y. Platelet miR-587 may be Used as a Potential Biomarker for Diagnosis of Patients with Acute Coronary Syndrome. Clinical Laboratory. 2020.
76. Su X, Lee L, Li X, Lv J, Hu Y, Zhan S, Cao W, Mei L, Tang Y-M, Wang D, Krauss RM, Taylor KD, Rotter JI, Yang H. Association Between Angiotensinogen, Angiotensin II Receptor Genes, and Blood Pressure Response to an Angiotensin-Converting Enzyme Inhibitor. Circulation. 2007. p. 725–732.
77. Tang C-M, Zhang M, Huang L, Hu Z-Q, Zhu J-N, Xiao Z, Zhang Z, Lin Q-X, Zheng X-L, -Yang M, Wu S-L, Cheng J-D, Shan Z-X. CircRNA_000203 enhances the expression of fibrosis-associated genes by derepressing targets of miR-26b-5p, Col1a2 and CTGF, in cardiac fibroblasts. Scientific Reports. 2017.
78. Chen X, Zhou L, Peng N, Yu H, Li M, Cao Z, Lin Y, Wang X, Li Q, Wang J, She Y, Zhu C, Lu M, Zhu Y, Liu S. MicroRNA-302a suppresses influenza A virus–stimulated interferon regulatory factor-5 expression and cytokine storm induction. Journal of Biological Chemistry. 2017. p. 21291–21303.
79. Hamada-Tsutsumi S, Naito Y, Sato S, Takaoka A, Kawashima K, Isogawa M, Ochiya T, Tanaka Y. The antiviral effects of human microRNA miR-302c-3p against hepatitis B virus infection. Alimentary Pharmacology & Therapeutics. 2019. p. 1060–1070.
80. Peng N, Yang X, Zhu C, Zhou L, Yu H, Li M, Lin Y, Wang X, Li Q, She Y, Wang J, Zhao Q, Lu M, Zhu Y, Liu S. MicroRNA-302 Cluster Downregulates Enterovirus 71–Induced Innate Immune Response by Targeting KPNA2. The Journal of Immunology. 2018. p. 145–156.
81. Li G, Song Y, Li Y-D, Jie L-J, Wu W-Y, Li J-Z, Zhang Q, Wang Y. Circulating miRNA-302 family members as potential biomarkers for the diagnosis of acute heart failure. Biomarkers in Medicine. 2018. p. 871–880.
82. Braga L, Ali H, Secco I, Giacca M. Non-coding RNA therapeutics for cardiac regeneration. *Cardiovasc Res* 2020;
83. Wang J, Huang R, Xu Q, Zheng G, Qiu G, Ge M, Shu Q, Xu J. Mesenchymal Stem Cell–Derived Extracellular Vesicles Alleviate Acute Lung Injury Via Transfer of miR-27a-3p. Critical Care Medicine. 2020. p. 1.
84. Zhao X-R, Zhang Z, Gao M, Li L, Sun P-Y, Xu L-N, Qi Y, Yin L-H, Peng J-Y. MicroRNA-27a-3p aggravates renal ischemia/reperfusion injury by promoting oxidative stress via targeting growth factor receptor-bound protein 2. *Pharmacol Res* 2020;**155**:104718.
85. Lv X, Yan J, Jiang J, Zhou X, Lu Y, Jiang H. MicroRNA-27a-3p suppression of peroxisome proliferator-activated receptor-γ contributes to cognitive impairments resulting from sevoflurane treatment. Journal of Neurochemistry. 2017. p. 306–319.
86. Vegter EL, Ovchinnikova ES, Veldhuisen DJ van, Jaarsma T, Berezikov E, Meer P van der, Voors AA. Low circulating microRNA levels in heart failure patients are associated with atherosclerotic disease and cardiovascular-related rehospitalizations. Clinical Research in Cardiology. 2017. p. 598–609.
87. Ovchinnikova ES, Schmitter D, Vegter EL, Maaten JM ter, Valente MAE, Liu LCY, Harst P van der, Pinto YM, Boer RA de, Meyer S, Teerlink JR, O'Connor CM, Metra M, Davison BA, Bloomfield DM, Cotter G, Cleland JG, Mebazaa A, Laribi S, Givertz MM, Ponikowski P, Meer P van der, Veldhuisen DJ van, Voors AA, Berezikov E. Signature of circulating microRNAs in patients with acute heart failure. European Journal of Heart Failure. 2016. p. 414–423.