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In Silico Approach to Identify the Relationships between COVID-19 and Coronary Artery Disease/Rheumatoid Arthritis

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HIGHLIGHTS

- Common hub genes, miRNAs, TFs and shared mechanisms were identified in mild and severe COVID-19-CAD patients and mild and severe COVID-19-RA patients.
- Mild and severe forms of COVID-19 differ in potential biomarkers, mechanisms, miRNAs and TFs in CAD and RA patients.
- First study investigating the potential shared mechanisms, biomarkers, TFs and miRNAs between COVID-19 and CAD patients and COVID-19 and RA patients.

Abstract: Global public has been threatened by the coronavirus disease 2019 (COVID-19) pandemic which led to nearly 15 million deaths around the world. People with complex and chronic diseases usually have more severe COVID-19 symptoms than the general population. Mounting evidence indicates individuals with coronary artery disease (CAD) and rheumatoid arthritis (RA) have worse COVID-19 outcomes yet the underlying mechanism still needs to be explored. The aim of our study is to reveal *in silico* evidence for the molecular mechanisms shared by COVID-19, CAD and RA pathogenesis which may aggravate the COVID-19 disease severity. Public datasets (GSE164805 and GSE23561) were downloaded from the Gene Expression Omnibus (GEO) database and analyzed for differential expression analysis (DEG). Identified differential expressed genes (DEGs) were further analyzed to find common DEGs, common pathways, hub genes, transcription factors (TFs) and microRNAs (miRNAs). Our study identified common hub genes, miRNAs, TFs and shared mechanisms in both mild and severe COVID-19-CAD patients and mild and severe COVID-19-RA patients. We also uncovered that mild and severe forms of COVID-19 differ in potential biomarkers, mechanisms, miRNAs and TFs in both CAD and RA patients. Our study is the first study investigating the potential shared mechanisms, biomarkers, TFs and miRNAs between COVID-19 and CAD patients and COVID-19 and RA patients. Our results could shed on light to the patient management strategies with CAD with COVID-19 and patients with RA with COVID-19 based on the severity of the COVID-19 disease.

Keywords: COVID-19; coronary artery disease; rheumatoid arthritis; differentially expressed genes; bioinformatics; transcription factors; microRNAs.

INTRODUCTION

The coronavirus (COVID-19) disease is caused by severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) accounting for nearly 15 million deaths around the world between 2019-2022 [1]. It is still a global public health problem as the novel variants of the virus evolve and different patterns of the symptoms have been caused by novel forms of the disease. The SARS-Cov-2 virus targets type 2 alveolar cells, esophagus, ileum epithelial cells, and myocardium resulting in respiratory system problems such as pneumonia or acute respiratory distress syndrome (ARDS), and other complex diseases of the nervous system and cardiovascular system. Although many people with COVID-19 show mild symptoms, people with complex and chronic diseases usually have more severe COVID-19 symptoms than the general population [2]. Mounting evidence indicates individuals with coronary artery disease (CAD) and rheumatoid arthritis (RA) have worse COVID-19 outcomes yet the mechanism underlying behind the aforementioned association still needs to be explored [3, 4, 5]. Thus, it is important to discover the shared pathological mechanisms between these diseases and COVID-19.

Coronary artery disease (CAD) is a condition in which the heart muscle cannot perform its function and tissue damage occurs due to occlusion of the coronary arteries for various reasons. CAD is still one of the leading reasons of death all over the world. Recently, a recent meta-analysis showed that cardiovascular diseases (CVD) impact the death rates and the severity of COVID-19 patients suggesting underlying cardiovascular problems can worsen COVID-19 outcomes [6]. However, COVID-19 patients manifest several cardiovascular complications associated with common molecular mechanisms involved in both diseases such as alterations of Angiotensin-converting enzyme 2 (ACE2) signaling pathways and systemic inflammation [7,8,9]. These findings suggest that COVID-19 and the cardiovascular system have a bidirectional association, yet the mechanisms underlying molecular mechanisms warrant further investigation.

Rheumatoid arthritis (RA) is a chronic inflammatory disorder occurring when the patient's immune system attacks the tissue called "synovium". RA can have devastating effects on several organs, such as the heart, skin, eyes and blood vessels [10]. Patients with RA are usually prone to getting certain infections, so the risk of having COVID-19 infection is higher in RA patients than in non-RA patients [11]. However, there is a lack of evidence for understanding the possible common mechanisms and biomarkers contributing to the pathogenesis of both diseases. Thus, the detection of common biological processes and molecular markers is crucial for discovering new therapeutic options for CAD patients and RA patients with COVID-19 disease. The acquired knowledge can also be utilized for translational and clinical applications in the future coronavirus-originated viral infections.

In our study, we conducted a bioinformatics study to determine potential mechanisms and key biomarkers, TFs and miRNAs shared between COVID-19, CAD and RA by using an integrative bioinformatics approach. We also aimed to investigate if the severity of the COVID-19 will result in any differences in shared potential mechanisms and biomarkers between COVID-19-CAD patients and COVID-19-RA patients. Discovering the potential mechanisms and biomarkers can facilitate to develop novel personalized treatment options for patients with CAD and RA.

MATERIAL AND METHODS

Datasets

The public microarray datasets used in the study were obtained from the Gene Expression Omnibus (GEO, http://www.ncbi.nlm.nih.gov/geo) database [12]. GSE164805 dataset includes 10 COVID-19 patients (5 severe and 5 mild) and 5 controls. GSE23561 dataset includes 6 CAD patients, 6 RA patients, and 9 controls (Table 1). The datasets included in the study were evaluated based on specific criteria. The criteria used to select the datasets were: (i) blood samples were studied; (ii) case-control study; (iii) no drug use and studies that did not meet the criteria were excluded. As a result, two data sets were included in the study and used in the subsequent analyses. The workflow of the study is shown in Figure 1.

Table 1. Dataset information used in the study

GEO Accession	Sample type	Sample groups	Experiment Type	Microarray Platform
GSE164805	PBMCs	5 severe, 5 mild COVID-19 patients - 5 Control	"Expression profiling by array"	"Arraystar human lncRNA V5 microarray"
GSE23561	Peripheral blood	6 CAD - 9 Control	"Expression profiling by array"	"Human 50K Exonic Evidence-based oligonucleotide array"
GSE23561	Peripheral blood	6 RA - 9 Control	"Expression profiling by array"	"Human 50K Exonic Evidence-based oligonucleotide array"

PBMCs: peripheral blood mononuclear cells; CAD: coronary artery disease; RA: rheumatoid arthritis.

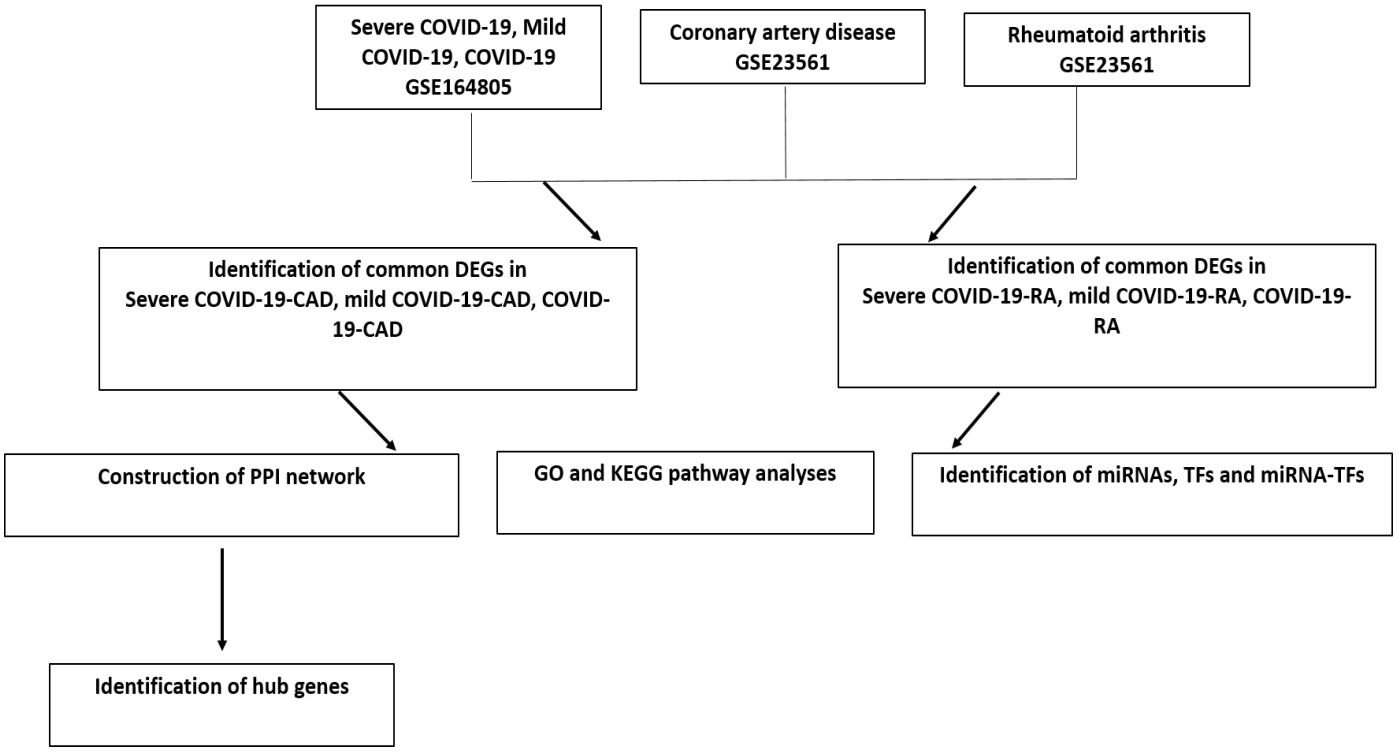

Figure 1. Workflow of the study

Identification of DEGs and common DEGs among COVID-19 and CAD and COVID-19 and RA

DEGs were analyzed by the GEO2R platform [13]. DEGs were identified in GSE164805 dataset in three groups: Severe COVID-19 patients and controls, mild COVID-19 patients, and all COVID-19 (mild and severe) patients and controls. DEGs were also identified in the GSE23561 dataset in two groups: CAD patients and controls, RA patients and controls. The criteria used for identifying DEGs is p-value < 0.01, log2FC ≥ 1 (up-regulated DEGs) or p-value < 0.01 and log2FC ≤ -1 (down-regulated DEGs). Common DEGs for severe COVID-19-CAD, mild COVID-19-CAD, COVID-19-CAD, severe COVID-19-RA, mild COVID-19-RA, COVID-19-RA were shown by the Venn diagrams.

Protein-protein interaction (PPI) network analysis and identification of top 10 hub genes

Common DEGs for each group were transferred to the Search Tool for the Interacting Genes (STRING) database (https:// stringdb.org/) to analyze the protein-protein interactions (PPI) [14]. > 0.7 PPI combined score was selected to create the PPI network. Then, the Cytohubba plugin in the Cytoscape (v3.9.1) software was used to visualize and identify the hub genes filtered by Degree term [15]. The "hub genes" identified as common for COVID-19 and CAD and COVID-19 and RA were identified as the top 10 genes with the highest value according to the Degree term in topological analysis.

Functional enrichment analysis

The Database for Annotation, Visualization and Integrated Discovery (DAVID, (https://david.ncifcrf.gov/) database was used for Gene Ontology (GO) function analysis ("biological processes (BP)", "molecular functions (MF)" and "cellular components (CC)") and Kyoto Encylopedia of Genes and Genomes (KEGG) pathway analysis [16]. The GO database is a bioinformatics tool created to serve as a resource for biomedical research by classifying genes and their products according to functional information obtained from experimental studies in a wide variety of organisms. GO ontology terms enable the identification of genes and gene products in three key biological domains [Molecular function (GO-MF), Biological process (GO-BP) and Cellular component (GO-CC) shared by all organisms. GO enrichment analysis with GO ontology terms is widely used to investigate the functions of genes and proteins in molecular processes. KEGG pathway analysis allows us to investigate the function of genes and their interactions at the molecular level and categorize them in distinct cellular pathways. Functional and pathway analysis were performed for each group separately.

Identification of TFs and miRNAs

Correlations between hub genes and miRNAs, hub genes and TFs and TF-miRNA were predicted. TFs-hub gene interaction was created by TRRUST v.2 database [17] and miRNA-hub gene interaction was created by the microRNA Data Integration Portal (mirDIP) v4.1 database [18] in each group. TRUSST v2 is a useful tool to discover the significant TFs in human diseases. MirDIP v4.1 database is a useful database including millions of human miRNA-hub gene predictions from the 30 different sources. Finally, TF-miRNA interactions and functional enrichment analyses of the miRNAs by their regulating TF were analyzed by the TransmiR v2.0 database [19]. The TransmiR database is utilized to find potential regulatory relations between TFs and miRNAs by collecting data from the literature, ChIP-seq derived and the predicted TF-miRNA regulations.

RESULTS

Identification of DEGs

DEGs were analyzed by the GEO2R platform. DEGs were identified in GSE164805 dataset in three groups: Severe COVID-19 patients and controls, mild COVID-19 patients and all COVID-19(mild and severe) patients and controls. DEGs were also identified in the GSE23561 dataset in two groups: CAD patients and controls, RA patients and controls. The criteria used for identifying DEGs is p-value < 0.01, log2FC≥1 (up-regulated DEGs) or p-value<0.01 and log2FC ≤ -1 (down-regulated DEGs). Common DEGs for severe COVID-19 and CAD, mild COVID-19 and CAD, COVID-19 and CAD, severe COVID-19 and RA, mild COVID-19 and RA, COVID-19 and RA were determined by the Venny diagram tool (Figure 2).

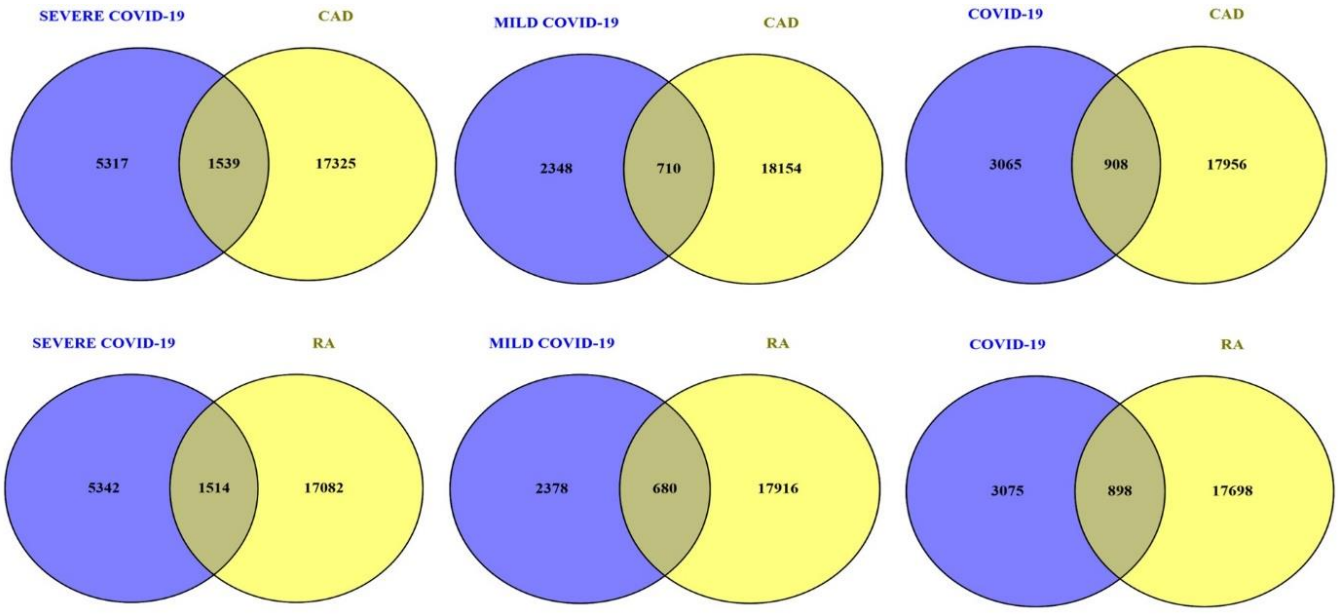

Figure 2. Venn diagrams showing the DEGs (adj p < 0.01, log2FC< -1, log2FC> 1) identified in the datasets.

PPI Network analysis and top 10 hub gene detection

Protein-Protein Interaction (PPI) analysis of common DEGs was performed in the STRING database and a statistically significant PPI network was established for each dataset. The PPI enrichment values for the datasets are given below: Severe COVID-19-CAD (*p*=1.56e-07), Mild COVID-19-CAD (*p*=0.01), COVID-10-CAD (*p*=5.46e-05), Severe COVID-19-RA (*p*=1.59e-05), Mild COVID-19-RA (*p*=0.018), COVID-19-RA (*p*=0.00426)

The top 10 hub genes for severe COVID-19-CAD are *ACTB, UBC, PRKACB, ESR1, CD44, MMP9, IL4, IFNG, CCNB1, RAC1*, for mild COVID-19-CAD are *ACTB, IL1B, CCL2, IGF1, IL1A, CXCL1, RPL11, ASPM, CCNB1, CSF2* and for COVID-19-CAD are *ACTB, IL1B, MMP9, IGF1, SCCNB1, RAC1, SNCA, CSF2, PLCG1, IL1A. ACTB* and *CCNB1* are the common hub genes between severe COVID-19-CAD and mild COVID-19-CAD.

The top 10 hub genes for hub genes for severe COVID-19-RA are *ACTB, UBC, ESR1, RHOA, NOTCH1, PRKACB, CD44, CTLA4, MMP9, DLG4*, for mild COVID-19-RA are *ACTB, IL1B, CCL2, IGF1, IL1A, RPL11, IL13, CCNB1, CXCL1, RPS2* and for COVID-19-RA *are ACTB, IL1B, RHOA, NOTCH1, MMP9, DLG4, IGF1, RAC1, CCT3, IL13. ACTB* is the only common hub gene between mild COVID-19-RA and severe COVID-19-RA.

GO and KEGG Pathway Enrichment Analysis

The top 5 GO terms and KEGG pathway terms for severe COVID-19 and CAD, mild COVID-19 and CAD and COVID-19 and CAD are shown in Table 2. “Potassium ion transmembrane transport”, “regulation of presynapse assembly” and “G-protein coupled receptor signaling pathway” are the top significant GO terms in BP category for severe COVID-19 and CAD. “Plasma membrane”, “Extracellular region” and “Integral component of plasma membrane” are the most significant GO terms in CC category for severe COVID-19 and CAD. “Heparin binding”, “Interleukin-1 receptor activity” and “Extracellular matrix structural constituent” are the most significant GO terms in MF category for severe COVID-19 and CAD. “Amoebiasis”, “Insulin secretion” and “Primary immunodeficiency” are the most significant KEGG pathways for severe COVID-19 and CAD (Table 2). “Response to drug”, “monocyte chemotaxis” and “neutrophil chemotaxis” are the top significant GO terms in BP category for mild COVID-19 and CAD. “Extracellular region”, “Cytosol” and “Cytosolic large ribosomal unit” are the top significant GO terms in CC category for mild COVID-19 and CAD. “Chemokine activity”, “ATP binding” and “Protein serine/threonine/tyrosine kinase activity” are the top significant GO terms in MF category. The “Rheumatoid arthritis”, “Cytokine-cytokine receptor interaction” and “Ameobiasis” are the most significant KEGG pathways for mild COVID-19 and CAD (Table 2). “Response to drug”, “Neuron migration” and “Signal transduction” are the top significant GO terms in BP category for COVID-19 and CAD. “Plasma membrane”, “Extracellular region” and “Glutamatergic synapse” are the top significant GO terms in CC category for COVID-19 and CAD. “Heparin binding”, “Identical protein binding” and “Protein binding” are the top significant GO terms in MF category for COVID-19 and CAD. “Regulation of actin cytoskeleton”, “Insulin secretion” and “Serotonergic synapse” are the most significant KEGG pathways for severe COVID-19 and CAD (Table 2).

Table 2. The top 5 GO terms (BP, MF, CC) and KEGG pathways for Severe COVID-19 and CAD, Mild COVID-19 and CAD, all COVID-19 and CAD

	Category	Term	Count	p-value
Severe COVID-19 and CAD	**GOTERM_BP**	Potassium ion transmembrane transport	25	1,8E-5
	GOTERM_BP	Regulation of presynapse assembly	11	7,5E-5
	GOTERM_BP	G-protein coupled receptor signaling pathway	118	1,6E-4
	GOTERM_BP	Bicarbonate transport	12	2,2E-4
	GOTERM_BP	Outflow tract morphogenesis	12	5,0E-4
	GOTERM_CC	Plasma membrane	462	3,4E-11
	GOTERM_CC	Extracellular region	207	1,8E-6
	GOTERM_CC	Integral component of plasma membrane	150	3,0E-6
	GOTERM_CC	Neuron projection	45	3,1E-4
	GOTERM_CC	Glutamatergic synapse	45	4,9E-4
	GOTERM_MF	Heparin binding	27	4,6E-4
	GOTERM_MF	Interleukin-1 receptor activity	5	9,1E-4
	GOTERM_MF	Extracellular matrix structural constituent	22	1,4E-3
	GOTERM_MF	Growth factor activity	24	3,0E-3

Cont. Table 2

Severe COVID-19 and CAD	**GOTERM_MF**	Heme binding	22	4,3E-3
	KEGG PATHWAY	Amoebiasis	21	3,7E-4
	KEGG PATHWAY	Insulin secretion	17	2,5E-3
	KEGG PATHWAY	Primary immunodeficiency	10	4,1E-3
	KEGG PATHWAY	Dilated cardiomyopathy	17	7,5E-3
	KEGG PATHWAY	Cocaine addiction	11	7,9E-3
Mild COVID-19 and CAD	**GOTERM_BP**	Response to drug	22	5,6E-4
	GOTERM_BP	Monocyte chemotaxis	8	6,3E-4
	GOTERM_BP	Neutrophil chemotaxis	10	1,4E-3
	GOTERM_BP	Eosinophil chemotaxis	5	2,0E-3
	GOTERM_BP	Chemokine-mediated signaling pathway	9	2,1E-3
	GOTERM_CC	Extracellular region	95	1,7E-3
	GOTERM_CC	Cytosol	211	3,1E-3
	GOTERM_CC	Cytosolic large ribosomal subunit	8	3,2E-3
	GOTERM_CC	Cytosolic ribosome	9	3,8E-3
	GOTERM_CC	Cytoplasm	208	4,5E-3
	GOTERM_MF	Chemokine activity	9	3,1E-4
	GOTERM_MF	ATP binding	76	2,0E-3
	GOTERM_MF	Protein serine/threonine/tyrosine kinase activity	28	2,5E-3
	GOTERM_MF	Cytokine activity	15	6,9E-3
	GOTERM_MF	Protein kinase activity	23	1,3E-2
	KEGG PATHWAY	Rheumatoid arthritis	15	1,6E-5
	KEGG PATHWAY	Cytokine-cytokine receptor interaction	25	6,7E-4
	KEGG PATHWAY	Amoebiasis	13	7,0E-4
	KEGG PATHWAY	Coronavirus disease-COVID-19	20	2,2E-3
	KEGG PATHWAY	Chemokine signaling pathway	17	4,1E-3
COVID-19 and CAD	**GOTERM_BP**	Response to drug	27	2,0E-4
	GOTERM_BP	Neuron migration	15	2,6E-4
	GOTERM_BP	Signal transduction	76	4,2E-4
	GOTERM_BP	Inflammatory response	32	9,8E-4
	GOTERM_BP	Embriyonic camera-type eye development	5	1,2E-3
	GOTERM_CC	Plasma membrane	260	4,0E-5
	GOTERM_CC	Extracellular region	124	1,3E-4
	GOTERM_CC	Glutamatergic synapse	30	8,9E-4
	GOTERM_CC	Integral component of plasma membrane	85	1,7E-3
	GOTERM_CC	Cytosol	265	2,7E-3
	GOTERM_MF	Heparin binding	17	4,4E-3
	GOTERM_MF	Identical protein binding	99	5,0E-3
	GOTERM_MF	Protein binding	592	6,3E-3
	GOTERM_MF	Growth factor activity	15	1,7E-2
	GOTERM_MF	GTPase activator activity	22	1,7E-2
	KEGG PATHWAY	Regulation of actin cytoskeleton	24	4,9E-4
	KEGG PATHWAY	Insulin secretion	12	3,4E-1
	KEGG PATHWAY	Seratonergic synapse	14	4,5E-3
	KEGG PATHWAY	Amoebiasis	13	4,5E-3
	KEGG PATHWAY	Retrograde endocannabinoid signaling	16	6,6E-3

The top 5 GO terms and KEGG pathway terms for severe COVID-19 and RA, mild COVID-19 and RA and COVID-19 and RA are given in Table 3. “Viral transfection”, Neuron migration” and “Potassium ion transmembrane transport” are the top significant GO terms in BP category for severe COVID-19 and RA. “Plasma membrane”, “integral component of plasma membrane” and “extracellular region” are the top significant GO terms in CC category for severe COVID-19 and RA. “Protein binding”, “identical protein binding” and NAD(P)+ nucleosidase activity” are the top significant GO terms in MF category. “cGMP-PKG signaling pathway”, “Vascular smooth muscle contraction” and “Proteoglycans in cancer” are the most significant KEGG pathways for severe COVID-19 and RA (Table 3). “Immune response”, “Enamel mineralization” and “Response to drug” are the top significant GO terms in BP category for mild COVID-19 and RA. “Extracellular region”, “Cytosolic ribosome” and “Cytoplasm” are the top significant GO terms in CC category for mild COVID-19 and RA. “Chemokine activity”, “Protein serine/threonine/tyrosine kinase activity”

and “Cytokine activity” are the top significant GO terms in MF category for mild COVID-19 and RA. “Rheumatoid arthritis”, “Coronavirus disease-COVID-19”, “Cytokine-cytokine receptor interaction” are the most significant KEGG pathways for mild COVID-19 and RA (Table 3). “Neuron migration”, “Response to drug” and “Inflammatory response” are the top significant GO terms in BP category for COVID-19 and RA. “Plasma membrane”, “Extracellular region” and “Cytosol” are the top significant GO terms in CC category for COVID-19 and RA. “Identical protein binding”, “Protein binding” and “D1 dopamine receptor binding” are the top significant GO terms in MF category for COVID-19 and RA. “cGMP-PKG signaling pathway”, “Vascular smooth muscle contraction” and “Wnt signaling pathway” are the most significant KEGG pathways for COVID-19 and RA (Table 3).

Table 3. The top 5 GO terms (BP, MF, CC) and KEGG pathways for Severe COVID-19 and RA, Mild COVID-19 and RA, all COVID-19 and RA

	Category	Term	Count	p-value
Severe COVID-19 and RA	**GOTERM_BP**	Viral transcription	21	1,1E-4
	GOTERM_BP	Neuron migration	21	1,2E-4
	GOTERM_BP	Potassium ion transmembrane transport	23	1,3E-4
	GOTERM_BP	Actin filament organization	23	3,2E-4
	GOTERM_BP	Peptide antigen assembly with MHC class II protein complex	7	5,7E-4
	GOTERM_CC	Plasma membrane	452	4,6E-10
	GOTERM_CC	Integral component of plasma membrane	145	1,7E-5
	GOTERM_CC	Extracellular region	195	8,3E-5
	GOTERM_CC	Cell projection	27	3,2E-4
	GOTERM_CC	Focal adhesion	49	8,3E-4
	GOTERM_MF	Protein binding	1001	2,2E-5
	GOTERM_MF	Identical protein binding	164	4,0E-4
	GOTERM_MF	NAD(P)+ nucleosidase activity	7	7,0E-4
	GOTERM_MF	NAD+ nucleotidase, cylic ADP-ribose generating	7	7,0E-4
	GOTERM_MF	Interleukin-1 receptor activity	5	8,9E-4
	KEGG PATHWAY	cGMP-PKG signaling pathway	28	1,0E-3
	KEGG PATHWAY	Vascular smooth muscle contraction	23	2,4E-3
	KEGG PATHWAY	Proteoglycans in cancer	31	2,8E-3
	KEGG PATHWAY	Primary immunodeficiency	10	4,2E-3
	KEGG PATHWAY	Cell adhesion molecules	24	4,2E-3
Mild COVID-19 and RA	**GOTERM_BP**	Immune response	30	6,5E-4
	GOTERM_BP	Enamel mineralization	5	7,7E-4
	GOTERM_BP	Response to drug	21	8,1E-4
	GOTERM_BP	Nuclear-transcribed mRNA catabolic process, nonsense-mediated decay	12	1,5E-3
	GOTERM_BP	SRP-dependent cotranslational protein targeting to membrane	10	2,1E-3
	GOTERM_CC	Extracellular region	94	5,4E-4
	GOTERM_CC	Cytosolic ribosome	10	6,8E-4
	GOTERM_CC	Cytoplasm	203	1,6E-3
	GOTERM_CC	Cell surface	33	4,3E-3
	GOTERM_CC	Cytosol	198	8,4E-3
	GOTERM_MF	Chemokine activity	8	1,2E-3
	GOTERM_MF	Protein serine/threonine/tyrosine kinase activity	25	9,5E-3
	GOTERM_MF	Cytokine activity	14	1,1E-2
	GOTERM_MF	Ubiquitin protein ligase activity	20	1,1E-2
	GOTERM_MF	Interleukin-1 receptor activity	3	2,1E-2
	KEGG PATHWAY	Rheumatoid arthritis	14	4,6E-5
	KEGG PATHWAY	Coronavirus disease-COVID-19	21	5,3E-4
	KEGG PATHWAY	Cytokine-cytokine receptor interaction	23	2,0E-3
	KEGG PATHWAY	Amoebiasis	11	5,3E-3
	KEGG PATHWAY	Type I diabetes mellitus	7	5,4E-3
	GOTERM_BP	Response to drug	26	4,6E-4

Cont. Table 3

COVID-19 and RA	**GOTERM_BP**	Inflammatory response	32	9,1E-4
	GOTERM_BP	Signal transduction	74	9,8E-4
	GOTERM_BP	Embryonic camera-type eye development	5	1,2E-3
	GOTERM_CC	Plasma membrane	263	6,9E-6
	GOTERM_CC	Extracellular region	125	5,7E-5
	GOTERM_CC	Cytosol	268	7,0E-4
	GOTERM_CC	Cytosolic ribosome	11	2,3E-3
	GOTERM_CC	Glutamatergic synapse	27	6,0E-3
	GOTERM_MF	Identical protein binding	104	6,4E-4
	GOTERM_MF	Protein binding	591	3,1E-3
	GOTERM_MF	D1 dopamine receptor binding	4	8,2E-3
	GOTERM_MF	MHC class II receptor activity	4	1,8E-2
	GOTERM_MF	Cytokine activity	16	2,2E-2
	KEGG PATHWAY	cGMP-PKG signaling pathway	19	1,7E-3
	KEGG PATHWAY	Vascular smooth muscle contraction	16	2,8E-3
	KEGG PATHWAY	Wnt signaling pathway	18	4,1E-3
	KEGG PATHWAY	Lipid and atherosclerosis	21	5,4E-3
	KEGG PATHWAY	Regulation of actin cytoskeleton	21	6,3E-3

Identification of transcription factors and miRNAs regulating the common DEGs

We identified several TFs that may regulate the common DEGs that were listed in the Table 4. The top TF for severe COVID-19-CAD is GATA3, RELA is for mild COVID-19-CAD, JUN is for all COVID-19-CAD groups. RELA, NFKB1, SP1, BRCA1, STAT1, JUN are identified as common TFs between severe COVID-19-CAD and mild COVID-19-CAD (Table 4).

Table 4. Transcription factors of the hub genes in COVID-19 and CAD

	Transcription factor	**p-value**	**Q-value**	**List of Overlapped genes**
Severe COVID-19 and CAD	GATA3	8.95e-07	2.33e-05	*ESR1,IFNG,IL4*
	IRF1	2.2e-06	2.86e-05	*IFNG,MMP9,CCNB1*
	IKBKB	3.79e-06	3.28e-05	*CD44,MMP9*
	HDAC1	6.01e-06	3.67e-05	*ESR1,MMP9,CD44*
	STAT5B	7.07e-06	3.67e-05	*IFNG,ESR1*
	RELA	1.23e-05	4.71e-05	*MMP9,IL4,IFNG,CCNB1*
	NFKB1	1.27e-05	4.71e-05	*IFNG,CCNB1,MMP9,IL4*
	TBX21	1.66e-05	5.11e-05	*IL4,IFNG*
	KLF5	1.97e-05	5.11e-05	*CCNB1,MMP9*
	STAT5A	1.97e-05	5.11e-05	*IFNG,ESR1*
	SNAI2	2.29e-05	5.42e-05	*CD44,MMP9*
	PTTG1	2.64e-05	5.73e-05	*RAC1,CCNB1*
	MTA1	6.93e-05	0.000134	*ESR1,MMP9*
	SP1	7.2e-05	0.000134	*CD44,UBC,MMP9,ESR1*
	CIITA	0.000117	0.000202	*MMP9,IL4*
	TWIST1	0.000149	0.000242	*ESR1,CD44*
	MYCN	0.000247	0.000378	*CD44,IFNG*
	EP300	0.000383	0.000544	*IFNG,MMP9*
	BRCA1	0.000397	0.000544	*ESR1,CCNB1*
	USF1	0.000517	0.000671	*CCNB1,IFNG*
	TFAP2A	0.000616	0.000763	*MMP9,CCNB1*
	STAT1	0.000861	0.00102	*MMP9,IFNG*
	YY1	0.00101	0.00114	*IFNG,IL4*
	STAT3	0.00243	0.00264	*MMP9,IFNG*
	JUN	0.00267	0.00278	*MMP9,IFNG*
	TP53	0.00323	0.00323	*CCNB1,ESR1*

Cont. Table 4

Mild COVID-19 and CAD	RELA	3.11e-09	2.11e-08	*IL1B,CCL2,CSF2,IL1A,CCNB1,CXCL1*
	NFKB1	3.24e-09	2.11e-08	*IL1B,CCNB1,IL1A,CSF2,CXCL1,CCL2*
	BRCA1	1.55e-08	6.71e-08	*CXCL1,ASPM,CCNB1,IGF1*
	JUN	7.55e-07	2.45e-06	*IL1B,CCL2,CSF2,IL1A*
	HMGA1	3.42e-05	8.9e-05	*CXCL1,IL1B*
	REL	5.81e-05	0.000126	*CCL2,IL1B*
	HDAC2	8.81e-05	0.000164	*CCL2,IGF1*
	KLF4	0.000195	0.000317	*IL1B,CCNB1*
	CEBPA	0.000318	0.000459	*IGF1,CCL2*
	SPI1	0.00047	0.000611	*IL1B,CCL2*
	STAT1	0.000861	0.00102	*CCL2,IL1B*
	E2F1	0.00217	0.00235	*IL1B,CCNB1*
	SP1	0.0246	0.0246	*CCL2,CXCL1*
COVID-19 and CAD	JUN	4.56e-07	4.56e-06	*IL1B,MMP9,CSF2,IL1A*
	RELA	7.5e-06	2.57e-05	*MMP9,IL1B,CSF2,IL1A*
	NFKB1	7.7e-06	2.57e-05	*IL1B,IL1A,MMP9,CSF2*
	NFKBIA	3.44e-05	8.61e-05	*IL1B,MMP9*
	ETS2	9.96e-05	0.000199	*CSF2,MMP9*
	SIRT1	0.000226	0.000376	*IL1B,MMP9*
	EP300	0.000307	0.000398	*IGF1,MMP9*
	FOS	0.000318	0.000398	*MMP9,IL1A*
	ETS1	0.000611	0.000679	*CSF2,MMP9*
	STAT1	0.000691	0.000691	*MMP9,IL1B*

Moreover, 10 TFs for severe COVID-19-RA, 15TFs for mild COVID-19-RA, and 12 TFs for all COVID-19-RA were identified that may regulate the common DEGs for COVID-19 and RA (Table 5). 7 common TFs (IKBKB, HDAC1, KLF5, SNAI2, MTA1, SP1, and TWIST1) were identified between severe COVID-19-CAD and severe COVID-19-RA. 13 common TFs (RELA, NFKB1, BRCA1, JUN, HMGA1, REL, HDAC2, KLF4, CEBPA, SPI1, STAT1, E2F1 and SP1) were identified between mild COVID-19-CAD and mild COVID-19-RA. 7 common TFs (JUN, RELA, NFKB1, NFKBIA, SIRT1, EP300 and STAT1) were identified between COVID-19-CAD and COVID-19-RA**.**

Table 5. Transcription factors of the hub genes in COVID-19 and RA

	Transcription factor	**p-value**	**Q-value**	**List of Overlapped genes**
Severe COVID-19 and RA	LMO4	2.53e-06	1.89e-05	*ESR1,DLG4*
	IKBKB	3.79e-06	1.89e-05	*CD44,MMP9*
	HDAC1	6.01e-06	2.00e-05	*ESR1,MMP9,CD44*
	KLF5	1.97e-05	4.59e-05	*NOTCH1,MMP9*
	SNAI2	2.29e-05	4.59e-05	*CD44,MMP9*
	MTA1	6.93e-05	0.000103	*ESR1,MMP9*
	SP1	7.2e-05	0.000103	*CD44,UBC,MMP9,ESR1*
	LEF1	0.000102	0.000127	*ESR1,CTLA4*
	TWIST1	0.000149	0.000166	*ESR1,CD44*
	SIRT1	0.000281	0.000281	*NOTCH1,MMP9*
Mild COVID-19 and RA	RELA	2.35e-07	1.83e-06	*IL1B,CCL2,IL1A,CCNB1,CXCL1*
	NFKB1	2.43e-07	1.83e-06	*IL1B,CCNB1,IL1A,CXCL1,CCL2*
	BRCA1	3.09e-06	1.54e-05	*CXCL1,CCNB1,IGF1*
	HMGA1	3.42e-05	0.000124	*CXCL1,IL1B*
	JUN	5.56e-05	0.000124	*IL1B,CCL2,IL1A*
	AHR	5.81e-05	0.000124	*IL13,IL1B*
	REL	5.81e-05	0.000124	*CCL2,IL1B*
	HDAC2	8.81e-05	0.000165	*CCL2,IGF1*
	KLF4	0.000195	0.000325	*IL1B,CCNB1*
	CEBPA	0.000318	0.000477	*IGF1,CCL2*
	CEBPB	0.00044	0.000587	*IL1B,IL13*
	SPI1	0.00047	0.000587	*IL1B,CCL2*
	STAT1	0.000861	0.000994	*CCL2,IL1B*
	E2F1	0.00217	0.00233	*IL1B,CCNB1*
	SP1	0.0246	0.0246	*CCL2,CXCL1*

Cont. Table 5

COVID-19 and RA	SIRT1	1.83e-06	2.2e-05	*IL1B,NOTCH1,MMP9*
	KLF5	1.97e-05	0.000106	*NOTCH1,MMP9*
	PTTG1	2.64e-05	0.000106	*RAC1,RHOA*
	NFKBIA	4.3e-05	0.000129	*IL1B,MMP9*
	AHR	5.81e-05	0.000139	*IL13,IL1B*
	STAT6	0.000158	0.000315	*RHOA,IL13*
	EP300	0.000383	0.000657	*IGF1,MMP9*
	CEBPB	0.00044	0.00066	*IL1B,IL13*
	STAT1	0.000861	0.00115	*MMP9,IL1B*
	JUN	0.00267	0.00321	*IL1B,MMP9*
	RELA	0.0105	0.0106	*MMP9,IL1B*
	NFKB1	0.0106	0.0106	*IL1B,MMP9*

We also investigate the miRNA regulators of the identified hub genes of the common DEGs. Key miRNA regulators targeting the hub genes were listed in the Table 6 and Table 7 for COVID-19-CAD and COVID-19-RA, respectively.

Table 6. Shared miRNAs targeting the hub genes for COVID-19 and CAD

	miRNAs	**Target genes**
Severe COVID-19 and CAD	"hsa-miR-3622a-3p", "hsa-miR-3622b-3p"	*PRKACB, CD44, IFNG, ESR1*
	"hsa-miR-4715-5p"	*RAC1, IFNG, ESR1, PRKACB*
	"hsa-miR-548n"	*CCNB1, ACTB, PRKACB, ESR1*
	"hsa-let-7a-2-3p"	*CCNB1, RAC1, ACTB*
	"hsa-miR-130a-3p", "hsa-miR-130b-3p", "hsa-miR-20b-5p", "hsa-miR-454-3p", "hsa-miR-301a-3p", "hsa-miR-301b-3p", "hsa-miR-1306-3p"	*ESR1, UBC, PRKACB*
	"hsa-miR-183-5p"	*PRKACB, CCNB1,CD44*
	"hsa-miR-1915-3p", "hsa-miR-216a-5p", "hsa-miR-6772-5p", "hsa-miR-6764-5p"	*CD44, ESR1, ACTB*
	"hsa-miR-199a-3p"	*CD44, PRKACB, CCNB1*
	"hsa-miR-223-3p", "hsa-miR-548b-5p"	*PRKACB, CD44, RAC1*
	"hsa-miR-298", "hsa-miR-300", "hsa-miR-4264"	*ESR1, PRKACB, RAC1*
	"hsa-miR-302a-3p", "hsa-miR-302d-3p", "hsa-miR-3651", "hsa-miR-548b-3p", "hsa-miR-520a-3p", "hsa-miR-520d-3p", "hsa-miR-660-5p"	*ESR1, PRKACB, CD44*
	"hsa-miR-3130-5p"	*CD44, CCNB1, ESR1*
	"hsa-miR-4482-5p"	*CCNB1, CD44, ESR1*
	"hsa-miR-509-3p", "hsa-miR-518a-5p", "hsa-miR-4427", "hsa-miR-361-5p", "hsa-miR-4524a-3p"	*PRKACB, RAC1, ESR1*
	"hsa-miR-548ao-5p", "hsa-miR-548ax", "hsa-miR-3131"	*PRKACB, CCNB1, ESR1*
	"hsa-miR-585-5p"	*CD44, ESR1, RAC1*
	"hsa-miR-659-3p"	*RAC1, PRKACB, CD44*
	"hsa-miR-6794-5p"	*ESR1, ACTB, CCNB1*
Mild COVID-19 and CAD	"hsa-miR-1-3p", "hsa-miR-206"	*IGF1, ACTB, CCL2*
COVID-19 and CAD	"hsa-let-7a-2-3p"	*SNCA, AC1, ACTB*
	"hsa-miR-31-3p", "hsa-miR-486-5p"	*IGF1, PLCG1, ACTB*
	"hsa-miR-3174", "hsa-miR-4521"	*SNCA, IGF1, RAC1*
	"hsa-miR-509-3p"	*IL1A, RAC1, IGF1*

Of note, 25 common miRNAs (hsa-miR-3622a-3p, hsa-miR-3622b-3p, hsa-miR-4715-5p, hsa-miR-130a-3p, hsa-miR-130b-3p, hsa-miR-454-3p, hsa-miR-301a-3p, hsa-miR-301b-3p, hsa-miR-1306-3p, hsa-miR-183-5p, hsa-miR-1915-3p, hsa-miR-216a-5p, hsa-miR-6772-5p, hsa-miR-6764-5p, hsa-miR-199a-3p, hsa-miR-302a-3p, hsa-miR-302d-3p, hsa-miR-3651, hsa-miR-548b-3p, hsa-miR-520a-3p, hsa-miR-520d-3p, hsa-miR-660-5p, hsa-miR-509-3p, hsa-miR-361-5p, hsa-miR-4524a-3p) were identified between severe COVID-19-CAD and severe COVID-19-RA. hsa-miR-206 is the common miRNA between the mild COVID-19-CAD and mild COVID-19-RA while hsa-miR-509-3p is the only common miRNA between COVID-19-CAD and COVID-19-RA (Table 7).

Table 7. Shared miRNAs targeting the hub genes for COVID-19 and RA

	miRNAs	**Target genes**
Severe COVID-19 and RA	"hsa-miR-6817-5p"	*NOTCH1, CTLA4, RHOA, ESR1*
	"hsa-miR-7978"	*CTLA4, RHOA, PRKACB, CD44*
	"hsa-miR-548n"	*ACTB, NOTCH1, PRKACB, ESR1*
	"hsa-miR-12124"	*DLG4, ESR1, RHOA*
	"hsa-miR-1226-3p", "hsa-miR-22-3p"," hsa-miR-361-5p", "hsa-miR-3646", "hsa-miR-6750-5p", "hsa-miR-4524a-3p", "hsa-miR-449b-3p", "hsa-miR-4715-5p"	*RHOA, PRKACB, ESR1*
	"hsa-miR-130a-3p", "hsa-miR-130b-3p", "hsa-miR-454-3p"	*ESR1, UBC, PRKACB*
	"hsa-miR-183-5p"	*PRKACB, NOTCH1, CD44*
	"hsa-miR-185-5p"	*RHOA, PRKACB, CD44*
	"hsa-miR-1915-3p"	*CD44, ESR1, ACTB*
	"hsa-miR-199a-3p"	*CD44, PRKACB, RHOA*
	"hsa-miR-200b-3p", "hsa-miR-200c-3p", "hsa-miR-125a-3p", "hsa-miR-2116-5p"	*PRKACB, RHOA, NOTCH1*
	"hsa-miR-301a-3p", "hsa-miR-301b-3p"	*ESR1, UBC, PRKACB*
	"hsa-miR-302a-3p", "hsa-miR-302d-3p", "hsa-miR-3622a-3p", "hsa-miR-3622b-3p", "hsa-miR-373-3p", "hsa-miR-3651", "hsa-miR-1306-3p", "hsa-miR-660-5p"	*ESR1, PRKACB, CD44*
	"hsa-miR-3132"	*RHOA, CD44, ESR1*
	"hsa-miR-3152-5p"	*CTLA4, ESR1, NOTCH1*
	"hsa-miR-3650"	*CD44, CTLA4, RHOA*
	"hsa-miR-4529-5p", "hsa-miR-6877-5p"	*RHOA, ESR1, CD44*
	"hsa-miR-4687-5p", "hsa-miR-4724-5p", "hsa-miR-4640-5p", "hsa-miR-509-3p", "hsa-miR-5004-3p"	*PRKACB, NOTCH1, ESR1*
	"hsa-miR-4741"	*RHOA, NOTCH1, CTLA4*
	"hsa-miR-4769-3p"	*CLTA4, NOTCH1, ESR1*
	"hsa-miR-4778-3p", "hsa-miR-6787-3p"	*CLTA4, PRKACB, CD44*
	"hsa-miR-520d-3p"," hsa-miR-548b-3p", "hsa-miR-520a-3p"	*ESR1, PRKACB, CD44*
	"hsa-miR-5590-5p"	*ESR1, CLTA4, NOTCH1*
	"hsa-miR-577"	*RHOA, CD44, NOTCH1*
	"hsa-miR-6499-3p"	*ESR1, NOTCH1, CTLA4*
	"hsa-miR-656-5p", "hsa-miR-6717-5p", "hsa-miR-2355-5p"	*ESR1, CD44, NOTCH1*
	"hsa-miR-6764-5p", "hsa-miR-216a-5p"	*ESR1, CD44, ACTB*
	"hsa-miR-6772-5p"	*CD44, ACTB, ESR1*
	"hsa-miR-6778-5p"	*CD44, RHOA, DLG4*
	"hsa-miR-6786-3p"	*NOTCH1, DLG4, CD44*
	"hsa-miR-6815-3p", "hsa-miR-4267"	*CD44, RHOA, CTLA4*
	"hsa-miR-8071"	*NOTCH1, ACTB, CD44*
Mild COVID-19 and RA	"hsa-miR-206"	*IGF1, ACTB, CCL2*
COVID-19 and RA	"hsa-miR-1237-5p"	*NOTCH1, DLG4, RAC1*
	"hsa-miR-125a-3p"	*RHOA, IGF1, NOTCH1*
	"hsa-miR-146a-5p"	*RAC1, RHOA, CCT3*
	"hsa-miR-191-3p"	*ACTB, NOTCH1, RAC1*
	"hsa-miR-2115-3p"	*IGF1, RHOA, RAC1*
	"hsa-miR-3147"	*IL1B, IGF1, NOTCH1*
	"hsa-miR-4268"	*NOTCH1, RHOA, IGF1*
	"hsa-miR-4697-5p"	*DLG4, RAC1, NOTCH1*
	"hsa-miR-4715-5p"	*RHOA, RAC1, IGF1*

Cont. Table 7

	"hsa-miR-509-3p"	*RAC1, IGF1, NOTCH1*
	"hsa-miR-549a-5p"	*ACTB, IL1B, NOTCH1*
	"hsa-miR-577"	*RHOA, NOTCH1, IGF1*
	"hsa-miR-6499-3p"	*NOTCH1, IGF1, RAC1*
	"hsa-miR-6729-5p"	*NOTCH1, RAC1, IL13*
	"hsa-miR-6817-5p"	*NOTCH1, IGF1, RHOA*

Finally, we analyzed the miRNA-TF interactions by TransmiR database and enrichment analyses revealed 7 TFs that were possibly affecting the regulation of miRNAs for severe COVID-19-CAD. Eleven TFs were found to be possibly affecting the regulation of miRNAs for mild COVID-19-CAD and 2 TFs were possibly affecting the regulation of miRNAs for COVID-19-CAD (Table 8).

Table 8. Enrichment analyses of the miRNAs controlled by transcription factors for COVID-19-CAD

	Transcription factors	**p-value**	**Enriched miRNAs**
Severe COVID-19 and CAD	NF-7	0.00665096	"hsa-miR-301b", "hsa-miR-130b"
	PPARA	0.00665096	"hsa-miR-301a", "hsa-miR-454"
	IL6	0.01269176	"hsa-miR-301b", "hsa-miR-301a"
	SREBF2	0.019999058	"hsa-miR-183"," hsa-miR-301a", "hsa-miR-454"
	NR1H4	0.02040112	"hsa-miR-199a", "hsa-let-7a-2"
	BARX1	0.02639011	"hsa-miR-301a", "hsa-miR-454"
	ZNF486	0.04828694	"hsa-miR-548b"
Mild COVID-19 and CAD	MRF4	0.00000535	"hsa-miR-1", "hsa-miR-206"
	MYF5	0.00000535	"hsa-miR-1", "hsa-miR-206"
	MYOG	0.00001498	"hsa-miR-1", "hsa-miR-206"
	EGFR	0.00516528	"hsa-miR-1"
	SLUG	0.00516528	"hsa-miR-1"
	MYOCD	0.00619673	"hsa-miR-206"
	TNFSF12	0.00825803	"hsa-miR-206"
	NFE2L2	0.00861861	"hsa-miR-1", "hsa-miR-206"
	FOXO3	0.01031719	"hsa-miR-1"
	AP-1	0.01442908	"hsa-miR-206"
	MYOD1	0.02747683	"hsa-miR-1", "hsa-miR-206"
COVID-19 and CAD	BMP2	0.01214575	"hsa-miR-31"
	SOX4	0.0162-19433	"hsa-miR-31"

Furthermore, 18 TFs were found to be possibly affecting the regulation of miRNAs for severe COVID-19-RA; 7 TFs were possibly affecting the regulation of miRNAs for mild COVID-19-RA and 8 TFs were possibly affecting the regulation of the miRNAs for all COVID-19-RA (Table 9). PPARA and SREBF2 are the two common TFs possibly affecting the regulation of the miRNAs for severe COVID-19-CAD and severe COVID-19-RA. MRF4, MYF5, MYOG, MYOCD, THNFSF12 and AP-1 are the 6 common TFs possibly affecting the miRNAs for mild COVID-19-CAD and mild COVID-19-RA.

Table 9. Enrichment analyses of the miRNAs regulated by transcription factors for COVID-19-RA

	Transcription factors	**p-value**	**Enriched miRNAs**
Severe COVID-19 and RA	AKT2	0.00112157	"hsa-miR-200b", "hsa-miR-200c", ""hsa-miR-22
	PPARA	0.0025678	"hsa-miR-200c"," hsa-miR-301a", "hsa-miR-454"
	PTEN	0.00626717	"hsa-miR-302d", "hsa-miR-22", "hsa-miR-302a"

Cont. Table 9

	PRAME	0.0083903	“hsa-miR-1306”,” hsa-miR-6786”, “hsa-miR-301a”, “hsa-miR-454”, “hsa-miR-3651”, “hsa-miR-185”
	TWIST1	0.01097577	“hsa-miR-200b”, “hsa-miR-200c”, “hsa-miR-373”, “hsa-miR-199a”
	ETV4	0.015154	hsa-miR-200b, hsa-miR-125a
	SLUG	0.015154	“hsa-miR-200b”, “hsa-miR-200c”
	LEF1	0.01785253	“hsa-miR-183”, “hsa-miR-373”, “hsa-miR-302d”, “hsa-miR-302a”
	ASCL2	0.02076698	“hsa-miR-200b”, “hsa-miR-200c”
	BMP4	0.02076698	“hsa-miR-200b”, “hsa-miR-200c”
	PELP1	0.02076698	“hsa-miR-200b”, “hsa-miR-200c”
	SIP1	0.02076698	“hsa-miR-200b”, “hsa-miR-200c”
	SIX1	0.02076698	“hsa-miR-200b”, “hsa-miR-200c”
	TGFB1	0.02695805	“hsa-miR-200b”, “hsa-miR-200c”, “hsa-miR-199a”, “hsa-miR-302a”
	MBD2	0.03411573	“hsa-miR-4524a”, “hsa-miR-373”
	HDAC4	0.03473674	“hsa-miR-200b”, “hsa-miR-200c”, “hsa-miR-185”
	SREBF2	0.03534006	“hsa-miR-183”, “hsa-miR-6750”, “hsa-miR-301a”, “hsa-miR-454”
	SOX17	0.04174892	“hsa-miR-302d”, “hsa-miR-302a”
Mild COVID-19 and RA	MRF4	0.00206932	“hsa-miR-206”
	MYF5	0.00206932	“hsa-miR-206”
	MYOCD	0.00310398	“hsa-miR-206”
	MYOG	0.00362131	“hsa-miR-206”
	TNFSF12	0.00413864	“hsa-miR-206”
	AP-1	0.00724263	“hsa-miR-206”
	PHOX2B	0.02586653	“hsa-miR-206”
COVID-19 and RA	TLR2	0.00014459	“hsa-miR-146a”, “hsa-miR-125a”
	HOXD10	0.01312898	“hsa-miR-146a”
	EGR3	0.01748602	“hsa-miR-146a”
	IL1B	0.01748602	“hsa-miR-146a”
	ETV4	0.02183343	“hsa-miR-125a”
	DEK	0.0261712	“hsa-miR-146a”
	TNFSF12	0.03481781	“hsa-miR-146a”
	FOXP3	0.03912666	“hsa-miR-146a”

DISCUSSION

Emerging data suggest patients with chronic complex diseases such as CAD and RA are prone to develop severe COVID-19 symptoms due to underlying pathological conditions. Thus, it is of great importance to scrutinize the molecular abnormalities that exacerbate the COVID-19 disease severity in patients with complex diseases. As a result, understanding the interplay between COVID-19 and accompanying diseases is critical for disease management and developing efficient treatment strategies.

In our study, we conducted a comprehensive bioinformatics analysis to better understand the shared mechanisms, common genes, TFs and miRNAs between CAD and COVID-19 as well as between RA and COVID-19. In addition, we also identified if the severity of COVID-19 differentially affects the specific shared mechanisms and biomarkers between COVID-19-CAD patients and COVID-19-RA. We grouped the datasets into three categories for each disease by filtering the samples: Severe COVID-19-CAD, Mild-COVID-19-CAD, COVID-19-CAD, severe COVID-19-RA, mild COVID-19-RA and COVID-19-RA.

First, we identified 1539 common DEGs in severe COVID-19-CAD, 710 common DEGs in mild COVID-19-CAD datasets and 908 common DEGs in COVID-19-CAD. We identified 1514 common DEGs in severe COVID-19-RA, 680 common DEGs in mild COVID-19-RA and 898 common DEGs in COVID-19-RA. Second, we created the PPI network to identify the top 10 hub genes in the PPI network. We successfully determined top 10 hub genes that could serve as possible diagnostic and therapeutic biomarkers for COVID-19 and CAD. The top 10 hub genes for severe COVID-19-CAD are *ACTB, UBC, PRKACB, ESR1, CD44, MMP9, IL4,*

IFNG, CCNB1, RAC1, for mild COVID-19-CAD are *ACTB, IL1B, CCL2, IGF1, IL1A, CXCL1, RPL11, ASPM, CCNB1, CSF2* and for COVID-19-CAD are *ACTB, IL1B, MMP9, IGF1, SCCNB1, RAC1, SNCA, CSF2, PLCG1, IL1A.* ACTB and *CCNB1* are the common hub genes for severe COVID-19-CAD and mild COVID-19-CAD. Most of the hub genes were different in mild and severe COVID-19-CAD patients suggesting that severity of COVID-19 results in different mechanisms. Previous studies also discovered that the severity of COVID-19 resulted in different biomarkers [20]. The top 10 hub genes for severe COVID-19-RA are *ACTB, UBC, ESR1, RHOA, NOTCH1, PRKACB, CD44, CTLA4, MMP9, DLG4*, for mild COVID-19-RA are *ACTB, IL1B, CCL2, IGF1, IL1A, RPL11, IL13, CCNB1, CXCL1, RPS2* and for COVID-19-RA are *ACTB, IL1B, RHOA, NOTCH1, MMP9, DLG4, IGF1, RAC1, CCT3, IL13. ACTB* is the only common hub gene for severe COVID-19-RA and mild COVID-19-RA.

Functional enrichment analysis revealed top 5 GO terms in BP, MF and CC and KEGG pathways that were also different in mild and severe COVID-19-CAD patients. The only shared KEGG pathway was “ameobiasis” in mild and severe COVID-19-CAD patients. The identified pathways (Rheumatoid arthritis, Coronavirus disease-COVID-19, cytokine-cytokine receptor interaction and type I diabetes mellitus) associated with RA are consistent with the recent *in silico* research [21]. The top 5 GO terms in BP, MF and CC, and KEGG pathways were also different in mild and severe COVID-19-RA patients. This confirms the idea that mild and severe forms of COVID-19 may have different molecular mechanisms. The only shared GO term in the CC category was “extracellular region” in mild and severe COVID-19-RA patients. cGMP-PKG signaling pathway is one of the top 5 common KEGG pathways between COVID-19 and RA. Recently, cGMP-PKG signaling pathway was shown to be related with COVID-19 which was suggested for utilization in the treatment of the COVID-19 patients with RA [22].

Finally, we identified potential shared miRNAs and TFs in all groups to discover the potential transcriptional and post-transcriptional regulators of COVID-19-CAD and COVID-19-RA datasets. We also studied the miRNA-TF interactions in all groups. GATA3, IRF1 and IKBKB are the top TFs that were likely involved in the regulations of the hub genes in the severe COVID-19-CAD patients. RELA, NFKB1 and BRCA1 are the op TFs that were likely involved in the regulations of the hub genes in mild COVID-19-CAD patients. RELA, NFKB1, SP1, BRCA1, STAT1 and JUN are the common TFs between severe COVID-19-CAD and mild COVID-19-CADOverall, JUN, RELA and NFKB1 are the top TFs that were likely involved in the regulations of the hub genes in COVID-19-CAD group. Our results confirmed the results of recent research that cardiac biomarkers have important roles in COVID-19 diagnosis and treatment [23]. LMO4, IKBKB and HDAC1 are the top TFs that were likely involved in the regulations of the hub genes in severe COVID-19-RA. RELA, NFKB1 and BRCA1 are the top TFs that were likely involved in the regulations of the hub genes in mild COVID-19-RA. JUN, RELA, NFKB1, NFKBIA, SIRT1, EP300 and STAT1 are the common TFs between severe COVID-19-RA and mild COVID-19-RA. Overall, SIRT1, KFL5 and PTTG1 are the top TFs that were likely involved in the regulations of the hub genes in COVID-19-RA group. One recent study also discovered that RELA, JUN, STAT1 and E2F1 are among the top TFs in COVID-19-RA patients and this study confirms our results [21]. Overall, our results confirm that COVID-19 and RA share several mechanisms especially related to immunologic pathways [24].

We also discovered many miRNAs targeting at least three hub genes in all groups. The miRNAs targeting hub genes were also different between severe and mild COVID-19 and CAD and RA. Finally, we checked TF-miRNA interactions in each group. NF-7, PPARA and IL6 are the top TFs that were likely involved in the regulations of the miRNA in severe COVID-19-CAD. BMP2 and SOX4 are the only TFs that were likely involved in the regulations of the miRNAs in COVID-19-CAD. MRF4, MYF5 and MYOG are the top TFs that were likely involved in the regulations of the “mild” COVID-19-CAD. Overall, different TFs affecting the miRNAs were detected between severe and mild forms of COVID-19 and CAD patient that suggest the severity of COVID-19 may have different shared mechanisms. AKT2, PPARA and PTEN are the top TFs that were likely involved in the regulations of the miRNA in “severe” COVID-19-RA. MRF4, MYF5 and MYOCD are the top TFs that were likely involved in the regulations of the mild COVID-19-RA. TLR2, HOXD10 and EGR3 are the top TFs that were likely involved in the regulations of the miRNAs in COVID-10-CAD. Overall, different TFs affecting the miRNAs were detected between severe and mild forms of COVID-19 and CAD that imply the severity of COVID-19 may have been triggered by distinct shared mechanisms. Overall, the analysis of TF-hub gene and TF-miRNA discovered different TFs affect miRNAs and hub genes.

In our study, we found that IL-6 is one of the most significant TFs that may affect the “severe” COVID-19-CAD patients. Cytokines are the main cytokines of target cells that alter or regulate their activity

protein or glycoprotein structures are immunomodulators in the hematopoietic system. Cytokines exert their effects by binding specific ligands. Interleukin-6 (IL-6) is a pleiotropic pro-inflammatory cytokine with broad biological effects that are closely associated with tumor growth. Dysregulation of this cytokine is

associated with chronic inflammation and multifactorial autoimmune disorders. Previously, IL-6 has been found to be an important biomarker in COVID-19 [2]. Elevated levels of IL-6 were linked to the severity and disease progression of severe COVID-19 [2, 25, 26]. So, *IL-6* may be a key biomarker in CAD patients with severe COVID-19.

Although our *in silico* study determined several potential biomarkers, miRNAs, TFs and mechanisms between CAD and COVID-19, and RA and COVID-19, it has some limitations. First, different datasets with larger sample size would increase the validity of our results and *in silico* findings need to be confirmed by *in vivo* and *in vitro* experiments.

Our study highlights common hub genes, miRNAs, TFs and shared mechanisms in both mild and severe COVID-19-CAD and mild and severe COVID-19-RA. Our result also points out that mild and severe forms of COVID-19 differ in potential biomarkers, mechanisms, miRNAs and TFs in both CAD and RA patients. Our study is the first study that investigates the potential shared mechanisms, biomarkers, TFs and miRNAs between COVID-19 and CAD patients and COVID-19 and RA patients. Our results would contribute to further research approaches to develop treatment strategies for patients with CAD with COVID-19 and patients with RA with COVID-19 based on the severity of the COVID-19 disease.

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