OXFORD

Pathogenetic profiling of COVID-19 and SARS-like viruses

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Abstract

The novel coronavirus (2019-nCoV) has recently emerged, causing COVID-19 outbreaks and significant societal/global disruption. Importantly, COVID-19 infection resembles SARS-like complications. However, the lack of knowledge about the underlying genetic mechanisms of COVID-19 warrants the development of prospective control measures. In this study, we employed whole-genome alignment and digital DNA–DNA hybridization analyses to assess genomic linkage between 2019-nCoV and other coronaviruses. To understand the pathogenetic behavior of 2019-nCoV, we compared gene expression datasets of viral infections closest to 2019-nCoV with four COVID-19 clinical presentations followed by functional enrichment of shared dysregulated genes. Potential chemical antagonists were also identified using protein–chemical interaction analysis. Based on phylogram analysis, the 2019-nCoV was found genetically closest to SARS-CoVs. In addition, we identified 562 upregulated and 738 downregulated genes (adj. $P \leq 0.05$) with SARS-CoV infection. Among the dysregulated genes, SARS-CoV shared ≤ 19 upregulated and ≤ 22 downregulated genes with each of different COVID-19 complications. Notably, upregulation of *BCL6* and *PFKFB3* genes was common to SARS-CoV, pneumonia and severe acute respiratory syndrome, while they shared *CRIP2*, *NSG1* and *TNFRSF21* genes in downregulation. Besides, 14 genes were common to different SARS-CoV comorbidities that might influence COVID-19 disease. We also observed similarities in pathways that can lead to COVID-19 and SARS-CoV diseases. Finally, protein–chemical interactions suggest cyclosporine, resveratrol and quercetin as promising drug candidates against COVID-19 as well as other SARS-like viral infections. The pathogenetic analyses, along with identified biomarkers, signaling pathways and chemical antagonists, could prove useful for novel drug development in the fight against the current global 2019-nCoV pandemic.

Key words: 2019-nCoV; coronavirus; COVID-19; microarray; SARS-CoV-2; comorbidities

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Submitted: 2 April 2020; **Received (in revised form):** 23 June 2020

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Background

During the past century, several human coronaviruses have emerged, causing severe respiratory illness and global outbreaks in humans [1]. Coronaviruses (CoVs) are a large group of positive-sense single-stranded RNA viruses belonging to the *Coronaviridae* family [1, 2]. Six species of coronavirus have been identified as human pathogens [1, 3]. The spectrum of clinical manifestations ranges from mild to severe infection of the respiratory tract [3]. Four species OC43, HKU1, NL63 and 229E cause common cold in immune-compromised patients [1, 3]. Notably, two other coronaviruses, namely, severe acute the respiratory syndrome coronavirus (SARS-CoV) and Middle East respiratory syndrome coronavirus (MERS-CoV), have plagued the general public and caused global outbreaks in 2003 and 2012, respectively [1, 2]. In the late December 2019, however, a novel coronavirus (2019-nCoV), which may have evolved through host-induced natural selection [4], was first identified in patients with viral pneumonia in Wuhan City, China [1, 2]. As of 30 April 2020, there has been a total of 3 200 414 confirmed 2019-nCoV cases and a death toll of 226 893 across 210 countries and territories (https://worldometers.info/coronavirus/). At the time of article writing, Bangladesh had 7103 confirmed cases with 163 deaths (http://corona.gov.bd/). The number of cases is still increasing worldwide with 4.5% global mortality rate [5, 6]. On 7 January 2020, newly evolved SARS-CoV-2 was first identified in Wuhan City, China [1]. The virus was initially named as 2019 novel coronavirus (2019-nCoV) by the World Health Organization [7]. However, based on established taxonomic methods, the virus has been recognized as a sister to SARS-CoV, hence, formally designated as SARS-CoV-2 by the Coronavirus Study Group (CSG) of the International Committee on Taxonomy of Viruses. The disease outbreak is named as coronavirus disease 19 (COVID-19) [7].

The SARS-CoV-2 genome encodes for four major proteins, i.e. spike (S), envelope (E), membrane (M) and nucleocapsid (N) proteins. The S-protein of SARS-CoVs facilitates the viral entry upon binding to angiotensin-converting enzyme 2 (ACE2) expressed in human lung cells [8, 9]. However, the binding affinity of S-protein towards ACE2 is higher in SARS-CoV-2 due to the translational alteration of five out of six vital amino acids in the active site of its S-protein allowing improved hydrophobic and salt–bridge interactions [8]. Upon binding, viral membrane fuses with host cells. After fusion occurs, transmembrane serine protease 2 (TMPRSS2) present on the host cell surface activates the ACE2-S-protein complex followed by the conformational changes that allow the virus to enter the cells [10].

The viral entry induces the host's immune response by exposing itself to antigen-presenting cells (APCs) which have different types of pattern recognition receptors (PRRs) such as toll-like receptors (TLRs) [11]. For instance, TLR4 recognizes the S-protein and activate NF-κB transcription factors and pathogen-activated protein kinase (MAPK) pathway to induce proinflammatory proteins [10]. Meanwhile, other TLRs such as TLR3 could detect an RNA genome that eventually ends up activating the IRF3 and NF-κB transcription factors to induce proinflammatory cytokines such as IFN-α which in turn form complexes with its receptor, IFNAR, thereby, initiating the JAK-STAT pathways [10]. Further, JAK1 and TYK2 kinases phosphorylate STAT1 and 2 followed by its complexation with IRF9, and together they migrate into the nucleus to initiate the transcription of IFN-stimulated genes (ISGs) and lead to suppression of viral replication and prevent the severity of the disease [12]. However, excess releasing of pro-inflammatory cytokines (i.e. IFN-α, IFN-γ, IL-6, IL-18, TNF-α, TGFβ, etc.) and chemokines (i.e. CCL2, CCL5, CXCL8, CXCL10, etc.) from immune effector cells causes cytokine storms which will eventually lead to acute respiratory distress syndrome (ARDS) [10, 13]. However, for better survival and host infections, SARS-CoVs developed immune invasion strategies such as the formation of double vesicles during APC recognition by PRRs. In addition, it has several other proteins such as Nsp1 which can suppress INF-α activity through host translational machinery inactivation and inhibition of STAT1 phosphorylation [12].

Human coronavirus disease is a zoonosis, infection of animal origins. Most coronaviruses are originated from bats where they are non-virulent [14]. SARS-CoV-2 shares 88–92% genomic similarities with the most bat SARS-like coronaviruses, except W1V1 which showed ~96% sequence homology [14]. This bat coronavirus (W1V1) can use bat, civet and human ACE2 as a receptor for cell entry. Intriguingly, convalescent sera from SARS patients were able to neutralize WIV1 [14]. Therefore, it has been proposed that the bat is the original host of SARS-CoV-2 [15]. However, viral transmission from bats to humans requires several intermediate reservoirs [14]. In support, several studies have linked pangolins, cats, dogs and hamsters with SARS-CoV-2 infection and transmission [16–20]. These indicate the widespread prevalence of SARS-CoV-2 across animals as well as the potential threats to humans [21]. Preliminary evidence indicated that the COVID-19 outbreak started at the Wuhan seafood market. However, several recent studies suggest otherwise. For instance, among the first 41 cases, 14 had no prior contact with the market. In another study, five out of seven cases had no link with the market. Therefore, the virus could have amplified in the market, but it might neither have been the site of origin nor the only source of the outbreak [15].

The COVID-19 outbreak in China reiterates the significant and continued threat of zoonotic diseases caused by coronaviruses to the global population [1]. Unlike most other strains, the newly emerged 2019-nCoV causes SARS-like severe respiratory illness [2]. Like SARS-CoV infection, most infected patients develop pneumonia with prominent fever, shortness of breath, invasive lung lesions, anosmia and sometimes diarrhea [22–25]. The rate of severity and recovery from the COVID-19 is involved with the age, biological sex and other health conditions of the infected patients [6]. For instance, the majority of the COVID-19 patients are male (54.3%), while the fatality is more frequent (15%) in older age groups (above 80 years old) than in the younger population [6, 26]. In most cases, however, symptoms are similar to seasonal flu [27]. Nevertheless, in a recent Chinese study, 80% of cases were identified as asymptomatic, making early diagnosis difficult [28]. As a result, these undetected asymptomatic patients could be a major source of contagion [28]. Furthermore, the transmission of SARS-CoV-2 from humans to humans is highly frequent in family, health care and community setting [15]. The transmission occurs via respiratory tract droplets or fomites while fecal–oral spread is also possible. The estimated basic reproduction number (R_O) of SARS-CoV-2 ranged between 2.0 and 2.8 [15]. Therefore, early detection is crucial for reducing viral shedding as well as for effective treatment [28]. Despite the knowledge and experience related to SARS-CoV and MERS-CoV, major gaps related to the epidemiology, clinical manifestations and pathogenetic behaviors of this emerging 2019-nCoV remain to be fulfilled [2]. Since no vaccine or antiviral drugs are available, present treatments are based on managing symptoms and complications. Therefore, insight into biomarkers and molecular mechanisms that transform the 2019-nCoV infection into COVID-19 disease and related complications is necessary to
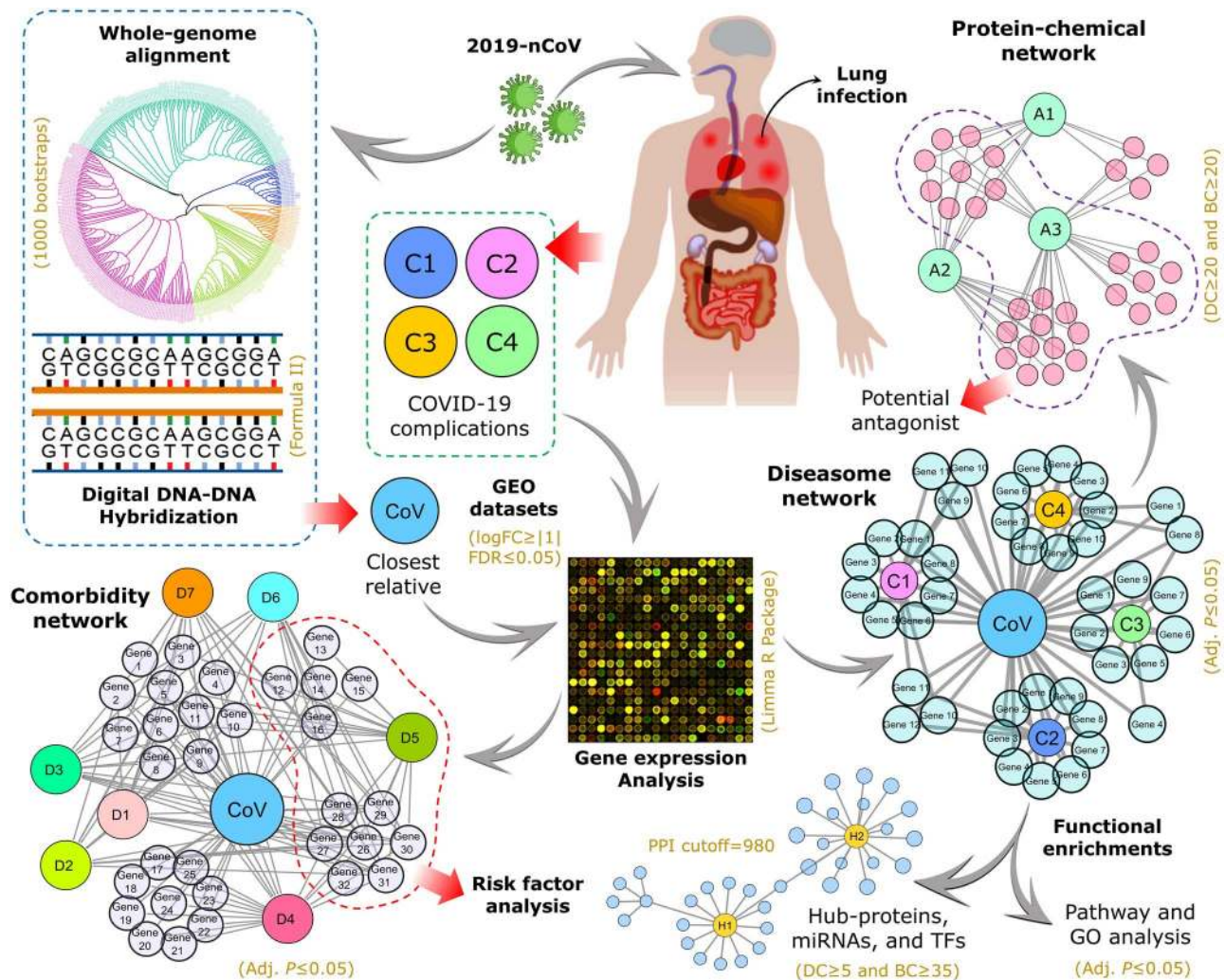
Figure 1. The overall experimental workflow applied in this study. Following establishing SARS-CoV as the closest relative of 2019-nCoV through whole-genome alignment (1000 bootstrap tests), the microarray datasets related to SARS-CoV infection and four COVID-19 complications (i.e. SARS, PNA, SHOB and DRA) were retrieved and analyzed to identify differentially expressed genes (DEGs). Herein, Limma R package was employed where logFC≥|1| was used to distinguish differential expression, and adjusted *P*-value ≤0.05 was set as cutoff for statistical significance. Then, the DEGs from SARS-CoV infection were subjected to validation with gold-standard databases (i.e. OMIM disease, OMIM expanded and dbGaP) for comorbidity and risk factor assessment followed by comparative analysis with the disease datasets. Finally, we used the shared DEGs for network-based interactome analysis and functional enrichments in terms of protein–protein interactions (PPI confidence cutoff = 980), gene–biomarker associations (DC≥5 and BC≥35) and protein–chemical interactions (DC≥20 and BC≥20). For pathway analysis, we considered BioCarta (2016), Reactome (2016), KEGG (human; 2019) and WikiPathways (human; 2019) databases, while Biological Process (2018), Molecular Function (2018) and Human Phenotype Ontology databases were used for gene ontological evaluation. In both cases, adjusted P-value ≤0.05 was considered as significant.

evaluate the clinical significance and in assessing the severity and prognosis of the disease.

In this study, we aimed to explore the molecular pathogenesis of COVID-19. As the 2019-nCoV shares characteristics with SARS-CoV in terms of genomic and pathogenic features, existing SARS-CoV experimental data is used to study molecular aspects of 2019-nCoV. To accomplish this, we first validated the genetic linkage among 2019-nCoV and other coronaviruses using phylogenetic analysis. We employed a multistep statistical method to determine differentially expressed genes (DEGs), regulatory networks and gene–disease associations as illustrated in Figure 1. Using the shared DEGs, we identified hubproteins and regulatory biomarkers, i.e. transcription factors (TFs) and microRNAs (miRNAs). Furthermore, gene ontology and pathway analyses were performed to determine significant regulatory checkpoints related to disease complications. Finally, we screened potential chemical antagonists useful in the fight against COVID-19 disease.

Materials and methods

Datasets

The complete genomes used in this study were collected from the Virus Pathogen Database and Analysis Resource (ViPR; https://viprbrc.org/). To investigate the impact of COVID-19 at the molecular level, we employed gene expression microarray datasets that were obtained from the National Center for Biotechnology Information (NCBI) Gene Expression Omnibus (http://www.ncbi.nlm.nih.gov/geo/). Due to the genetic closeness and unavailability of COVID-19-related datasets, however, we used microarray datasets related to SARS-CoV infection and four COVID-19 complications, i.e. pneumonia (PNA), severe acute respiratory syndrome (SARS), shortness of breath (SHOB) and diarrhea (DRA). In this study, five different datasets with accession numbers GSE30589, GSE103119, GSE14841, GSE137268 and GSE1739 were used [29–33]. The SARS-CoV dataset (GSE30589) is an Affymetrix microarray data involving

33 infected and 9 mock-infected patients with 3 hybridizations. The SARS dataset (GSE1739) is a gene expression profile derived from peripheral blood mononuclear cells (PBMCs) of 10 patients and 4 healthy controls using Affymetrix HG-Focus target array platform. The PNA dataset (GSE103119) is an Illumina humanHT-12 v4.0 gene expression array of 152 patients with community-acquired pneumonia and 20 healthy patients. The SHOB dataset (GSE137268) is a gene expression array of 54 asthma patients and 15 healthy individuals developed using Illumina humanRef-8 v2.0 bead chip. The DRA dataset (GSE14841) is an Affymetrix human genome U133 plus 2.0 array from jejunal mucosa of five patients with diarrhea-irritable bowel syndrome and four healthy volunteers.

Methods

To determine the relationship between 2019-nCoV and other coronaviruses, we created a comprehensive phylogram that includes the complete genomes of 389 human beta-coronaviruses (Supplementary File S1). The tree was inferred with the FastME program integrated with ViPR database using genomic data with 1000 bootstrap replications. Then, Newick-formatted phylogram was redesigned with the interactive tree of life (iTOL) online tool (https://itol.embl.de/). In addition, the genomic distance and G+C difference were calculated through digital DNA–DNA hybridization (dDDH) using Genome-to-Genome Distance Calculator (GGDC) server v2.1 with default settings [34]. For this analysis, we considered reference genomes of 2019-nCoV (NC_045512.2), SARS-CoV (NC_004718.3), MERS-CoV (NC_019843.3), CoV-OC43 (NC_006213.1) and CoV-HKU1 (NC_006577.2); and formula-2 derived dDDH and G+C difference scores were emphasized as per the server's recommendation [34].

Evaluation of gene expression microarray is very efficient when it comes to determining underlying molecular mechanisms that lead to the development of disease [35]. Using the Limma R package [36], we analyzed the selected microarray datasets as described earlier [35, 37]. Briefly, we categorized the samples in each dataset into two groups (treatment and control) followed by log transformation with a series of statistical methods (i.e. B- and t-statistics) and P-value adjustment with Benjamini and Hochberg method (false discovery rate). Herein, genes were considered as significantly upregulated or downregulated when expressed at adjusted P-value ≤ 0.05 with logFC score greater or lesser than (or equal to) $+1$ or -1, respectively.

To determine gene–disease associations, we applied neighborhood-based benchmark and topological methods. Thus, we built a comorbidity network from the gene–disease association where the node in the network can be either a disease or a gene. This network is a type of bipartite graph in which diseases are connected when they share at least 1 significant dysregulated gene. Let a particular set of human diseases be D and a set of human genes be G; a comorbidity network then attempts to find whether gene $g \in G$ is associated with disease $d \in D$. If G_i and G_j, the sets of significant up- and downregulated genes, are associated with diseases i and j, respectively, then the number of shared dysregulated genes (n^g_{ij}) associated with both diseases i and j is as follows:

$$n^g_{ij} = N\left(G_i \cap G_j\right) \ldots \ldots (1)$$

Co-occurrence indicates the number of common genes in the comorbidity network. Based on the Jaccard similarity index, the common neighbors are found. For each node pair with the edge (E), the prediction point is as follows:

$$E\left(i,j\right) = \frac{N\left(G_i \cap G_j\right)}{N\left(G_i \cup G_j\right)} \ldots \ldots (2)$$

The protein–protein interaction (PPI), regulatory biomarkers (i.e. TFs and miRNAs), protein–drug interaction (PDI) and protein–chemical interaction (PCI) networks were constructed using NetworkAnalyst tool [38]. Experimentally validated STRING interactome with 980 confidence cutoff was used for PPI network. For TFs and miRNAs, we employed experimentally verified TF target genes from the JASPAR database [39] and miRNA target genes from TarBase [40] and miRTarBase [41] via NetworkAnalyst [38]. Similarly, PDI and PCI were evaluated based on DrugBank v5.0 and Comparative Toxicogenomics Database, respectively [42, 43]. All interactions were visualized and customized in Cytoscape v3.7.2 [44].

The degree centrality (DC) and betweenness centrality (BC) were applied to filter out potential regulatory biomarkers and chemical antagonists. Nodes with a high degree act as important 'hubs' in a network, while high betweenness indicates nodes as important 'bottlenecks' in a network. Briefly, the DC of a node v in a network can be defined as the total number of nodes that are directly connected to node v in that network which can also be expressed as follows:

$$DC\left(v\right) = \sum_{j \in G} \frac{a_{vj}}{n-1}, \ldots (3)$$

where n represents the total number of nodes in the network and a_{vj} represents that node v and j are directly connected. BC represents the total number of times node v appears in the shortest path between other nodes. It is also defined as follows:

$$BC\left(v\right) = \sum_{i \neq j \neq v \in V} \frac{\sigma_{ivj}}{\sigma_{ij}} \ldots (4)$$

where σ_{ij} is the total number of shortest paths from node i to node j and σ_{ivj} is the total number of paths through node v.

Furthermore, we analyzed the pathways and gene ontology (GO) of the shared DEGs with the Enrichr web application [45] to understand how genetic determinants transform gene expressions in developing SARS-CoV complications. For pathways, we considered BioCarta (2016), Reactome (2016), KEGG (human; 2019) and WikiPathways (human; 2019) databases and GO Biological Process (2018), GO Molecular Function (2018) and Human Phenotype Ontology databases for GO analysis. Herein, an adjusted P-value ≤ 0.05 was considered as statistically significant.

Results

Human coronavirus phylogram

Whole-genome phylogenetic analysis revealed the relation among the different human beta-coronaviruses as shown in Figure 2. Importantly, the recent epidemic 2019-nCoV formed a sister clade with human SARS-CoV indicating their closest relationship with SARS-CoV at the genomic level. Moreover, as per the phylogenetic position, both SARS-CoV and 2019-nCoV share a common ancestral origin with MERS-CoV, which happened to be closely related to the CoV-HKU1 (Figure 2).
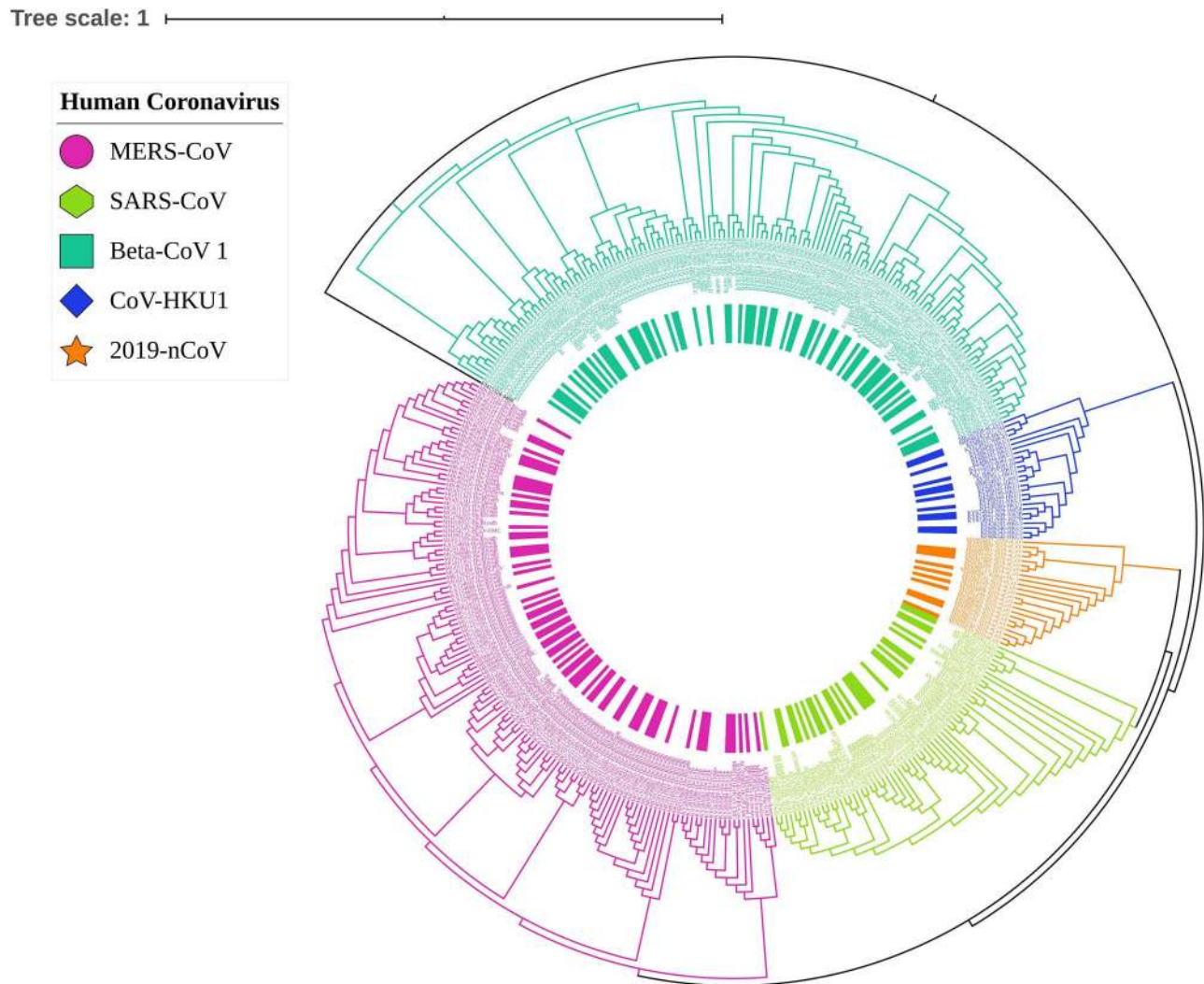
Figure 2. Phylogram of human coronavirus shows the respective position of newly emerged 2019-nCoV among the 389 beta-coronavirus. Different colors indicate the clades of different coronavirus where 2019-nCoV (orange) shared the same branch with SARS-CoV (lime). The whole-genome phylogram was inferred with 1000 bootstrap values in ViPR database and customized with the interactive tree of life (iTOL) web tool.

This result coincides with the estimated genomic distance and G+C content difference. In our study, the taxonomic distance between SARS-CoV and 2019-nCoV was 0.1985 rendering 22.10% of DNA–DNA hybridization. Moreover, the G+C content of 2019-nCoV was different from SARS-CoV, MERS-CoV and CoV-HKU1 by 2.79, 3.26 and 5.91%, respectively. Interestingly, the G+C difference between 2019-nCoV and CoV-OC43 was lower (1.18%) compared to the closest neighbors. Since the G+C difference is bound to $\leq 1\%$ within the same species, all these results suggest 2019-nCoV as a distinct coronavirus species.

Infectome and diseasome

To investigate the host response upon 2019-nCoV infection, we analyzed the gene expression pattern of SARS-CoV-infected tissues and healthy tissues using microarray data. We found that 1300 genes were differentially expressed as compared to healthy controls in which 562 genes were upregulated while 738 genes were downregulated significantly with infection (adj. $P \leq 0.05$). Similarly, we also analyzed the diseasome of

four COVID-19 complications to observe their association with SARS-CoV infection. We identified a large number of DEGs for each condition, i.e. 427 in DRA, 581 in SHOB, 997 in PNA and 728 in SARS (Supplementary File S2). Then, we performed a cross-comparative analysis to find common significant DEGs between each condition and SARS-CoV infection. We found a total of 87 unique shared DEGs in which SARS-CoV infection shares 18, 15, 21 and 40 genes with DRA, SHOB, PNA and SARS, respectively. To visualize their association, we construct infectome–diseasome relationship networks centered on the SARS-CoV infection, in which two diseases are comorbid if there exists one or more genes that are associated with both diseases (Figure 3).

Table 1 includes comprehensive details of important DEGs in terms of their expression pattern, associated conditions and pathogenic roles in disease development. Interestingly, SARS-CoV infection shared ≤ 19 upregulated and ≤ 22 downregulated genes with each different COVID-19 complication. Notably, upregulation of *BCL6* and *PFKFB3* genes was common to SARS-CoV, PNA and SARS (Figure 3A) while they shared *CRIP2*, *NSG1* and *TNFRSF21* genes in downregulation (Figure 3B). Nevertheless, less frequent COVID-19 conditions such as DRA

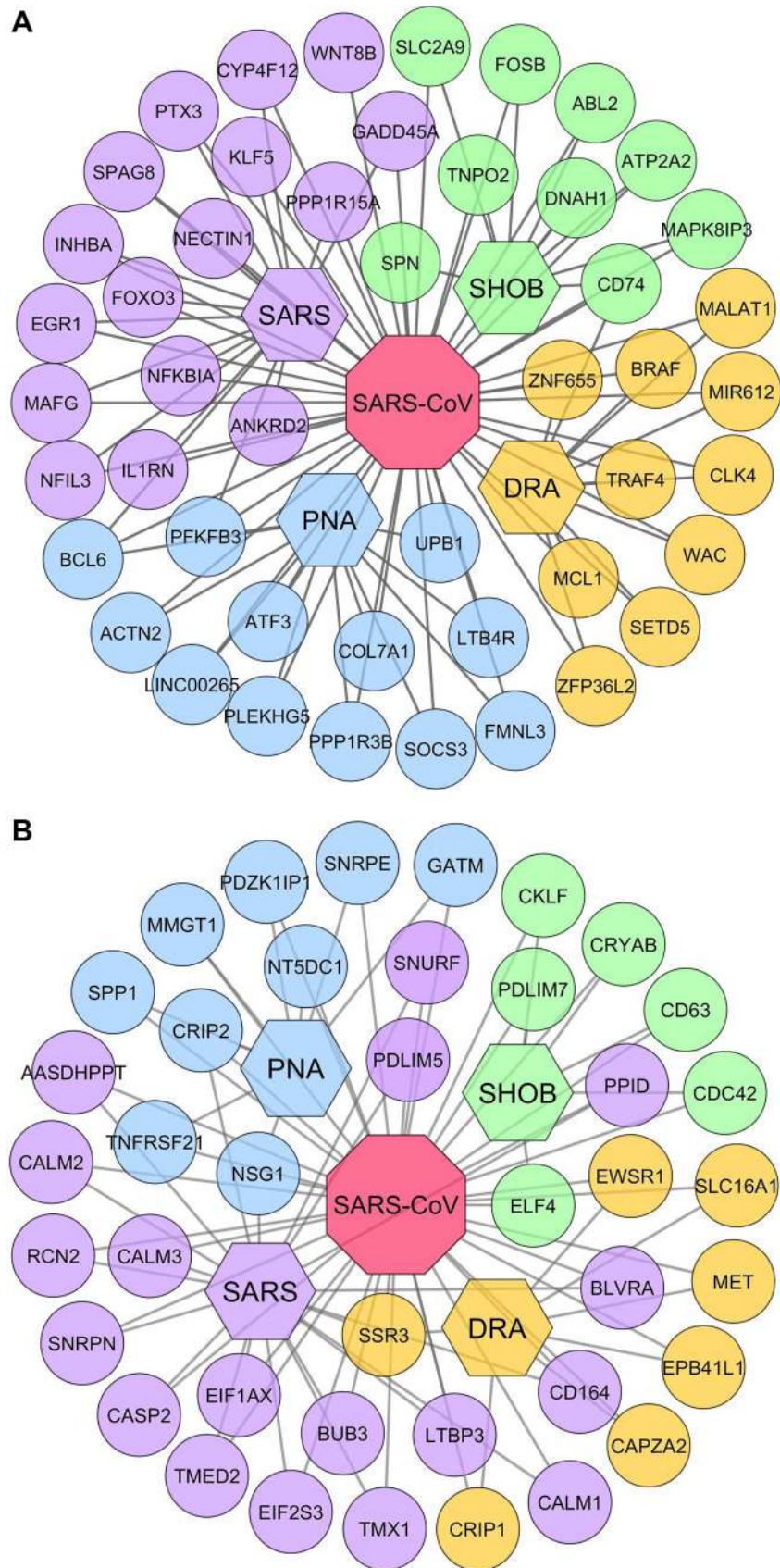**Figure 3**. Infectome–diseasome network. The figure showing the interconnection of the shared DEGs: (A) upregulated genes and (B) downregulated genes. The octagon-and hexagon-shaped nodes represent viral infection (SARS-CoV) and four COVID-19 diseases (i.e. SARS, PNA, SHOB and DRA), respectively, while circular nodes delineate the genes involved. Clusters of different colored nodes indicate the disease-wise gene group.

Table 1. List of shared DEGs with their expression pattern and pathogenetic mechanisms involved in the disease progression

Gene symbol	Name	Category	Expression pattern	Associated conditions	Pathogenetic mechanisms	Infectious agents	Reference
SSR3	Signal sequence receptor subunit 3	Protein	Down	VAD, SARS	Ssr3 is a B-cell activation-related gene and was found to express in plasma and memory B cells in the early stage of COVID-19 recovery. It also has the most complex interacting network with viral proteins.	SARS-CoV-2	[46, 47]
ABL2	Abelson tyrosine-protein kinase 2	Protein	Up	SHOB	Abl2 kinase activity is important for SARS-like viral entry and efficient replication.	SARS-CoV MERS-CoV SARS-CoV2	[48, 49]
CRIP2	Cysteine-rich intestinal protein 2	Protein	Down	PNA, SARS	Crip2 functions in the differentiation of airway smooth muscle and correlated negatively with ACE2 protein.	–	[50]
ATF3	Activating transcription factor 3	Protein	Up	PNA	Atf3 is involved in apoptosis, especially in renal tissue damage and upregulated in MERS and COVID-19 infection.	MERS-CoV SARS-CoV-2	[51, 52]
SPP1	Secreted phosphoprotein 1	Protein	Down	PNA	Spp1 is a significant pro-fibrotic gene which is involved in M2-like macrophage infiltration correlated to COVID-19 severity	SARS-CoV-2	[53]
BCL6	B-cell lymphoma 6	Protein	Up	PNA	Bcl6 regulates the inhibition of antiviral resistance in follicular Th cells, thereby increasing the susceptibility to viral infections.	HIV	[54]
TNFRSF21	TNF receptor superfamily member 21	Protein	Down	PNA, SARS	Tnfrsf21 activates the JNK and NF-κB pathways that eventually lead to a series of inflammatory reactions, and it also promotes cellular apoptosis.	–	[55]
COL7A1	Collagen-type VII alpha 1 chain	Protein	Up	PNA	Col7a1 is upregulated in idiopathic interstitial pneumonia and involved in cell–matrix interactions	–	[56]
FMNL3	Formin-like 3	Protein	Up	PNA	Fmnl3 controls cytoskeleton organization and cell morphogenesis and is implicated in various diseases.	PRRSV	[57, 58]
LINC00265	Long intergenic nonprotein coding RNA 265	lncRNA	Up	PNA	Linc00265 activates PI3K-AKT pathway which is required for viral entry into cells and regulates inflammation.	HSV VACV	[60–61]
LTB4R	Leukotriene B4 receptor	Protein	Up	PNA	Ltb4 overexpression triggers acute asthma attack, and it is important in inflammatory pathways and is highly prevalent in type 1 diabetic patients.	–	[62, 63]

(Continued)

Table 1. Continued

Gene symbol	Name	Category	Expression pattern	Associated conditions	Pathogenetic mechanisms	Infectious agents	Reference
CALM1	Calmodulin 1	Protein	Down	SARS	Calm1 regulates and modulates the cardiac ion channels and renin secretion. It is also involved in viral replication.	HIV	[64, 65]
SOCS3	Suppressor of cytokine signaling 3	Protein	Up	PNA	Socs3 controls the negative feedback mechanism of IL-6 which is highly prevalent in COVID-19 patients.	SARS-CoV-2	[66]
LTBP3	Latent-transforming growth factor beta binding protein 3	Protein	Down	SARS	Ltbp3 enhances lung alveolarization, but nsp12 protein of SARS-CoV-2 can modulate it otherwise.	SARS-CoV-2	[67]
EGR1	Early growth response 1	Protein	Up	SARS	Egr1 activates TGF-β1 promoter via ROS/p38 MAPK/STAT3 pathway by papain-like protease (PLpro) of SARS-like virus.	SARS-CoV	[68]
FOXO3	Forkhead box O3	Protein	Up	SARS	Foxo3 increases tissue-specific polyclonal cytotoxic T-cell expansion by reducing apoptosis and targeted by miR-223 for type 1 IFN production.	SARS-CoV-2 VSV	[69, 70]
GADD45A	Growth arrest and DNA-damage-inducible 45 alpha	Protein	Up	SARS	Gadd45a is a key modulator of the MAPK signaling pathway which promotes cell death and asthma in SARS-like disease.	SARS-CoV-2 SARS-CoV	[71, 72]
IL1RN	Interleukin-1 receptor antagonist	Protein	Up	SARS	Il1rn inhibits IL-1α and IL-1β, thereby modulating inflammatory responses in COVID-19, and remains elevated even after viral clearance.	SARS-CoV-2 MERS-CoV	[73]
INHBA	Inhibin subunit beta A	Protein	Up	SARS	Inhba overexpression induced by influenza virus causes acute respiratory distress-like syndrome in the lung.	H1N1	[75]
KLF5	Kruppel-like factor 5	Protein	Up	SARS	Klf5 regulates IFN-induced transmembrane proteins (IFITM1, 2 and 3) in A549 cells, and IFITM1 is involved in inflammatory bowel disease.	H5N1	[75, 76]
NECTIN1	Nectin cell adhesion molecule 1	Protein	Up	SARS	Nectin1 is a receptor for several viruses and also expressed in COVID-19.	SARS-CoV-2	[77]
NFIL3	Nuclear factor, interleukin-3 regulated	Protein	Up	SARS	Nfil3 plays a critical role in NK cell development and overexpressed in SARS-CoV infection.	SARS-CoV	[78, 79]
NFKBIA	NF-κB inhibitor alpha	Protein	Up	SARS	Nfkbia is a key modulator of IL-1 and TNF-α-related chemokine signaling, thereby contributing to infectious and inflammatory diseases.	SARS-CoV MERS-CoV	[80–82]

(Continued)

Table 1. Continued

Gene symbol	Name	Category	Expression pattern	Associated conditions	Pathogenetic mechanisms	Infectious agents	Reference
PPP1R15A	Protein phosphatase 1 regulatory subunit 15A	Protein	Up	SARS	Ppp1r15a is a translation regulator that controls cytokine production and is upregulated by coronavirus replication and also found in cells with high levels of SARS-CoV-2.	SARS-CoV-2 HCoV-229E	[83, 84]
CD74	Cluster of differentiation 74	Protein	Up	SHOB	Cd74 induces p44/p42 MAPK activation and MIF-mediated pulmonary inflammation. It contributes to the adaptive immunity impairment in COVID-19 patients.	SARS-CoV-2	[85, 86]
ELF4	E74-like ETS transcription factor 4	Protein	Down	SHOB	ELF4 contributes in NK cell development and function as well as cell cycle arrest in naive CD8+ cells upon viral infection.	RSV	[87]
SLC16A1	Solute carrier family 16 member 1	Protein	Down	VAD	Slc16a1 is downregulated in patients with inflammatory bowel disease in response to cytokines TNF-α and IFN-γ. It also plays an important role in SARS-CoV-2 cell entry.	SARS-CoV-2	[88, 89]
BRAF	B-Raf proto-oncogene	Protein	Up	VAD	Braf regulates MAP kinase/ERKs signaling pathway affecting cell division, differentiation and secretion.	–	[90]
MALAT1	Metastasis-associated lung adenocarcinoma transcript 1	lncRNA	Up	VAD	Malat1 targets miR-155 to promote GATA3 and Th2 cytokine production that leads to severe asthma and loss of lung function.	–	[91]
MCL1	Myeloid cell leukemia sequence 1	Protein	Up	VAD	Mcl1 interacts with orf7a of coronavirus and initiates apoptosis process as antiviral defense, and it is upregulated in SARS-like coronaviruses.	SARS-CoV-2 SARS-CoV MERS-CoV	[92]
MIR612	microRNA 612	MiRNA	Up	VAD	miR-612 represses the transcription of IL-8 and ADARB2 and inhibits HIV production in concert with lncRNAs.	HIV	[93]
TRAF4	TNF receptor-associated factor 4	Protein	Up	VAD	Traf is upregulated by coronaviruses, and it connects IL-1R/Toll and TNF receptors activating of NF-κB and MAPK pathways.	SARS-CoV CoV-229E	[94]
ZNF655	Zinc finger protein 655	Protein	Up	VAD	Znf655 may be involved in cell cycle phase transition to facilitate delayed and lack of several antiviral mechanisms observed in COVID-19.	SARS-CoV-2	[95]

(Continued)

Table 1. Continued

Gene symbol	Name	Category	Expression pattern	Associated conditions	Pathogenetic mechanisms	Infectious agents	Reference
CRIP1	Cysteine-rich protein 1	Protein	Down	VAD	Crip1 helps TGF-β to inhibit the Th1 and Th2 differentiation, is involved in ACE2 expression and is downregulated by coronavirus.	SARS-CoV-2	[96, 97]
CALM2	Calmodulin 2	Protein	Down	SARS	Calm2 is involved in calcium signaling which is significantly downregulated in virus-infected ciliated cells.	SARS-CoV-2	[52]
EIF1AX	Eukaryotic translation initiation factor 1A X-linked	Protein	Down	SARS	Eif1ax enhances ribosome dissociation and stabilizes the binding of the initiator Met-tRNA to 40S ribosomal subunit.	SARS-CoV-2	[98]
MAFG	MAF BZIP transcription factor G	Protein	Up	SARS	Mafg is an important transcription factor and a hub-gene in COVID-19 and upregulated upon viral infection.	SARS-CoV-2	[99]
PTX3	Pentraxin 3	Protein	Up	SARS	Ptx3 plays an important role in host defense and inflammatory response in the lung and a protective role in pulmonary infection.	MHV-1 SARS-CoV	[100]
SPAG8	Sperm-associated antigen 8	Protein	Up	SARS	Spag8 is involved in fertilization and compromised in ACE2-positive cells which is targeted by SARS-CoV-2 leading to the disruption of spermatogenesis.	SARS-CoV-2	[101]
EWSR1	Ewing sarcoma breakpoint region 1	Protein	Down	VAD	Ewsr1 interacts with viral cis-acting replication element (CRE) for efficient replication and regulates cell cycle progression and DNA damage.	HCV	[102]
ATP2A2	ATPase sarcoplasmic/ER Ca2+ transporting 2	Protein	Up	SHOB	Atp2a2 is associated with cellular processes important in coronavirus replication.	SARS-CoV MERS-CoV	[103]
FOSB	FosB proto-oncogene	Protein	Up	SHOB	Fosb is a part of activator protein 1 which is central to cytokine storms inflicted by SARS-like viruses and upregulated by their N and 3b proteins.	SARS-CoV MERS-CoV	[104, 105]
MAPK8IP3	Mitogen-activated protein kinase 8-interacting protein 3	Protein	UP	SHOB	Mapk8ip3 plays an essential role in MAPK pathway to facilitate viral replication and can also trigger airway inflammation.	H7N9	[106]
PFKFB3	6-phosphofructo-2-kinase/fructose-2,6-biphosphatase 3	Protein	Up	PNA	Pfkfb3 enhances glycolysis in macrophage glycolysis to promote its antiviral defense (removal and engulfment) but can be resulted in accelerated sepsis while being accompanied by zhx2 protein.	RSV Lentivirus	[107, 108]

*Genes that are studied for their involvement in viral diseases, especially in SARS-like viral infection, were considered, and their roles in respective pathogenesis are tabulated in light of the cited literatures and GeneCards (https://www.genecards.org/), a human gene database.
Abbreviations: DRA, diarrhea; PNA, pneumonia; SHOB, shortness of breath; SARS, severe acute respiratory syndrome; Down, downregulation; Up, upregulation; RSV, respiratory syncytial virus; H7N9, influenza A strain H7N9; MHV-1, coronavirus murine hepatitis virus strain 1; HCV, hepatitis C virus; HIV, human immunodeficiency virus; H5N1, influenza A strain H5N1; H1N1, influenza A strain H1N1; VSV, vesicular stomatitis virus; VACV, vaccinia virus; PRRSV, porcine reproductive and respiratory syndrome virus; ACE2, angiotensin-converting enzyme 2; COVID-19, coronavirus disease 2019.
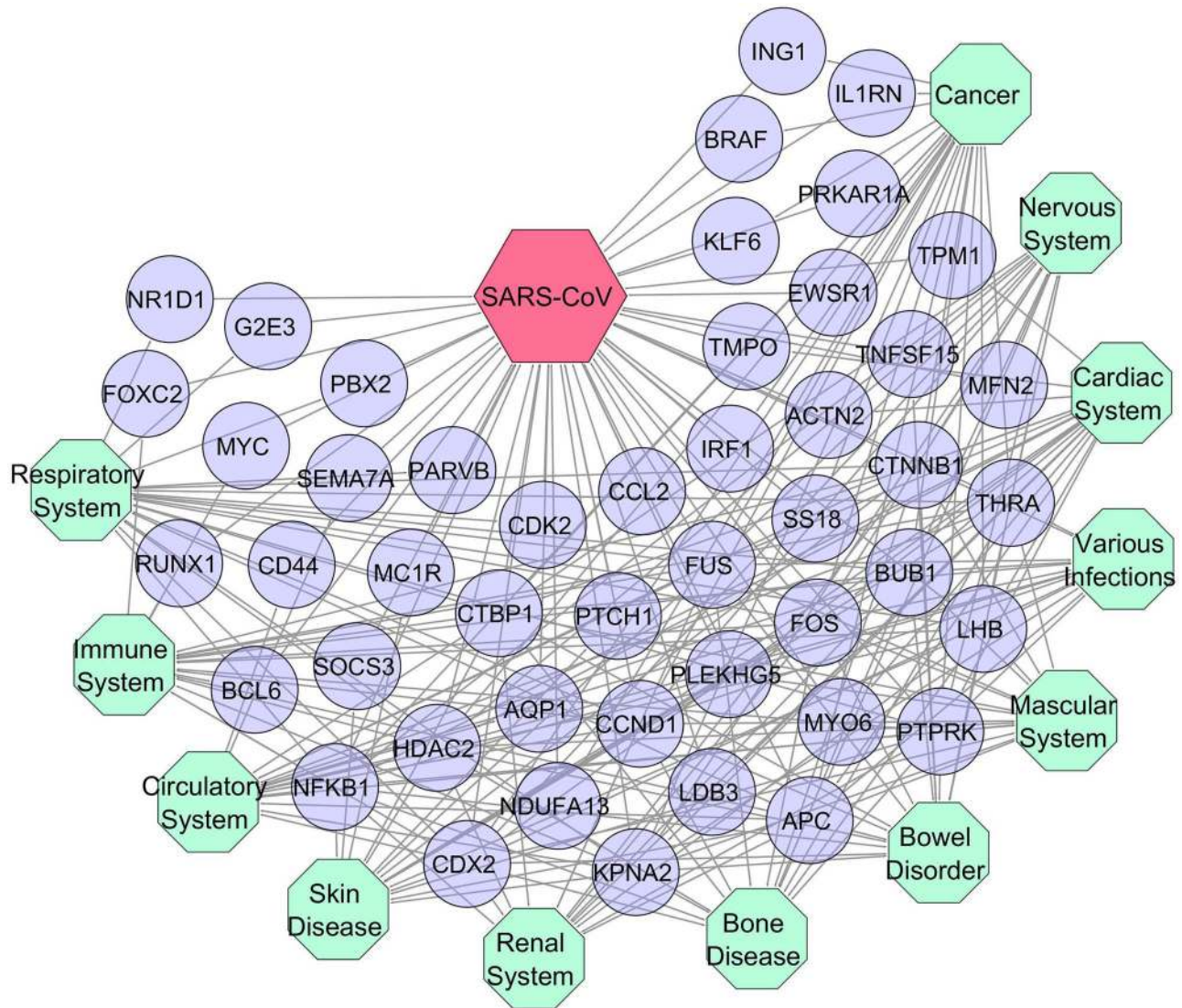
Figure 4. Comorbidity profile of SARS-CoV infection. Dysregulated genes showing association with various types of diseases and comorbidities. Herein, octagon-shaped nodes indicate the different affiliation of SARS-CoV-associated diseases (turquoise), while circular-shaped nodes represent different genes (violet) that are common with the other categories of disorders.

and SHOB shared upregulated *CD74* with SARS-CoV while the *SSR3* gene was downregulated in DRA, in SARS as well as in SARS-CoV infection. However, no downregulated gene related to SHOB was found in the three other conditions.

Comorbidities of SARS-CoV infection

We used genes that are significantly dysregulated upon SARS-CoV infection to construct gene–disease association networks (GDN) to explore the shared pathogenetic projections and comorbidities in COVID-19, as shown in Figure 4. This multi-relational GDN is a bipartite graph consisting of two disjointed sets of nodes in which one set represents the known genetic disorders while others correspond to the significantly altered genes for SARS-CoV infection. The information related to disorders, genes and their possible association that we embedded into the GDN was obtained from OMIM and dbGaP databases (Supplementary File S3). Based on the physiological system affected, we classified the associated disorders into 13

categories including SARS-CoV. Herein, the nodes in the GDN indicate either disease categories or involved genes, and two diseases are related to each other if they have at least one gene in common (Figure 4).

The number of interconnected genes between SARS-CoV and other diseases suggested that cardiac (20), immunological (18), cancer (23) and respiratory (19) diseases are greatly influenced by SARS-CoV and vice versa due to their higher number of shared genes (Figure 4). Interestingly, 14 genes were also common to all included categories of disease such as *APC*, *KPNA2*, *CTNNB1*, *CCND1*, *NFKB1*, *FOS*, *HDAC2*, *PTPRK*, *LHB*, *FUS*, *CTBP1*, *THRA*, *CDX2* and *BUB1* which further indicate their strong association with these diseases.

Functional enrichment

In complex pathological conditions, analysis of signaling pathways can shed light on the underlying molecular mechanisms linking multiple diseases to each other. Therefore, we

Table 2. Signaling pathways involved with the shared dysregulated genes

Pathways	Adj. P-value *	Associated genes
DRA		
Negative feedback regulation of MAPK pathway	5.38×10^{-3}	BRAF, MET
Bile salt and organic anion SLC transporters	1.25×10^{-2}	SLC16A1
Serotonin receptor 4/6/7 and NR3C signaling	1.69×10^{-2}	BRAF
Hemostasis	1.25×10^{-2}	CD74, SLC16A1, CAPZA2
Focal adhesion	1.34×10^{-2}	BRAF, MET
SHOB		
FOSB gene expression and drug abuse	3.74×10^{-3}	FOSB
EGFR downregulation	2.01×10^{-2}	CDC42
MAPK signaling pathway	2.01×10^{-2}	CDC42, MAPK8IP3
TGF-beta signaling pathway	4.29×10^{-3}	CDC42, FOSB
Oxidative damage	2.96×10^{-2}	CDC42
PNA		
Leukotriene receptors	5.24×10^{-3}	LTB4R
Interleukin-6 signaling	1.15×10^{-2}	SOCS3
PERK regulates gene expression	2.90×10^{-2}	ATF3
Osteopontin signaling	1.36×10^{-2}	SPP1
IL22 soluble receptor signaling pathway	1.15×10^{-2}	SOCS3
Regulation of IFN-γ signaling	1.46×10^{-2}	SOCS3
Interleukin-11 signaling pathway	4.52×10^{-2}	SOCS3
SARS		
Chemokine signaling pathway	4.25×10^{-2}	NFKBIA, FOXO3
NRF2 pathway	3.45×10^{-2}	EGR1, MAFG
Non-small cell lung cancer	7.72×10^{-3}	GADD45A, FOXO3
FoxO signaling pathway	2.32×10^{-3}	BCL6, GADD45A, FOXO3
Hypoxia and p53 in the cardiovascular system	4.12×10^{-2}	GADD45A

*Pathways that are relevant to the respective conditions and have adjusted *P*-value ≤ 0.05 were considered.
Abbreviations: DRA, diarrhea; PNA, pneumonia; SHOB, shortness of breath; and SARS, severe acute respiratory syndrome.

analyzed a total of 87 unique shared DEGs from four COVID-19 presentations to determine the significantly involved pathways (Supplementary File S4). Table 2 shows a disease-wise total of 22 significant pathways that are relevant and shared among 4 COVID-19 presentations and SARS-CoV disease with their associated genes and adjusted *P*-values. For example, we identified five pathways including chemokine signaling pathway NRF2 pathway, non-small cell lung cancer, FoxO signaling pathway and hypoxia and p53 in the cardiovascular system that are significantly expressed in SARS condition (Table 2). In addition, we observed five pathways related to SHOB that are FOSB gene expression and drug abuse, EGFR downregulation, MAPK signaling pathway, TGF-β signaling pathway and oxidative damage. In this study, PNA-related significant pathways were mostly associated with cytokine signaling (Table 2); and DRA shared five pathways with SARS-CoV disease.

In addition to pathways, GO properties were anticipated to determine the overrepresented ontological groups among the DEGs (Supplementary File S5). We found 25 significant GO groups that are linked to the highly expressed genes shared by SARS-CoV and COVID-19 diseases. The genes associated with their GO terms and adjusted *P*-values are presented in Table 3. Each of the complication PNA, SHOB and DRA shared six significant pathways with SARS-CoV, while SARS had seven pathways related to SARS-CoV disease (Table 3).

Among the 25 ontological features, notable GO terms were regulation of p38MAPK cascade (GO:1900744), regulation of NF-κB import into the nucleus (GO:0042345), protein kinase binding (GO:0019901), negative regulation of JAK-STAT cascade (GO:0046426), leukotriene receptor activity (GO:0004974), respiratory insufficiency due to muscle weakness (HP:0002747), respiratory failure (HP:0002878), regulation of epidermal growth

factor receptor signaling pathway (GO:0042058), MAPK cascade (GO:0000165) and regulation of ERK1 and ERK2 cascade (GO:0070372). These ontological features were common in SARS-CoV disease and COVID-19 complications. Therefore, they could either be risk factors or regulatory checkpoints in COVID-19 disease.

Hub-proteins and their interactions

Using the shared DEGs, 12 proteins having experimental evidence were characterized as seed and formed an interaction network with the other 122 proteins. These proteins are SOCS3, BUB3, EIF2S3, MET, MCL1, NFKBIA, GADD45A, ATF3, EGR1, FOSB, ATP2A and KLF5. Among these 12 proteins, 7 proteins have interactions with 8 or more other proteins and are identified as hub-proteins (Figure 5). In our study, the majority of hub-proteins were related to SARS such as NFKBIA, BUB3, EIF2S3 and GADD45A, while two proteins (MET and MCL1) belonged to DRA and a single protein, SOCS3, was associated with PNA. However, no hub-protein was found in the shared DEGs for SHOB complication. From Figure 5, we can see the interrelationships among the different diseases, hub-proteins and their protein networks. However, EGR1 of SARS and ATF3 of PNA were intermediately connected, while FOSB, KLF5 and ATP2A2 were non-hub proteins.

Transcription factors and microRNAs

We found 47 miRNAs and 28 TFs that might influence the differential expression of these genes thereby regulating disease progression. Among the 47 miRNAs, four human miRNAs (i.e. let-7c-5p, miR-27b-3p, miR-98-5p and miR-125a-5p) were found to

Table 3. Gene ontological features associated with the shared dysregulated genes

Terms	Pathways	Adj. P-value *	Associated genes
	DRA		
GO:0033674	Positive regulation of kinase activity	1.35×10^{-4}	CD74, TRAF4, MET
GO:0070372	Regulation of ERK1 and ERK2 cascade	1.32×10^{-3}	CD74, BRAF, MET, ZFP36L2
GO:0000165	MAPK cascade	1.86×10^{-3}	BRAF, MET, ZFP36L2, CD74
GO:0071277	Cellular response to calcium ion	3.71×10^{-2}	BRAF
GO:0035023	Regulation of Rho protein signal transduction	5.01×10^{-2}	MET
HP:0002020	Gastroesophageal reflux	3.98×10^{-2}	BRAF
	SHOB		
GO:0070296	Sarcoplasmic reticulum calcium ion transport	6.73×10^{-3}	ATP2A2
GO:0042058	Regulation of epidermal growth factor receptor signaling pathway	4.26×10^{-2}	CDC42
GO:0030544	Hsp70 protein binding	2.01×10^{-2}	SPN
GO:0008009	Chemokine activity	3.39×10^{-2}	CKLF
HP:0002747	Respiratory insufficiency due to muscle weakness	3.54×10^{-2}	CRYAB
HP:0002878	Respiratory failure	4.26×10^{-2}	CRYAB
	PNA		
GO:2000665	Regulation of interleukin-10/13 secretion	6.28×10^{-3}	TNFRSF21
GO:0071345	Cellular response to cytokine stimulus	1.15×10^{-2}	SOCS3, BCL6, TNFRSF21
GO:0006954	Inflammatory response	2.84×10^{-2}	LTB4R, TNFRSF21
GO:0046426	Negative regulation of JAK-STAT cascade	4.82×10^{-2}	SOCS3
GO:0004974	Leukotriene receptor activity	6.28×10^{-3}	LTB4R
HP:0002747	Respiratory insufficiency due to muscle weakness	4.92×10^{-2}	PLEKHG5
	SARS		
GO:0043068	Positive regulation of programmed cell death	1.54×10^{-4}	BCL6, GADD45A, CASP2, FOXO3, PPID
GO:0071345	Cellular response to cytokine stimulus	2.05×10^{-3}	EGR1, IL1RN, BCL6, FOXO3, TNFRSF21
GO:0034599	Cellular response to oxidative stress	2.22×10^{-2}	ANKRD2, FOXO3
GO:1900744	Regulation of p38MAPK cascade	5.08×10^{-2}	GADD45A
GO:0042345	Regulation of NF-κB import into nucleus	5.08×10^{-2}	NFKBIA
GO:0019901	Protein kinase binding	5.01×10^{-5}	PPP1R15A, CALM3, ANKRD2, FOXO3, PDLIM5, CALM1, CALM2
HP:0006530	Interstitial pulmonary disease	2.18×10^{-2}	IL1RN

*Pathways that are highly relevant to the respective conditions and have adjusted *P*-value ≤0.05 were considered.
Abbreviations: DRA, diarrhea; PNA, pneumonia; SHOB, shortness of breath; and SARS, severe acute respiratory syndrome.

be targeting the SARS-CoV-2 genome, while many others were associated with the development of COVID-19 symptoms, i.e. let-7b-5p, miR-155-5p, miR-186-5p, miR-16-5p, miR-27b-3p, miR-29a-3p, miR-30a-5p, etc., as depicted in Figure 6A. Among the 28 TFs, notable TFs are RELA, E2F1, STAT3, TP53, NFKB1, GATA3 and CREB1 as shown in Figure 6B.

Potential drugs and chemical agents

To identify potent drugs and antiviral agents, we used shared DEGs to analyze their interactions with different drugs and chemical agents. Using PDI approach, we found 13 drugs that act against the *MET* gene (Supplementary File S6). Besides, the PCI method predicted 31 potential chemical agents related to 52 genes as shown in Figure 7. Among the 13 drugs, most of them were kinase inhibitors such as antibiotic K-252A, cabozantinib, amuvatinib, crizotinib and SGX-523. Out of the total 31 chemicals, relevant chemical agents were cyclosporine, quercetin, resveratrol, (+)-JQ1 compound, SB-431542 and dorsomorphin.

Discussion

To understand the genetics of COVID-19, we developed a network-based framework where intra- and interconnections among genes and proteins may provide valuable information on their roles in developing certain diseases or conditions. In this study, we first established the genomic closeness and position of 2019-nCoV within the *Coronaviridae* family using the phylogenic approach. In the phylogram, both 2019-nCoV and SARS-CoV formed two clades in a single node which confirms their close relationship. However, it also revealed that 2019-nCoV is not descended from SARS-CoV; rather they have a common ancestral origin. This observation is in line with another recent study that led to the naming of 2019-nCoV as SARS-CoV-2 [7]. Due to higher genetic similarity, all SARS-like coronaviruses (i.e. 2019-nCoV, SARS-CoV, MERS-CoV and CoV-HKU1) formed a separate branch from all other coronaviruses included in Beta-CoV 1, such as HCoV-OC43. This relationship is in line with an earlier report [108]. Furthermore, a recent study reveals that 2019-nCoV shares the highest (79.7%) nucleotide sequence identity with SARS-CoV [109]. This high degree of genomic similarity could be responsible for their analogous pathogenic behavior and clinical disease presentation. Therefore, SARS-CoV might share pathogenic signatures and pathways involved in the progression of COVID-19 upon 2019-nCoV infection, thereby providing the basis for further investigation.

Based on this guiding principle, we identified 87 significant DEGs shared among SARS-CoV infection and 4 COVID-19-related disorders. These genes could be associated with
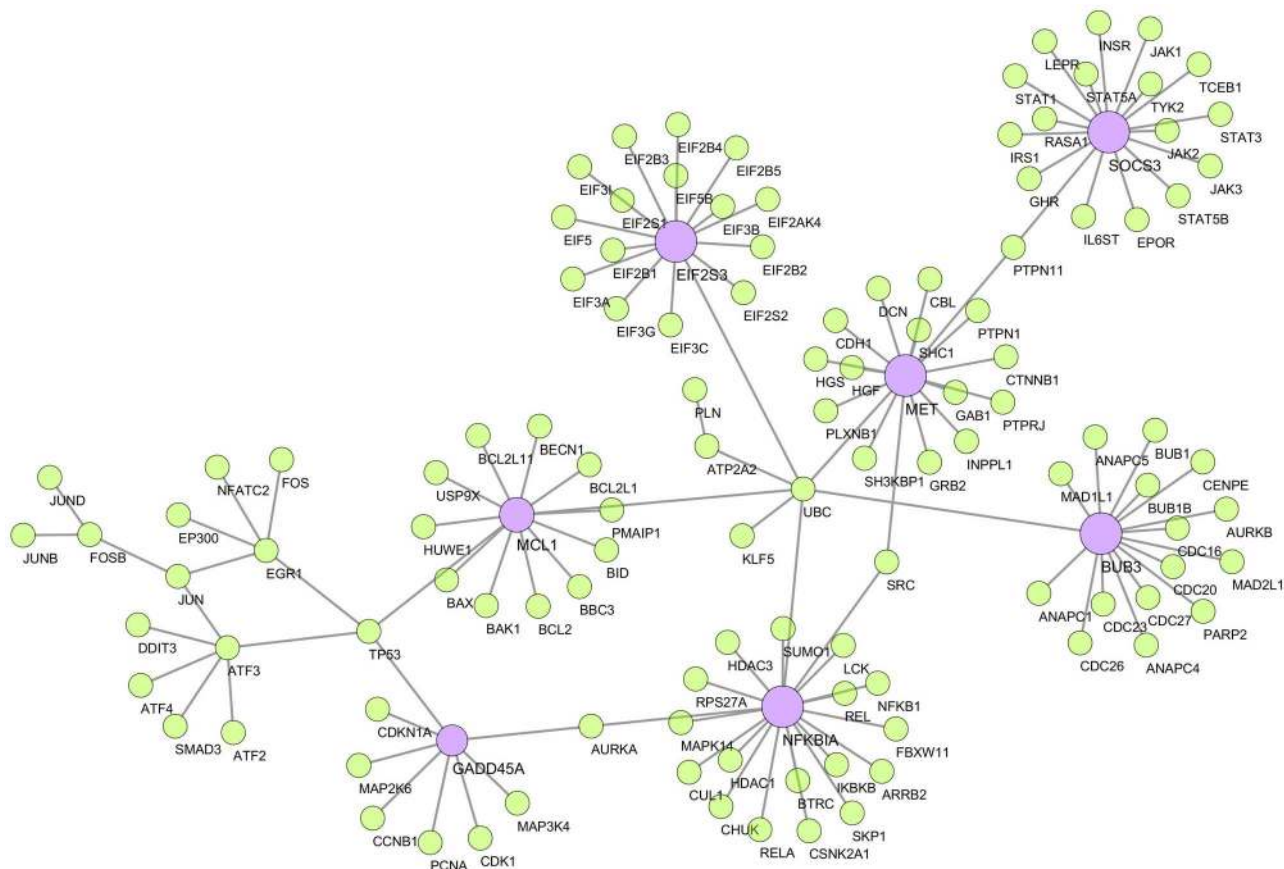
Figure 5. Protein–protein interaction network. The network was created using the experimentally validated STRING interactome with 980 as confidence cutoff. The seven large nodes (violet) indicate the hub-proteins, while smaller nodes (lime) and edges are the associated genes and interactions, respectively.

2019-nCoV infections as well. Therefore, we reviewed the molecular pathogenesis of 44 genes (out of 87 DEGs) that are involved in COVID-19 and other viral infections, especially SARS-like viruses. Intriguingly, the expression pattern and pathogenetic roles of most genes are indifferent to COVID-19 and other viral infections, except for few. In a recent study, for example, the increased level of inflammatory chemokines such as IL-1 and TNF-α has been observed in COVID-19 patients [22]. As we speculated, the upregulation of *NFKBIA* and *FOXO3* could be involved in this process by being the key modulators of IL-1 and TNF-α-related chemokine signaling [81, 110, 111]. In addition, the association of *NFKBIA* gene in SARS-CoV and MERS-CoV infections was found in a previous study [79]. Another gene *Abl2* is important for cell entry and replication of SARS-like viruses [47, 48]. Furthermore, *LTB4* is highly prevalent in type 1 diabetic patients, and overexpression of this gene triggers acute asthma attacks in SARS-related diseases [61, 62]. This also explains the increased susceptibility of diabetic patients to COVID-19. Therefore, they are not merely associated with SARS-CoV rather may have broader roles that might include their involvement in COVID-19 pathogenesis. In addition, we have characterized GO and pathways that are regulated by these genes. About 22 pathways were identified that are linked to SARS-CoV infection and could possibly be involved in COVID-19 as well. For instance, high expression of IL-6 signaling [22, 112] and IFN-γ production [113] was observed in COVID-19 sufferers. Therefore, these pathways could be linked to COVID-19 and may reveal important checkpoints for drug targets. We also identified

25 important GO terms that are related to the clinical conditions of COVID-19. Therefore, the regulation of these pathways might be important in COVID-19 progression. For example, interferon-MAPK pathway-mediated adaptive immune response against COVID-19 was observed through blood single-cell immune profiling [2]. Therefore, it is probable that COVID-19 disease progression might be mediated by those and other significant pathways.

To validate our findings, significant DEGs were employed to predict gene–disease association using benchmark databases. In doing so, we created a GDN network as comorbidities of SARS-CoV, to understand the possible COVID-19 projections and risk factors. Comorbidities may result from pathogenic relationships or common risk factors among concomitant disorders [114]. Apart from the respiratory disease, the anticipated comorbid profiles suggested a wide array of diseases involved in 2019-nCoV infection including cardiac, renal, brain and blood-related problems as well as different types of cancer. For example, 17 genes were found to be associated with blood diseases including thalassemia, thrombocytopenia, hypertension, thrombophilia and leukemia. Strikingly, a recent meta-analysis included thrombocytopenia as a comorbidity of COVID-19 [115]. Very recently, unusual blood clots have been found in multiple organs causing strokes and organ failures [116, 117]. This could be due to thrombophilia, an abnormal blood clotting disorder caused by altered expression of the associated genes upon 2019-nCoV infection. On the other hand, comorbidities related to brain diseases include encephalopathy, dementia and Leigh
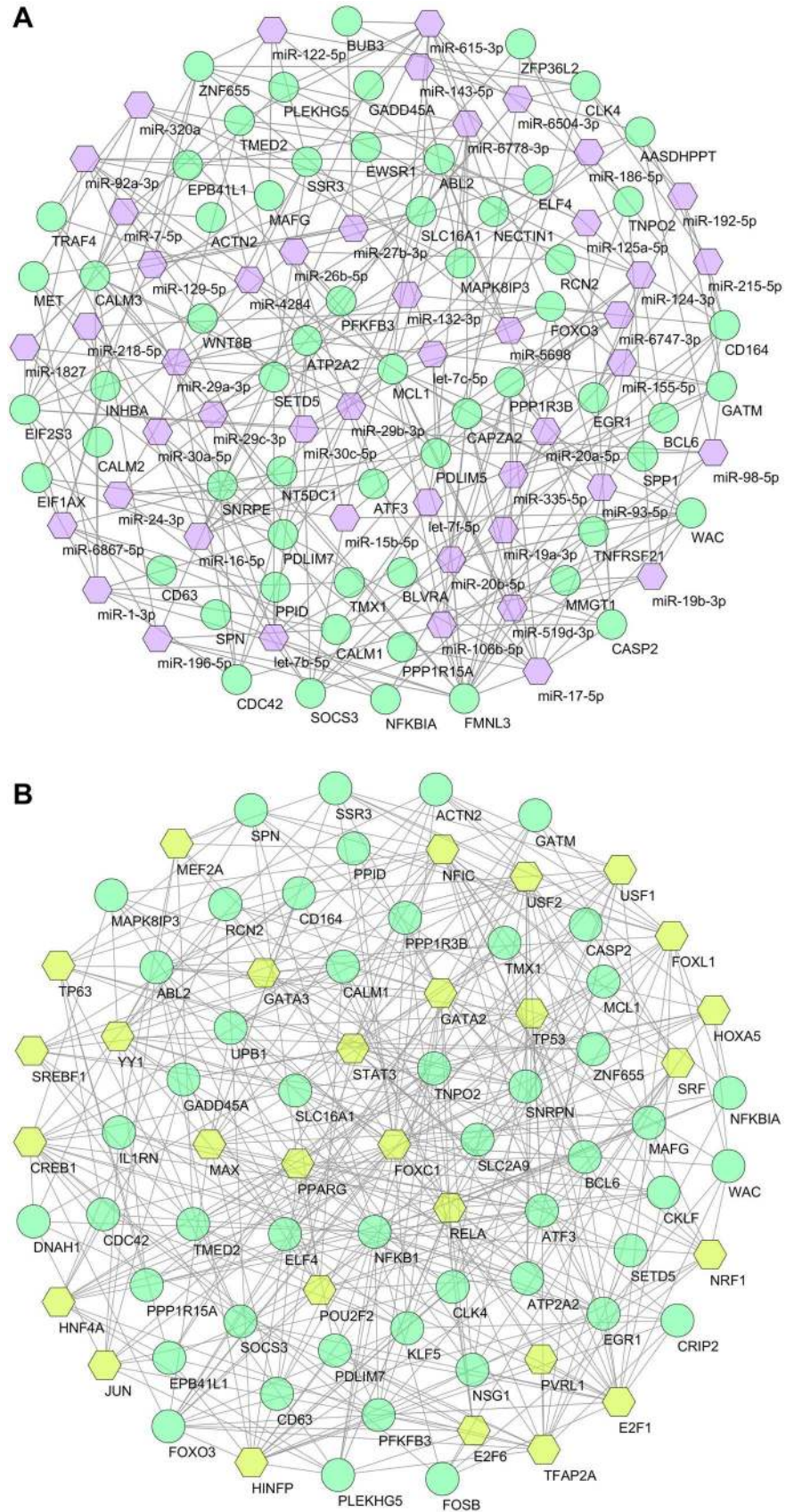
Figure 6. Regulatory networks of gene–biomarker interactions. (A) Interacting network of miRNAs and (B) interacting network of TFs. Both networks were filtered with degree centrality ≥5 and betweenness centrality ≥35, where circles are dysregulated genes (turquoise) and hexagons are associated miRNAs (violet) or TFs (lime).
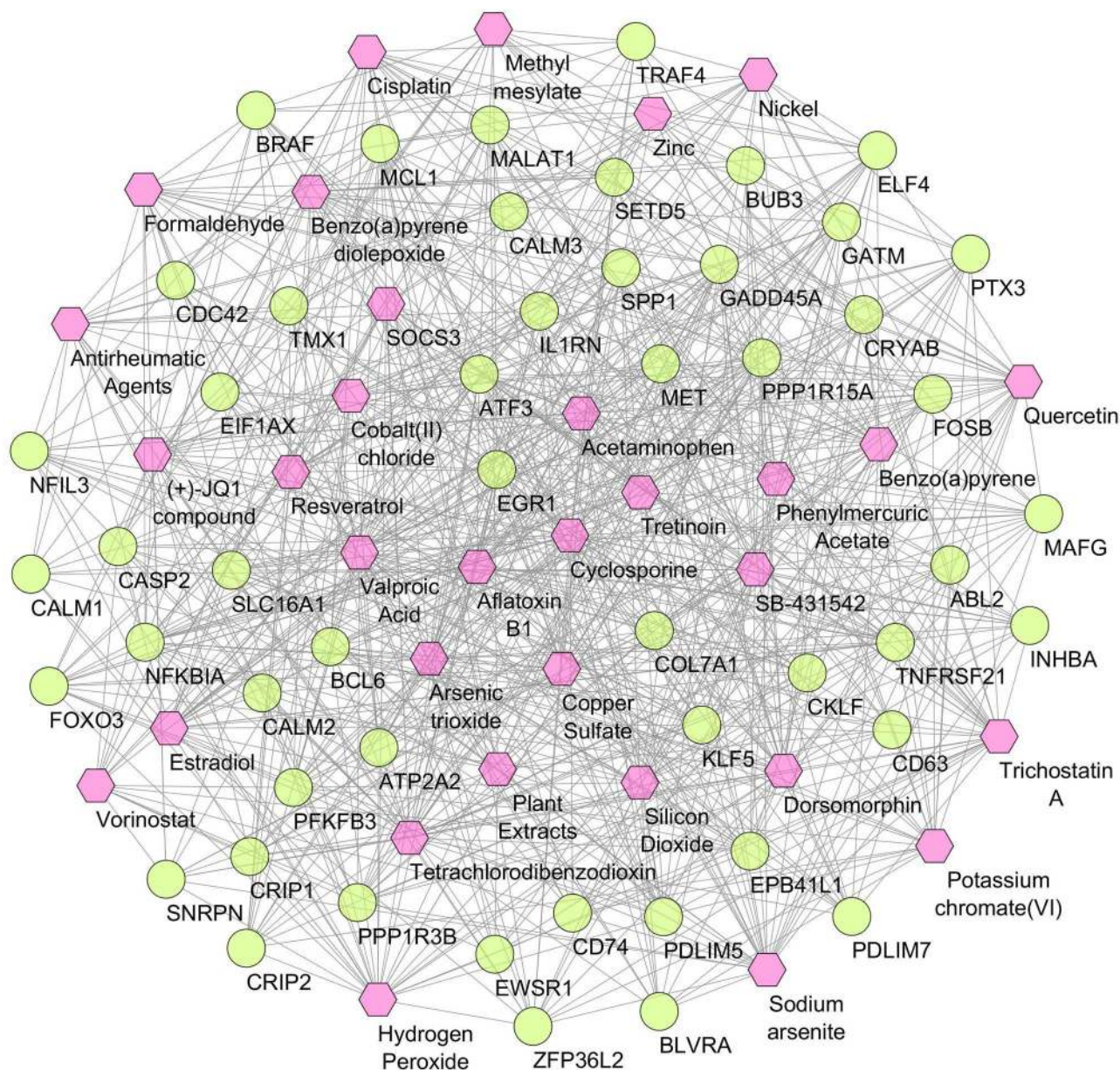
Figure 7. Protein–chemical interaction network. Of the total 83 nodes, circles represent the shared dysregulated genes (lime), while hexagons indicate the interacting chemical agents (pink). Degree centrality ≥20 and betweenness centrality ≥20 were considered for this network.

syndrome. Interestingly, a recent study suggested that COVID-19 could lead to acute hemorrhagic necrotizing encephalopathy, which is in line with our findings [118].

Furthermore, a study suggested hypertension as a potential risk factor in 2019-nCoV infection [119]. Two recent studies from China have shown a strong association between hypertension and COVID-19 deaths (Hazard ratio 1.70 to 3.05), raising concerns that hypertension is confounded by treatment with specific antihypertensive medications such as ACE inhibitors (ACEIs) and angiotensin receptor blockers (ARBs) [120, 121]. In response, the European Society of Cardiology Council on Hypertension made the following statement, 'The Council on Hypertension strongly recommends that physicians and patients should continue treatment with their usual anti-hypertensive therapy because there is no clinical or scientific evidence to suggest that

treatment with ACEIs or ARBs should be discontinued because of the COVID-19 infection [123]'. According to the American College of Cardiology (ACC) clinical bulletin, the fatality rate in COVID-19 is higher in patients with preexisting conditions such as cancer (5.6%), hypertension (6.0%), cardiovascular diseases (10.5%) and diabetes (7.3%) [123–125]. Furthermore, about 16.7% of patients developed arrhythmia, and 7.2% experienced an acute cardiac injury in addition to 2019-nCoV-associated complications [123]. These cardiovascular complications may result from various mechanisms, including relative ischemia, systemic inflammation and pathogen-mediated damage [126]. Interestingly, we identified 20 genes that are involved in different cardiovascular diseases and 16 genes related to diabetes mellitus (type 1 and 2). Evidence to date suggests that people who died of COVID-19 were more likely to have comorbidities such as

hypertension, diabetes, cardiovascular disease or chronic lung disease [127, 128]. Further research on these genes could reveal long-term cardiovascular implications of viral infection, thus closing the significant knowledge gaps in the recent COVID-19 outbreak.

Using the shared genes, a PPI network was constructed to pinpoint the key disease-modifying agents in COVID-19 in light of SARS-CoV pathobiology. Hubs are defined as proteins with eight or more interactions, while proteins with less than four interactions are named non-hubs and the rest are intermediately connected [129]. We identified seven hub-proteins (MCL1, NFKBIA, BUB3, MET, EIF2S3, GADD45A and SOCS3) involved in SARS-CoV and COVID-19 diseases. The protein NFKBIA regulates inflammatory responses and plays an important role in the pathogenesis of acute respiratory distress syndrome [130]. Another hub-protein GADD45A is known to play a significant role in lung injury due to inflammation [131, 132]. Furthermore, the expression of SOCS3 downregulated JAK2/STAT3 pathway to promote macrophage polarization that plays a key role in lung inflammation [133]. Therefore, these hub-proteins can be regarded as candidate biomarkers or, if their biological role in COVID-19 is confirmed, as potential drug targets. Furthermore, TFs control the rate of transcription [134], while miRNAs are key players in RNA silencing and regulation of gene expression at the posttranscription level [135]. Hence, both are essential to understand particular disease development. In this regard, this study uncovered relationships among the shared DEGs and their respective TFs and regulatory miRNAs. Herein, we identified several TFs, such as STAT3, NFKB1A, E2F1, TP53, GATA3 CREB1, which are known to involve in viral-mediated acute respiratory diseases [86, 136, 137]. Further, some miRNAs associated with lung diseases (i.e. let-7b-5p and let-7c-5p), asthma (i.e. miR-155-5p, miR-186-5p, miR-16-5p, miR-27b-3p) and pneumonia (i.e. miR-29a-3p and miR-30a-5p) were also found highly prevalent in COVID-19 [86, 138–140].

TFs and miRNAs usually target host proteins to alter their expression during the development of specific diseases. For coronavirus, for instance, miR-98 modulates the host inflammatory response by interacting with STAT3. For other respiratory viruses, let-7b and let-7c target STAT3, while miR-30a influences TP53 [86]. Both are regulatory TFs that are identified as important biomarkers in this study. Furthermore, miR-16 and miR-155 modulate inflammatory responses by targeting the MCL1 and NFKBIA hub-proteins [86]. Interestingly, we also identified four miRNAs (let-7c-5p, miR-125a-5p, miR-27b-3p and miR-98-5p) that are known to target different genes of 2019-nCoV [141, 142]. For instance, let-7c-5p targets ORF1ab gene of 2019-nCoV and inhibits viral replication [142]. Conversely, the spike protein from SARS-CoV may downregulate the expression of miR-98 in bronchoalveolar stem cells to control their differentiation and inflammatory cytokine production. Moreover, we identified two long noncoding RNA (lncRNA) in our shared DEGs, i.e. MALAT1 and LINC00265. These lncRNAs may act in concert with miRNAs to orchestrate the competing endogenous RNA (ceRNA) network in disease progression. For instance, upregulation of MALAT1 ceases miR-155 expression which promotes GATA3 and Th2 cytokine production leading to severe asthma and loss of lung function [90]. On the other hand, LINC00265 inhibits let-7a activity using ceRNA mechanism, which results in the upregulation of IL6 [143]. All these eventually lead to the deterioration of lung tissues and successful viral replication [144].

In addition to regulatory molecules, we identified 13 MET gene-targeting drugs using PDI method that are mostly kinase inhibitors. Kinases play a key role in the airway muscle

contractions and in the release of pro-inflammatory mediators [145]. Therefore, our results suggest plausible mechanisms, since most of the proteins are related to inflammatory responses. Nevertheless, we excluded these drugs since they have multiple known side effects including hypertension, diarrhea, anorexia and nausea [146]. Furthermore, we anticipated PCI network to identify the chemical agents that could act as potential antagonists. Among the total 31 chemical agents, we could find the potent antagonists by targeting the hub-proteins that are linked to COVID-19 progression as stated above. Previously, several drugs and chemical agents showed potent therapeutic action against 2019-nCoV. For example, remdesivir and chloroquine have been reported to inhibit 2019-nCoV and other SARS-like viruses [147]. Furthermore, a Japanese flu–drug favipiravir showed a significant protective effect against COVID-19 [148]. Moreover, a recent nonrandomized clinical trial showed a promising effect of hydroxychloroquine in concert with azithromycin against 2019-nCoV by inhibiting their genomic replication [149, 150]. Although hydroxychloroquine possesses less toxicity, prolonged and overdose usage of this anti-inflammatory agent can still cause poisoning [151]. Alternatively, the chemical agents identified in this study can be checked against 2019-nCoV or its disease form COVID-19. For example, we identified quercetin which is a potent antiviral and anti-inflammatory agent active against different strains of influenza virus (i.e. H1N1 and H3N2) [152]. Another identified chemical antagonist was resveratrol in our study. Interestingly, resveratrol is a prominent antiviral agent which showed antiviral activity against several human and animal viruses [153, 154]. Most importantly, Lin et al. showed resveratrol-mediated effective inhibition of MERS-CoV due to the downregulation of apoptosis [155]. As we stated earlier, overexpression of NF-κB is central to asthma and other pulmonary diseases [156]. Resveratrol inhibits the expression of NF-κB and related pathways, thereby subduing the conditions [154]. Another major identified compound is cyclosporine, which is known to inhibit the replication of RNA viruses including SARS-CoV, MERS-CoV and CoV-229E by targeting cyclophilins [157, 158]. Cyclophilins regulate the replication of RNA viruses and are usually overexpressed in viral infections as well as in inflammatory diseases such as asthma [157–159]. Importantly, a recent study includes resveratrol and cyclosporine A in the list of therapeutic options for 2019-nCoV [160]. Overall, these data are of clinical interest and may shed light on the cause and progression of COVID-19, as well as any new prospective therapeutic strategies.

Conclusion

The present study highlights molecular insight on potential biomarkers, regulatory components and molecular checkpoints that may help in developing therapeutics to combat the current 2019-nCoV pandemic. Based on gene expression analysis, we highlight significantly expressed genes with possible projections and risk factors associated with COVID-19, in the form of comorbidities. These identified biomarkers could be potential drug targets based on their role in regulatory checkpoints and disease progression. Furthermore, potent chemical agents that are identified could be used to target-specific disease-modifying factors. The comorbidity analysis presented here provides disease mechanistic insight and prognostic features relevant in COVID-19 disease. These data will aid in unlocking new opportunities for novel therapeutics to control this, as well as future viral outbreaks.

Key Points
- The 2019-nCoV and SARS-CoV have a common ancestral origin, and their pathobiological profiles could have a number of significantly dysregulated genes in common. These could be compared with upcoming COVID-19 transcriptomes to assess the distinct genetic mechanisms involved in a disease.
- Comorbidity profiles and associated genes could be evaluated for their roles in recently observed clinical presentations and future implications of COVID-19.
- In protein–protein network analyses, identified hub-proteins can lead us to co-expression partners related to normal and disease states, which can help in the assessment of risk factors related to COVID-19 complications.
- TFs and miRNAs identified as biomarkers can be used as prognostic signatures to differentiate patients and healthy individuals. Their role as disease-modifying traits can further be explored.
- Signaling pathways that are common to SARS-CoV and COVID-19 presentations can be used as molecular checkpoints to develop therapeutics against 2019-nCoV infection.
- Protein–chemical interactome analysis suggests cyclosporine, resveratrol and quercetin as potential chemical antagonists against COVID-19 as well as SARS-like viral infections.

Supplementary data

Supplementary data are available online at https://academic.oup.com/bib.

Authors' contributions

M.A.M. and Z.N. conceived and designed the study; Z.N. performed the computational analyses, prepared the illustrations and wrote the manuscript; H.K.R. helped in the experimental procedure and draft preparation; P.L., M.A.S., S.M.S.I. and M.A.M. contributed in the critical analysis and revision; M.A.M. supervised the whole study. All authors approved the final version of the manuscript.

Acknowledgements

We thank all those who have contributed in 2019-nCoV genome sequences deposited to the GISAID database (https://www.gisaid.org/) and provided ideas to the Virological website (http://virological.org/).

Conflict of interest

The authors declare no conflict of interests.

Ethical statement

Not applicable.

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