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ORIGINAL ARTICLE

# Uncovering the genetic links of SARS-CoV-2 infections on heart failure co-morbidity by a systems biology approach

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## Abstract

**Aims** The co-morbidities contribute to the inferior prognosis of COVID-19 patients. Recent reports suggested that the higher co-morbidity rate between COVID-19 and heart failure (HF) leads to increased mortality. However, the common pathogenic mechanism between them remained elusive. Here, we aimed to reveal underlying molecule mechanisms and genetic correlation between COVID-19 and HF, providing a new perspective on current clinical management for patients with co-morbidity.
**Methods** The gene expression profiles of HF (GSE26887) and COVID-19 (GSE147507) were retrieved from the GEO database. After identifying the common differentially expressed genes ($|\log2FC| > 1$ and adjusted $P < 0.05$), integrated analyses were performed, namely, enrichment analyses, protein–protein interaction network, module construction, critical gene identification, and functional co-expression analysis. The performance of critical genes was validation combining hierarchical clustering, correlation, and principal component analysis in external datasets (GSE164805 and GSE9128). Potential transcription factors and miRNAs were obtained from the JASPER and RegNetwork repository used to construct co-regulatory networks. The candidate drug compounds in potential genetic link targets were further identified using the DSigDB database.
**Results** The alteration of 12 genes was identified as a shared transcriptional signature, with the role of immune inflammatory pathway, especially Toll-like receptor, NF-kappa B, chemokine, and interleukin-related pathways that primarily emphasized in response to SARS-CoV-2 complicated with HF. Top 10 critical genes (TLR4, TLR2, CXCL8, IL10, STAT3, IL1B, TLR1, TP53, CCL20, and CXCL10) were identified from protein–protein interaction with topological algorithms. The unhealthy microbiota status and gut–heart axis in co-morbidity were identified as potential disease roads in bridging pathogenic mechanism, and lipopolysaccharide acts as a potential marker for monitoring HF during COVID-19. For transcriptional and post-transcriptional levels, regulation networks tightly coupling with both disorders were constructed, and significant regulator signatures with high interaction degree, especially FOXC1, STAT3, NF-κB1, miR-181, and miR-520, were detected to regulate common differentially expressed genes. According to genetic links targets, glutathione-based antioxidant strategy combined with muramyl dipeptide-based microbe-derived immunostimulatory therapies was identified as promising anti-COVID-19 and anti-HF therapeutics.
**Conclusions** This study identified shared transcriptomic and corresponding regulatory signatures as emerging therapeutic targets and detected a set of pharmacologic agents targeting genetic links. Our findings provided new insights for underlying pathogenic mechanisms between COVID-19 and HF.

**Keywords** Heart failure; COVID-19; Genetic links; Molecular mechanism; Computational biology

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# Introduction

Coronavirus (COVID-19) has emerged as a global epidemic, significantly impacting patients worldwide. There are over 486.8 million confirmed cases, and the deaths exceed 6.1 million since December 2019 (https://covid19.who.int/). The pathogen responsible for this pneumonia is SARS-CoV-2, a highly contagious coronavirus.[1] The human heart has higher expression of SARS-CoV-2 viral targets angiotensin-converting enzyme 2 (ACE2) than lung, increasing infection susceptibility and exacerbating pre-existing cardiovascular conditions through potential gene–pathway associations.[2–4]

Heart failure (HF) is a progressive disorder induced by compromised cardiac function and structure. Single-cell profiling showed elevated expression of ACE2 in pericytes of HF patients. The SARS-CoV-2 infection could cause myocardial aggressiveness, specifically increased vulnerability to the pericytes-mediated microcirculation disorder, increasing the risk of heart attack and severe consequences.[5,6] Multiple studies have revealed that HF, as a common complication, is associated with poor prognosis of COVID-19 patients.[7–9] And elevated levels of chronic HF markers in COVID-19 condition are linked to high mortality. Therefore, finding a genetic link between COVID-19 and HF mortality is critical.[2,10]

Angiotensin-converting enzyme inhibitors (ACEIs) and angiotensin II receptor blockers (ARBs) are regularly used for treating HF patients with COVID-19. Considering that ACEI/ARBs may upregulate ACE2, the SARS-CoV-2 entry point, the impact of those drugs on COVID-19 individuals with cardiovascular diseases is still debated.[11] To date, identifying an appropriate option for treating SARS-CoV-2 remains challenging due to the adverse cardiovascular events induced by promising antiviral therapies. Although several potential drug candidates, including immunomodulatory and antiviral therapy, have recently been evaluated, the therapeutic efficiency is unclear.[12] Because of the fact that SARS-Cov-2 use it as the entry point, ACE2 has been proposed as a promising therapeutic target for co-morbidity.[13] For instance, recombinant human ACE2 (rhACE2) has been demonstrated to prevent SARS-CoV-2 entry into target cells, and several investigations have indicated that infusing soluble rhACE2 could achieve cardioprotective properties, improving the prognosis of SARS-CoV-2 patients with cardiovascular co-morbidities.[14,15] Unfortunately, mono-targeted therapeutic efficacy is limited. As a result, a comprehensive review of the potential targets based on shared pathogenesis between COVID and HF could be beneficial for future therapy development.

So far, high-throughput sequencing analysis has been implemented to investigate SARS-CoV-2, exhibiting prominent data quality assessment in molecular pathogenic mechanisms.[16–18] The co-morbid effects of COVID-19 with multiple diseases[19–22] have been studied using bioinformatics, helping researchers establish optimal clinical management. However, the pathogenetic and genetic correlation between COVID-19 and HF is overlooked. Therefore, this study revealed molecule mechanisms underlying both disorders and pharmacologic agents targeting genetic links through a systems biology approach based on high-throughput data from biopsies samples. We hope this study could help researchers understand the genetic link between two diseases, which contributes to the future COVID-19 treatment.

# Methods

Please see the Supporting Information for a detailed methods section.

# Results

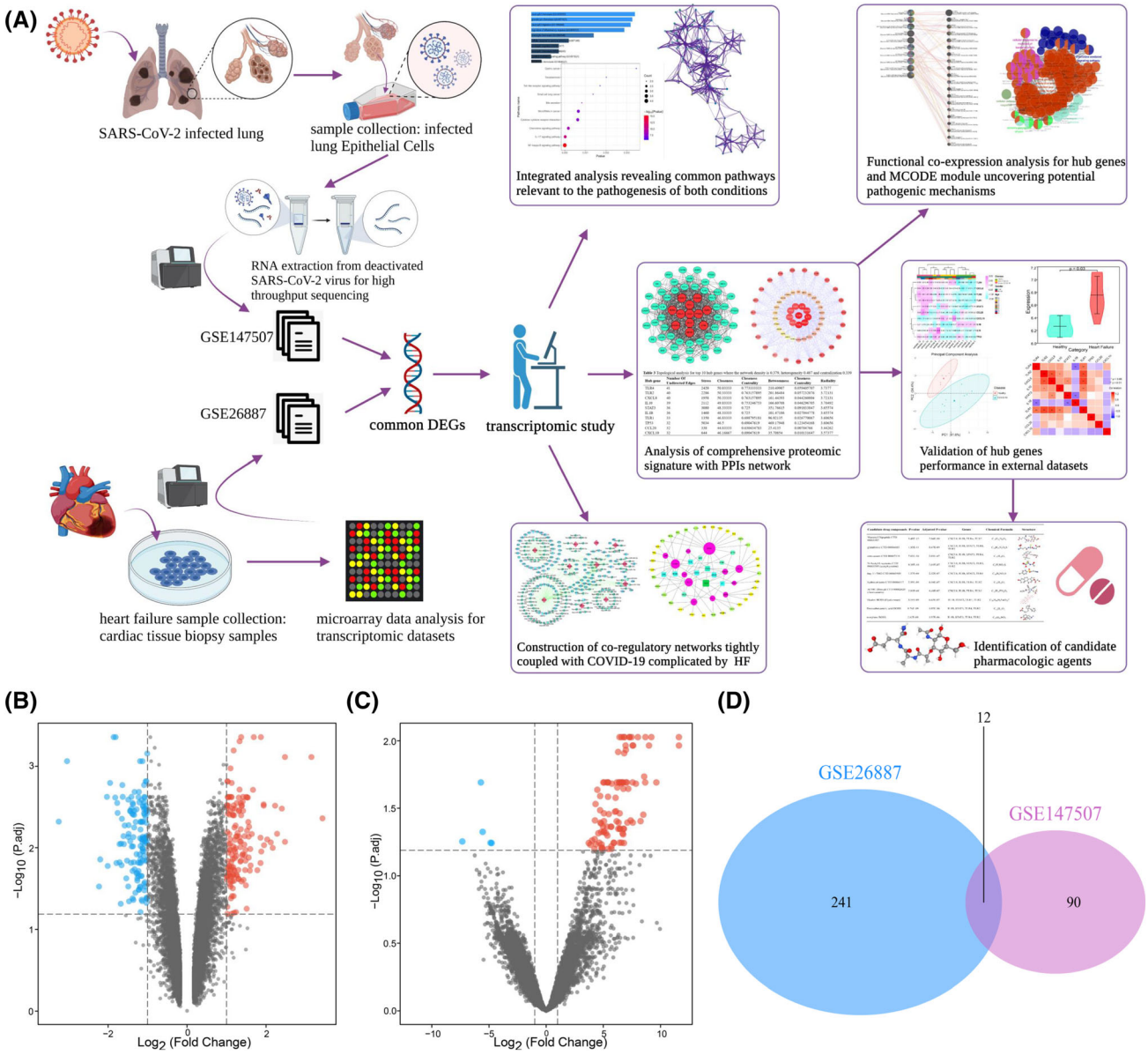
## Identification of shared transcriptomic signature between HF and COVID-19

As shown in the flow chart (*Figure 1A*), all data from two independent datasets (GSE147507: COVID-19 and GSE26887: HF) were obtained from the GEO database. Differentially expressed genes (DEGs) were extracted from both datasets using absolute log2FC cut-off 1 and adjusted *P*-value cut-off 0.05. There were 102 DEGs between SARS-CoV-2 infection patients and normal individuals. Meanwhile, a total of 253 DEGs were confirmed between HF and normal individuals. To provide a better visualization, the dysregulated genes for HF and COVID-19 were presented as volcano plots (*Figure 1B* and *1C*). We followed this up with a comparative analysis to determine the common DEGs between HF and SARS-CoV-2 infection responses and found 12 consensus genes (S100A8, ABCB1, S100A11, COX2, LY96, XCL1, RORA, CCL11, CCL4, S100A12, BCL2, and AQP9) that were integrated into the downstream analysis (*Figure 1D*).

## Analysis of functional characteristics relevant to common pathogenesis of both conditions

To interpret the functional overview of the shared transcriptomic signature between HF and COVID-19, the over-representation analysis was utilized to identify enriched functional terms via a set of databases (GO, Reactome, KEGG, WikiPathways, and BioCarta). The Top 10 GO terms for each of the three predominant categories of gene ontology (molecular functions, cellular component, and biological process) were listed in *Table S1*. As shown in *Figure 2A*, the biological process was highly associated with neutrophil chemotaxis, neutrophil migration, monocyte chemotaxis, regulation of inflammatory responses, and eosinophil chemotaxis. Top molecular function processes involving CCR

**Figure 1** (A) The predominant methodical workflow in the current study. Based on the cut-off criteria: absolute log2FC > 1 and adjusted *P* value < 0.05. DEGs in GSE26687 (B) and GSE147507 (C) were shown in the volcano plot, with red colour representing significantly high expression genes and blue dots representing significantly down-regulated genes. (D) Venn diagram depicted concordant genes from the intersection of two datasets GSE26687 and GSE147507.




chemokine receptor binding, chemokine activity, RAGE receptor binding, and Toll-like receptor binding were also identified. The cellular component analysis revealed significant enrichment of cytoplasmic vesicle lumen and secretory granule lumen in consensus genes.

Top enriched shared pathways were summarized in *Table S2*. Notably, shared genes for classic immune inflammatory pathways such as NF-κB, IL-17, and cytokine–cytokine receptor interaction were observed (*Figure 2B*). This coincides with the prominent early feature in SARS-CoV-2, activation of NF-κB caused by innate immunity activation, producing the initiation of cytokine and chemokine cascades.[23] It has been confirmed that after SARS-CoV infection, elevated levels of chemokines CCL2, CXCL10, IL-6, and IL-8 were associated with acute respiratory distress syndrome deterioration.[24] The chemokine pathway and Toll-like receptor signalling pathway, including Reactome, KEGG, WikiPathways, and GO, were among the top enriched overlapping pathways identified


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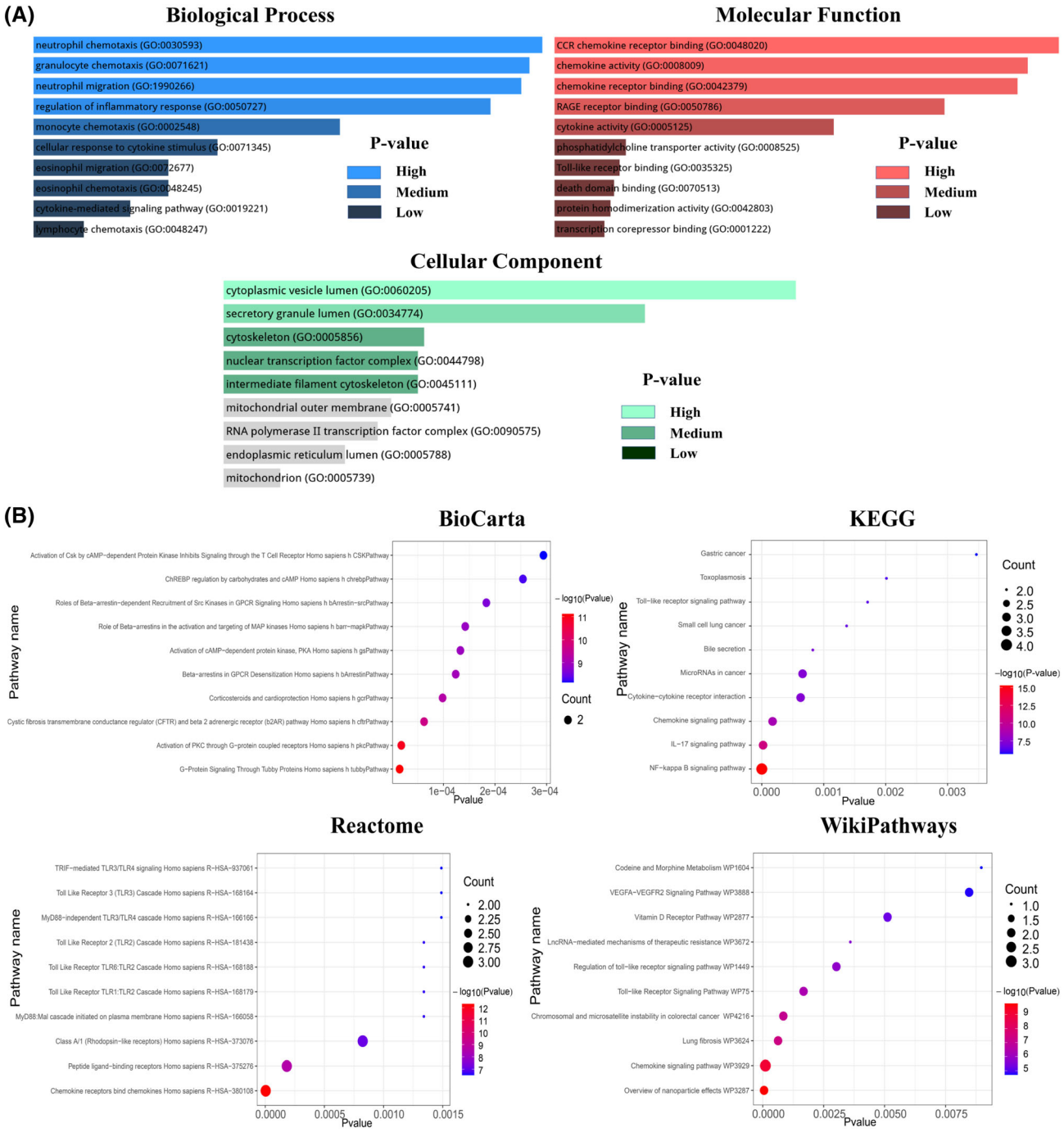
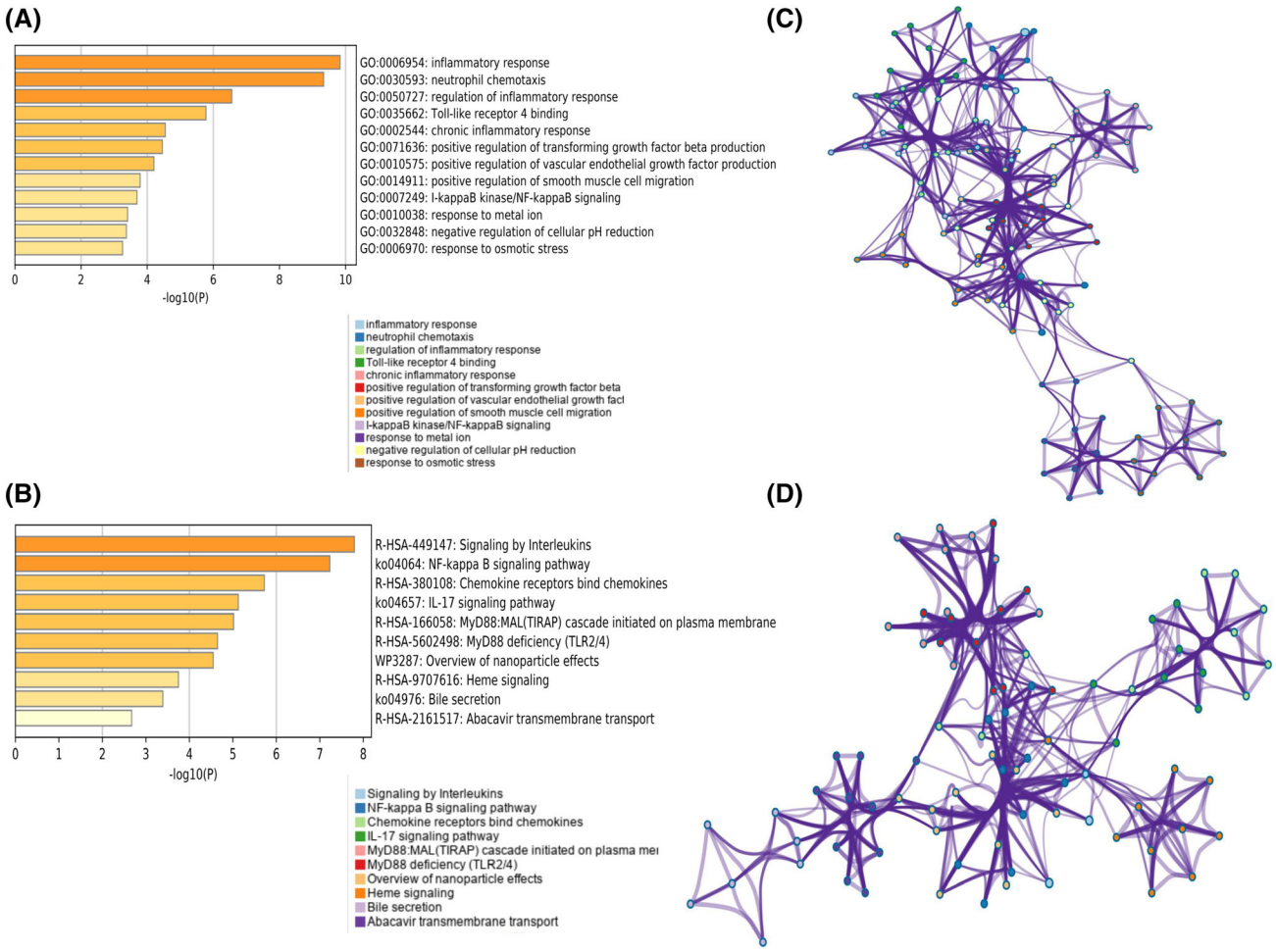
**Figure 2** (A) The bar plot showing the Top 10 GO pathways between COVID-19 and HF regarding molecular function, biological process, and cellular component based on the adj *P* value. (B) The top enrichment pathways from various databases were presented as bubble maps.




through multiple databases, which suggested that CCL11, CCL4, XCL1, and IL-17 might correlate with COVID-19 progression.

A functional annotation network using the Metascape was further analysed with overlapping genes to ensure the accuracy and reliability of the results. As shown in (*Figure 3A and 3B*), similarly, immune-related genes were up-regulated. Interestingly, neutrophil chemotaxis and inflammatory response presented significantly higher interactions with other network clusters, suggesting that

**Figure 3** (A) Identification of top GO clusters coloured by *P* values using Metascape enrichment analysis. (B) Bar chart showing the outcome of pathway enrichment analysis. (C) GO interaction network. The GO-enriched terms were represented by circle nodes, the size of which was proportional to the number of consensus genes related to the term, and the nodes of the same colour represented the same identity. Only terms with a similarity score of >0.3 were connected by edges. (D) The enrichment pathway cluster graph represented pathways as circle nodes coloured by each term identity. Moreover, node size scaled with the number of common DEGs falling into that pathway.

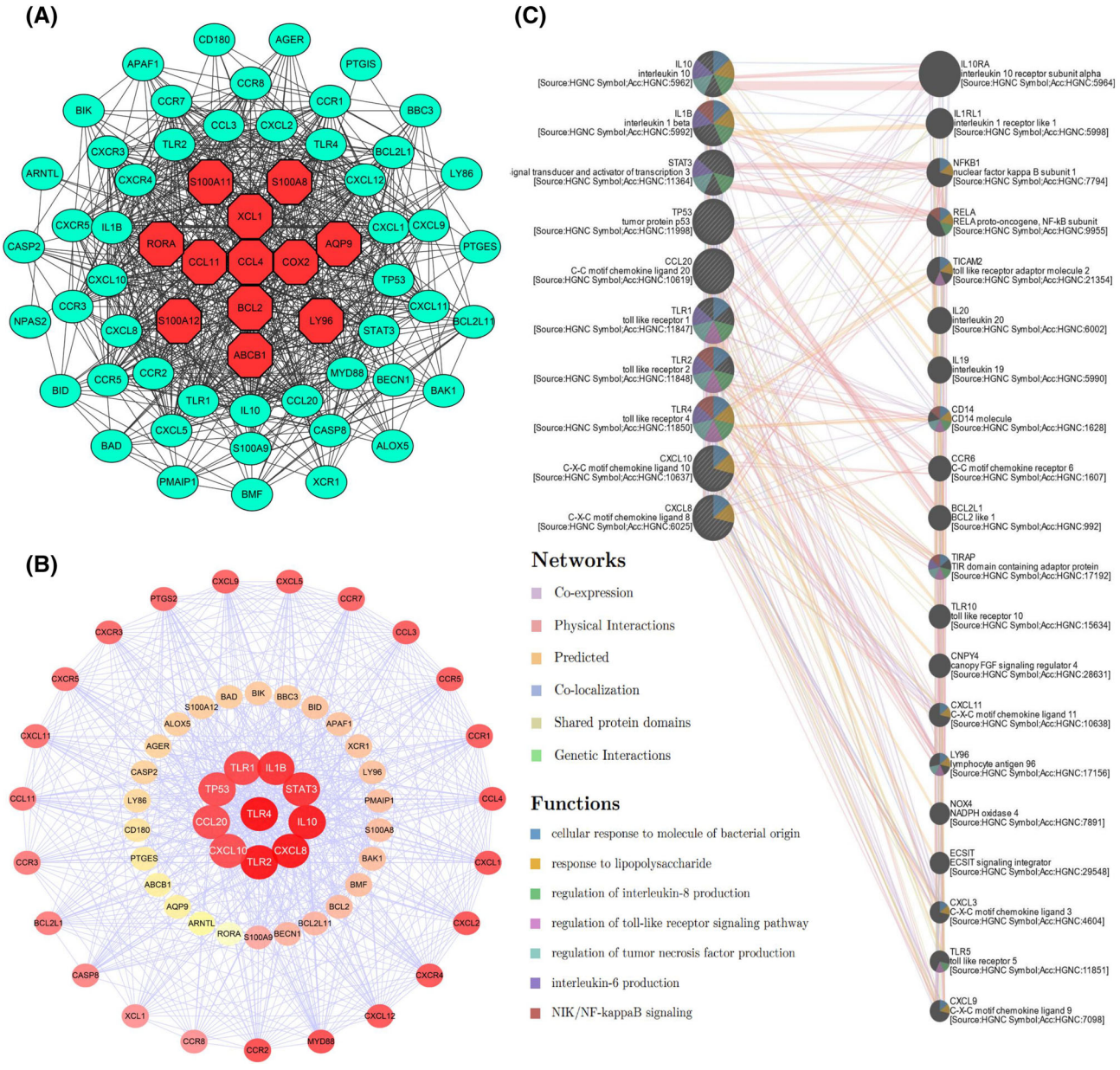



recruitment of neutrophil, monocyte, and granulocyte induced additional tissue damage, characterizing SARS-CoV-2 later phase. This potentially explains the pathogenesis of both disorders as reported previously, including acute inflammatory response triggered by an infection that could induce endothelial dysfunction, hyper-coagulability, and acute thrombosis that led to an increased risk of severe cardiac ischaemic injury (*Figure 3C*).[8] Furthermore, NF-κB and the interleukin pathway were at the core position (*Figure 3D*), indicating that NF-κB promoted cytokine release after SARS-CoV-2 infection. COVID-19-induced cytokine storm might indirectly cause or contribute to cardiac dysfunction, resulting in cardiomyocyte apoptosis and necrosis and increased vessel wall permeability accompanied by the formation of myocardial oedema. Eventually, decompensated HF occurred due to hypoxia and the subsequent adverse effect on cardiac output.[25]

## Protein–protein interaction analysis to establish functional networks

To benefit clinically and pharmacologically oriented research for further biological studies, protein–protein interaction (PPI) network with the perception of core genes and excavation of the gene cluster involved in both disorders was constructed, consisting of 62 nodes and 655 edges (*Figure 4A*). Each node corresponded to genes, including shared transcriptomic signature indicated by red, and edges represented the relationships. The association

**Figure 4** (A) PPI network constructed from identified common DEGs shared by two disorders (HF and COVID-19). Nodes in red represented 12 common DEGs, and edges indicated interconnections between different genes. The more central the PPI is, the more protein interacts with others, suggesting that the more critical functional role the gene played. (B) The screening of hub genes from consensus DEGs based on the PPI network related to *Figure 5* and construction of corresponding interaction network. Ten hub genes were highlighted in the most prominent nodes with white labels (TLR4, TLR2, CXCL8, IL10, STAT3, IL1B, TLR1, TP53, CCL20, and CXCL10), identified as highly interconnected nodes based on their degree value. The network contained 59 nodes and 649 edges, and the depth of the colour represented the degree value of 59 nodes (the darker the colour, the greater the degree). (C) The functional network generated by GeneMANIA. The genes in circles with a white slash at left represented identified 10 hub genes. Ten hub genes and 20 predicted genes were enriched in the network according to their functions associated with co-expression of 75.46%, physical interactions of 24.98%, predicted of 13.88%, co-localization of 10.23%, shared protein domains of 8.77%, and genetic interactions of 3.68%.




between both diseases could also be explored from PPI. The degree of interaction radiating inner to outer indicated CCL4 as the core of the network. Immense interactions among CCL4, COX2, and CCL11 confirmed that the SARS-CoV-2 leads to the specific gene alteration in the human heart and lung.

## Analysis of comprehensive proteomic signature

Various topological algorithms were used to identify the most corresponding components in the specific network. Based on the degree algorithm,10 hub genes (TLR4, TLR2, CXCL8, IL10, STAT3, IL1B, TLR1, TP53, CCL20, and CXCL10) were identified for further analysis and presented in *Figure 4B*. The hub gene network comprised 59 nodes and 649 edges, revealing TLR4 at the network's core. The topological analysis outcomes for 10 hub genes were reported in *Table S3*. Furthermore, four core genes (TLR4, TLR2, IL10, and STAT3) were confirmed by the overlap of the Top 10 genes according to seven ranking methods (*Table S4*). The Degree, MCC, MNC, BottleNeck, Closeness, and EcCentricity algorithms all confirmed that TLR4 had the highest connectivity. EPC algorithm indicated IL10 exhibited the highest whole network connectivity.

A co-expression network was established to describe the genetic interactions of 10 hub genes and their co-expressed genes based on GeneMANIA's functional annotation patterns (*Figure 4C*). A total of 20 predicted genes were included in this co-expression pattern based on the multiple properties of relationship (75.46% co-expression), (24.98% physical interactions), (13.88% predicted), (10.23% co-localization), (8.77% shared protein domains), and (3.68% genetic interactions). They might be involved in the coordinated pathways that cause HF in response to COVID-19. Unexpectedly, 17 of 30 predicted genes were highly related with cellular response to molecule of bacterial origin (adj. *P* value of 2.04E-23) and 13 with response to lipopolysaccharide (LPS) (adj. *P* value of 2.17E-17). It has been shown that there was a strong correlation between immune dysregulation caused by gut microorganisms and upregulation of host inflammatory response in SARS-CoV-2 patients.[26] Increasing evidence linked gut microorganisms to inflammatory conditions beyond the gut, including acute cardiac injury, and the highest expression of ACE2 in the digestive tract intestine. Therefore, a potential gut–heart axis was hypothesized to be one of the possible causes for a high incidence of cardiac involvement in COVID-19 patients.[27]

Next, MCODE algorithm was utilized to construct clusters representing the prominent modules from the comprehensive proteomic signature, yielding two highly dense modules. Among them, module1 (*Figure 5A*) (29 nodes and 381 edges) had a higher score (27.214) than module2 (23 nodes and 65 edges, score = 11.818). The module highlighted XCL1, CCL4, and CCL11 as shared DEGs between HF and COVID-19. Considering heart injury may be induced by the systemic inflammation in COVID-19, further research should be conducted to reveal the effect of the pharmacological targeting of these three critical inflammatory chemokines and their receptors. The biological interconnections for the key module collected 184 terms, grouped into six clusters. Cluster5 (cellular response from the molecular bacterial origin) and cluster6 (viral protein interaction with cytokine and cytokine receptors) were identified as top clusters (*Figure 5B and 5C*). The circular bar plot showed the top terms (*Figure 5D*). All terms were selected and rendered as a network, revealing the extensive cross-talk between Cluster5 and Cluster6 (*Figure 5E*). The integrated analysis for both hub gene set and key module provided encouraging preliminary data to our hypothesis from a bioinformatics perspective that SARS-Cov-2 may affect the potential gut–heart axis. Intestinal microbiome dysbiosis and gut leakage of microbial products induced by infection caused concomitant intestinal inflammation, which could contribute to HF among hospitalized COVID-19 patients.

## Validation of hub genes performance in two independent cohort of samples

The hub genes expression also showed a significant difference between COVID-19 peripheral blood samples and healthy controls (GSE164805 and GSE9128), demonstrating the reliability of hub genes shared between both disease states (*Figure 6A*). Interestingly, IL1B expression was higher in healthy controls, contrary to the former report that a plethora of IL1B has been observed in bronchoalveolar lavage fluid of COVID-19 patients.[28] Giamarellos-Bourboulis et al. have concluded that IL1B-driven macrophage activation syndrome was one of two critical patterns of immune dysfunction contributing to COVID-19 deterioration.[28] Given IL1B's short serum half-life, we examined its differential expression in the alveolar epithelium (GSE147507) and found that it up-regulated in COVID-19 patients ($P$ = 0.006). This finding may fit with evidence that IL1B was rarely detected and isolated in peripheral blood of COVID-19 patients.[29]

With the except of IL10, TLR1, TP53, and CCL20, hub genes similarly showed up-regulated in HF peripheral blood samples (*Figure 6B*). The hierarchical cluster algorithm confirming disease samples could be separated from controls based on hub genes to some extent (*Figure 6C and 6D*). Principal component analysis (PCA) showed a clear separation between samples from COVID-19 and health groups, where PC1 and PC2 explained 88.5% of the total variance. The distinct expression patterns of hub genes between HF and healthy control were also demonstrated through PCA (PC1 + PC2 = 90.0%). Spearman correlation matrix analysis was also performed to identify the hub gene pairs with obvious correlation at the expression levels in COVID-19 (*Figure 6E*) and HF (*Figure 6F*).

Five key genes (TLR4, TLR2, STAT3, IL1B, and CXCL8), differentially expressing in both diseases, were identified, most likely representing genetic link behind COVID-19 and co-morbid HF. The dimensionality of the key genes was further reduced to those explaining the largest variation

**Figure 5** (A) Densely interconnected regions (key module) composed of 29 nodes and 381 edges, obtained from the PPI network in *Figure 5* through MCODE. XCL1, CCL11, and CCL4 were also common DEGs between HF and COVID-19, highlighted in yellow circles. The shade of colours indicated genes' degree value, and the pinker the colour, the greater the degree value. (B) Donut chart summarizing the percentage of the functional terms included in each cluster. (C) Donut chart displaying the percentage of genes in the key module that are found in each cluster. (D) Circular bar plot showing representative terms per cluster ranked by the *P* value in -log base 10. (E) Construction of cross-link plot of significant functional categories defined by ClueGO, revealing the interconnection between each enriched term by calculating the kappa coefficients. The names of the leading subpopulation term were in bold, and the *P* value of each functional term was indicated by the size of the circle, with the larger circle representing a smaller *P* value.

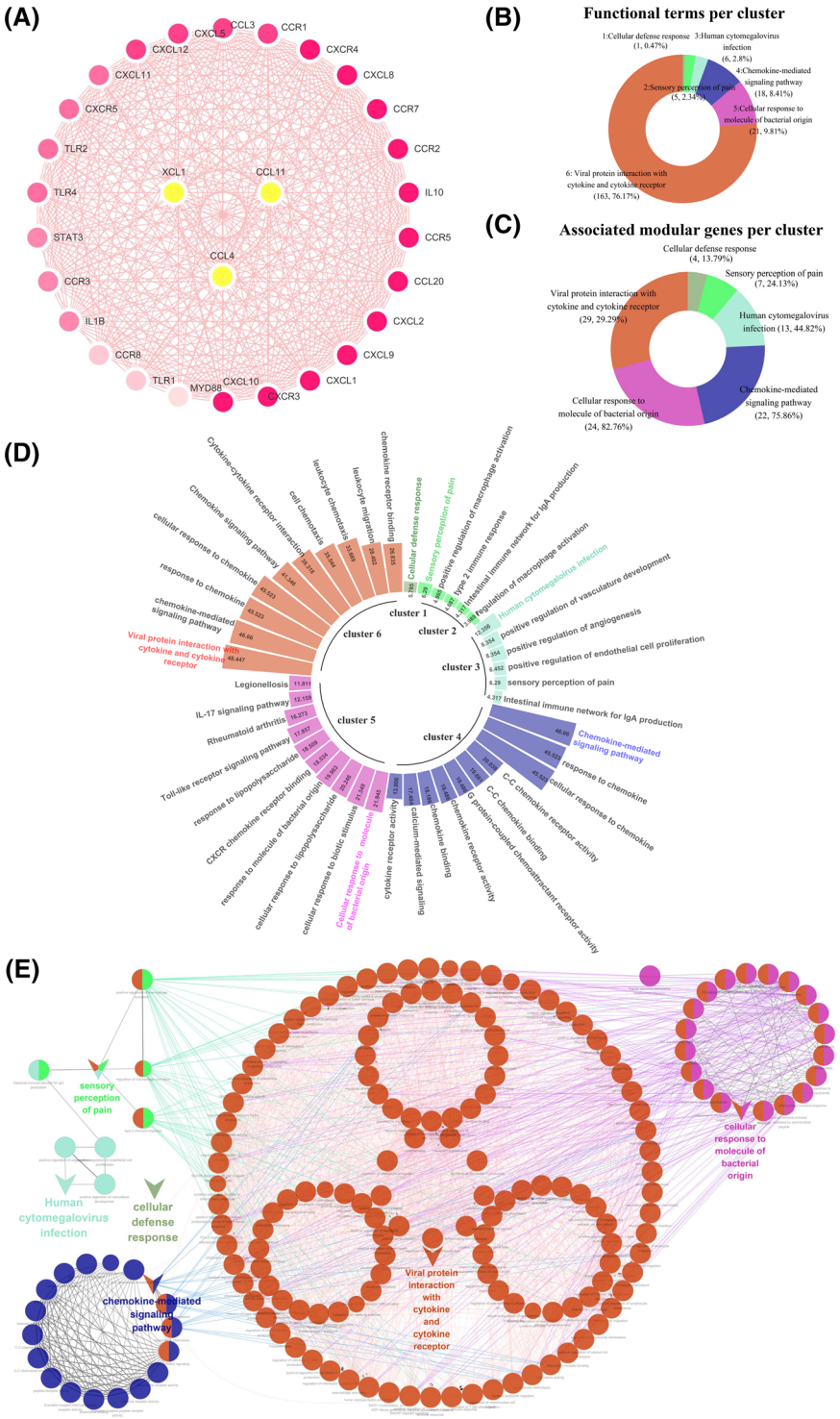


**Figure 6** Validation of hub genes performance in external datasets. The expression level of 10 hub genes in COVID-19 (A) and HF (B). The statistical analysis between the two sets of data was performed with Student's *t*-test, Welch's *t*-test, and Mann–Whitney *U* test comparison analysis. *P* values less than 0.05 were considered statistically significant. ns, no significant. (C) A heatmap shows results from a two-way hierarchical cluster analysis of 10 COVID-19 samples and five healthy control samples (columns) and hub genes (rows). Expression data were normalized and clustered using a hierarchical clustering method with Euclidean distance metric and complete linkage. Expression levels were depicted on a continuous scale from sky blue to purple. (D) Hierarchical clustering heatmap of hub genes based on eight HF samples and three control samples. (E) Spearman correlation matrix evaluated on the gene expression profile of hub genes in COVID-19. (F) Spearman correlation matrix showing the correlation of hub genes in HF. Colour scale, from blue to red, indicated from poor to solid correlation.

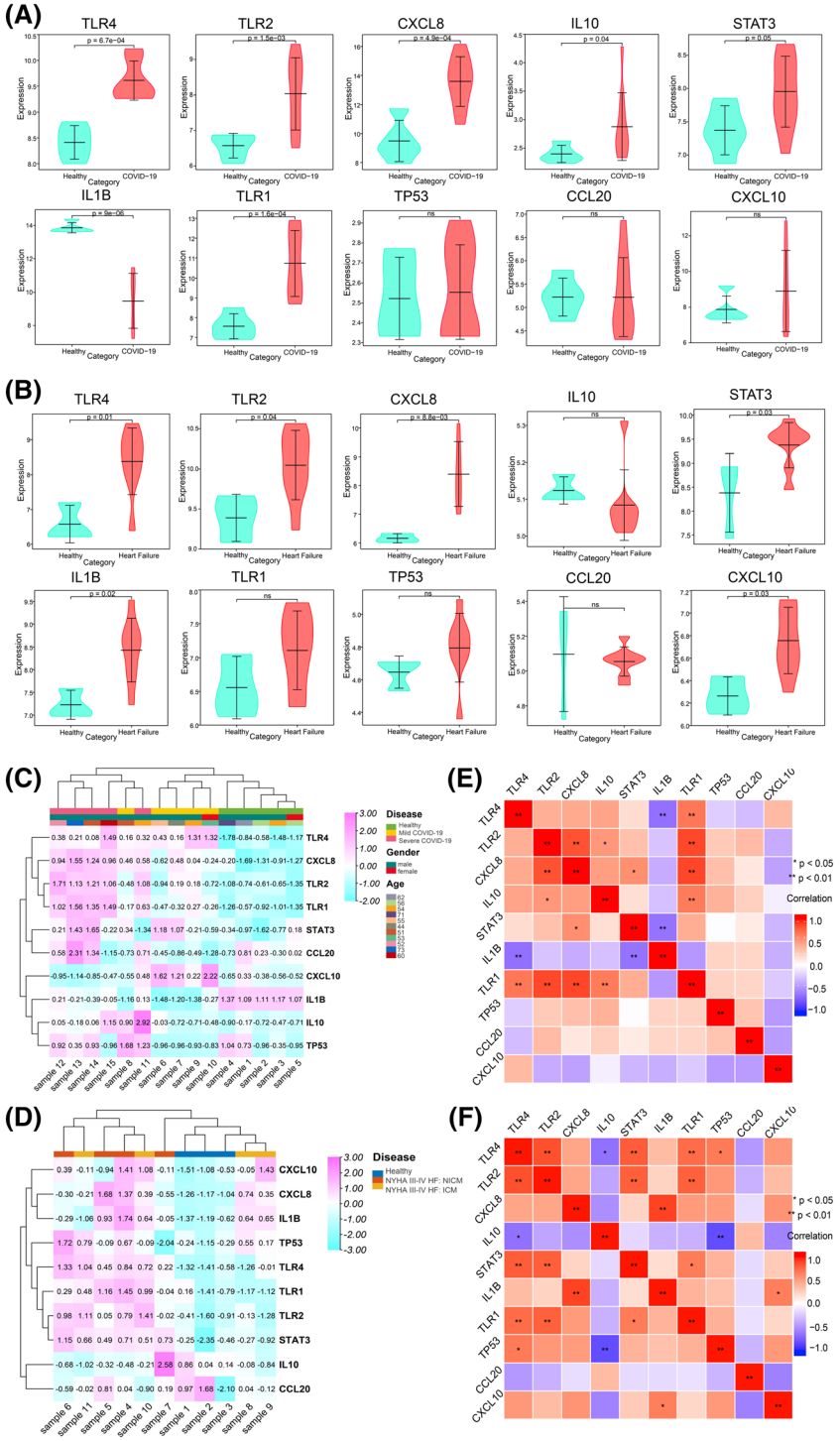




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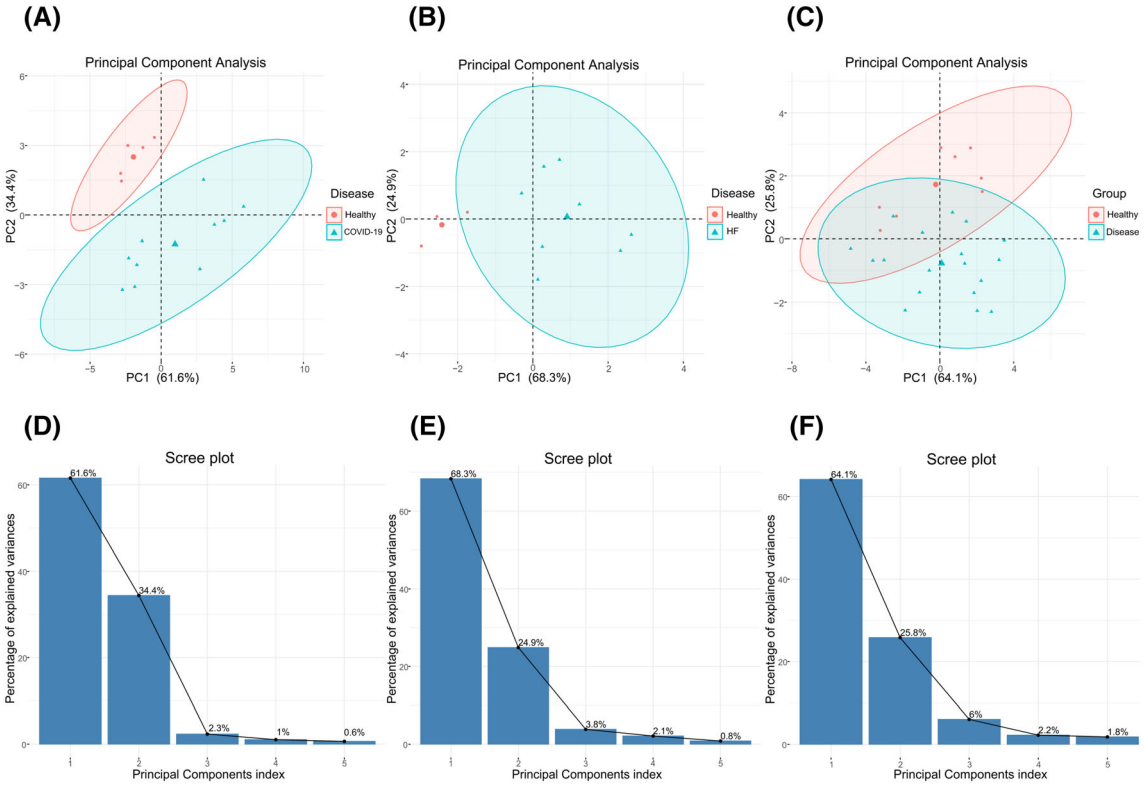
**Figure 7** PCA for five key genes (TLR4, TLR2, STAT3, IL1B, and CXCL8) in external datasets. Score plot of the first two principal components (PCs) of the COVID-19 (A) and HF (B). (C) Score plot of disease condition and healthy control based on five key genes in merged dataset. (D) Scree plot related to *Figure 7A* presenting explained variance per PC: PC1 and PC2 contributed 96% of total variance. (E) Scree plot related to *Figure 7B* presenting explained variance per PC: PC1 and PC2 contributed 93.2% of total variance. (F) Scree plot related to *Figure 7C* presenting explained variance per PC: The first two PCs explained 89.9% of the cumulative variability.



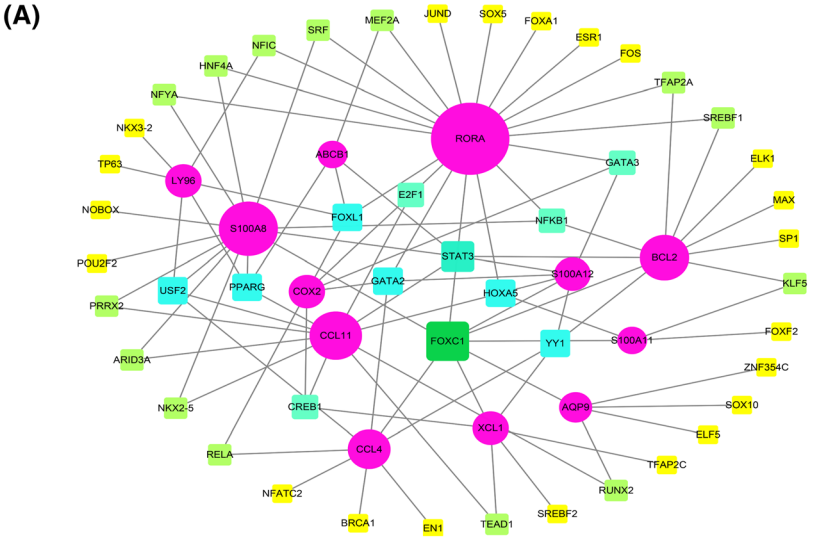

through PCA to evaluate the performance of our finding. Based on key genes, the distinct disease conditions were divided into different zones. The SARS-CoV-2 patients were concentrated in the lower and right quadrant, clearly separating from healthy controls (*Figure 7A*). Similarly, HF samples fell in the right part, whereas the control group was clustered in the opposite quadrant (*Figure 7B*). PC1 and PC2 explained 96.0% (*Figure 7D*) and 93.2% (*Figure 7E*) of the COVID-19 and HF dataset variability, respectively. Both datasets were further pooled and followed by their evaluation through PCA. The disease group (HF or COVID-19) and the healthy group were generally separated, implying the inherent pattern of key genes potentially indicative of both disease signatures (*Figure 7C and 7F*).

## Determination of regulatory signature and network

The integrated regulatory networks were utilized to identify involved crucial components and investigate the priority of regulatory interaction with key genes. Considering the interaction of TFs and miRNAs with genes might be a key reason for modulation of shared transcriptomic signature, 47 TF targets were predicted from JASPER database to generate the co-regulatory network (59 nodes and 98 edges; *Figure 8A*). Four critical genes with a higher degree (RORA, S100A8, CCL11, and BCL2) were identified as the core components, suggesting a robust interaction relationship involved in exacerbating the diseases process. Moreover, among 47 TFs, FOXC1 and STAT3 showed significantly enhanced communication with genes, which could be major drivers for the progression of COVID-19 complicated with HF.

To place shared transcriptomic signature and the regulators in a broader framework, we obtained 98 TFs and 137 miRNAs via the RegNetwork repository, establishing a vast network to present enhanced co-regulatory patterns (246 nodes and 310 edges; *Figure 8B*). RORA and BCL2, two of highly expressed DEGs in the core position of the network, were regulated by 37 TFs and 17 miRNAs as well as 34 TFs and 63 miRNAs, respectively. NF-κB1 was considered as the highest ranked TFs implicated in common transcriptional

**Figure 8** (A) TF–gene interaction network analysis. Shared genes were represented by the highlighted purple colour nodes, the size of which corresponded to the degree of association with TFs. Square nodes represented 47 TFs, and degree values indicated by a colour gradient from yellow (1) to green (8). The network was composed of 59 nodes and 98 edges. (B) TF–miRNA co-regulatory network analysis. The network was composed of 246 nodes and 310 edges, where the red diamond nodes indicated shared genes, blue square nodes represented 98 TFs, and green circle nodes corresponded to 137 miRNAs. (C) Predictive top 10 pharmacologic agent candidates for COVID-19 with HF.




| Candidate drug compounds | P-value | Adjusted P-value | Genes | Chemical Formula | Structure |
|---|---|---|---|---|---|
| Muramyl Dipeptide CTD 00005307 | 5.48E-12 | 7.04E-09 | CXCL8, IL1B, TLR4, TLR2 | $C_{19}H_{32}N_4O_{11}$ | |
| glutathione CTD 00006035 | 1.35E-11 | 8.67E-09 | CXCL8, IL1B, STAT3, TLR4, TLR2 | $C_{10}H_{17}N_3O_6S$ | |
| simvastatin CTD 00007319 | 7.85E-10 | 2.01E-07 | CXCL8, IL1B, STAT3, TLR4, TLR2 | $C_{25}H_{38}O_5$ | |
| N-Acetyl-L-cysteine CTD 00005305 (acetylcysteine) | 9.39E-10 | 2.01E-07 | CXCL8, IL1B, STAT3, TLR4, TLR2 | $C_5H_9NO_3S$ | |
| bay 11-7082 CTD 00003959 | 1.37E-09 | 2.52E-07 | CXCL8, IL1B, STAT3, TLR4 | $C_{10}H_9NO_2S$ | |
| hydrocortisone CTD 00006117 | 2.59E-09 | 4.16E-07 | CXCL8, IL1B, TLR4, TLR2 | $C_{21}H_{30}O_5$ | |
| ACMC-20mvek CTD 00002629 (Atorvastatin) | 2.93E-09 | 4.18E-07 | CXCL8, IL1B, TLR4, TLR2 | $C_{33}H_{35}FN_2O_5$ | |
| Healon BOSS (Hyaluronan) | 5.16E-09 | 6.63E-07 | IL1B, STAT3, TLR4, TLR2 | $C_{28}H_{44}N_2NaO_{23}^+$ | |
| Docosahexaenoic acid BOSS | 8.74E-09 | 1.02E-06 | IL1B, STAT3, TLR4, TLR2 | $C_{22}H_{32}O_2$ | |
| morphine BOSS | 2.62E-08 | 1.97E-06 | IL1B, STAT3, TLR4, TLR2 | $C_{17}H_{19}NO_3$ | |

regulation, with the degree of 6. Among the miRNAs targeting multiple shared DEGs, miR-181, miR-520, and miR-143 stood out as the most connected signatures. Furthermore, the three most significant TF genes (FOXC1, STAT3, and NF-κB1) showed a significant difference in expression across biopsies between disease and health (*Figure S1*).

### Identification of candidate pharmacologic agents

To facilitate further research on therapy development, we performed a drug target enrichment analysis using five key genes (CXCL8, STAT3, IL1B, TLR4, and TLR2) with good performance in the validation as genetic link targets. *Figure 8C* presented the Top 10 enriched agent candidates targeting genetic link, which could be a possible target to develop therapeutic strategy for co-morbidity. The analysis demonstrated that muramyl dipeptide (MDP) and glutathione (GSH) were the most statistically significant outcomes, most likely representing predominant pharmacological therapies available for the co-occurrence of infected SARS-CoV-2 and HF cases. Peptide therapeutics and molecular mimetics have emerged as promising treatment against pathogens comprising coronavirus and SARS-CoV-2, with over 140 peptides being investigated clinically.[30]

## Discussion

Among cardiac complications in COVID-19, HF is a leading cause of death.[31] Various myocardial aggression mechanisms were identified to be involved in SARS-CoV-2 infection complicated with HF such as direct myocardial injury and over-activated immune system induced by viral action,[32,33] direct and indirect inflammatory damage,[34,35] and oxygen supply–demand imbalance.[36] The drastically increased mortality rate is also closely related to pre-existing HF, and elevated sensitivity cardiac troponin I (hs-TnI)[37] and high D-dimer[10] on admission are considered as early prognostic predictors. This study focuses on finding shared transcriptional and regulatory signatures, possible interaction pathways, and novel potential therapeutic targets through a systems biology approach.

### Shared transcriptomic signature and functional characteristics relevant to pathogenesis of COVID-19 with HF

The specific gene alteration (LY96, S100A8, ABCB1, S100A11, RORA, XCL1, COX2, CCL11, CCL4, S100A12, BCL2, and AQP9) was identified as a shared transcriptomic signature involved in response to SARS-CoV-2 with HF. Immune inflammatory pathways were highly enhanced, and TLRs, NF-κB, and chemokine were confirmed as the primary pathways.

TLRs elicit innate immunities by identifying pathogenic microbial elements and bridging essential pro-inflammatory pathways.[38] Following myocardial aggression mechanisms due to viral action, damaged cardiomyocytes release endogenous danger signals, aggravating HF progression by binding to different TLRs.[39] The increased serum LY96 (myeloid differentiation factor 2 [MD2]) level correlated with a higher HF mortality rate, whose inhibition can reduce cardiac dysfunction and remodelling.[40] LY96 can activate TLR4 dimerization complex, inducing a broad array of inflammatory cytokines and gene expression in cardiac tissue,[41] in agreement with our Reactome analysis. TLR4/MD-2 complex can also bind to B-cell and T-cell antigenic epitopes within SARS-CoV-2 S protein, leading to downstream adaptive immune process-induced myocardial damage.[42] The modulation of TLR4/MD2 complex formation and signalling axis would be a potential therapeutic opportunity.

S100 family proteins, over-expressed in cardiac tissue, have proved to be prognostic biomarkers and potential therapeutic targets in HF and reliable danger host factors in COVID-19.[43,44] Neutrophil-derived calprotectin (S100A8/A9) increased inflammatory cells and platelet proliferation by activating NF-κB signalling through interaction with RAGE and TLR4, causing havoc on lung and heart tissue and promoting COVID-19 hyper-coagulability.[45] Similarly, persistent S100A8/A9 activation in cardiac macrophages and fibroblasts induces HF progression by elevating pro-inflammatory cytokines through activation of TLR and RAGE-dependent NF-κB signalling.[46]

COX2, playing diverse roles in inflammation and viral infections,[47] was identified as a predominant transcriptomic signature. SARS-CoV-2 can induce a robust up-regulation of COX2 in many human cell lines and mice respiratory systems.[48] COX2 also induces persistent HF inflammation and exerts diverse functional and morphological effects on cardiomyocytes.[49] Hypoxic ischaemia and inflammation under COVID-19 promote NF-κB-induced COX2, exacerbating myocardial remodelling.[50,51] COX2 inhibition could greatly alleviate the severity of COVID-19 by impairing the production of inflammatory cytokines and protective antibodies.[48] Therefore, by reducing inflammatory mediator release and ROS production, selective COX2 inhibitors may protect cardiomyocytes from SARS-CoV-2. However, considering adverse effects of long-term non-steroidal anti-inflammatory drug use, further evaluation is required in COVID-19 individuals with selective COX2 inhibition.[52]

Elevated chemokine levels, including identified CCL4, CCL11, and XCL1, aggravate COVID-19 condition severity, as bioactive prostaglandins could further exacerbate hyper-inflammatory pathology.[53] AQP9 was identified to be over-expressed in both disorders, suggesting a potential role in co-morbidity. It has been reported to be up-regulated in

HF patients with fluid retention and involved in pathological processes such as myocardial oedema.[54] This shared signature could, at least in part, open up new research avenues.

## The underlying role of microorganisms: Disease progression is more prevalent in individuals with unhealthy microbiota status linked to impaired immune response and pro-inflammatory condition

From a proteomic signature perspective, key signalling molecule detection represents viable targets for systemic molecular-based therapeutic strategies towards co-morbidity. Based on the cross-talk among co-expressed genes, we found that pathogenic linkage mainly originated from the cellular response to molecule of bacterial origin and response to LPS. Our analysis and accumulating evidence linked gut microbiome dysbiosis to an increase in inflammatory illnesses within and beyond the gut, a key feature in SARS-CoV-2 infection pathophysiology.[55]

The gastrointestinal tract develops immunological homoeostasis that protects the ecology of microbiota against pathogens via dynamic equilibrium, playing a pivotal role to facilitate finer regulation of the activation of various cells boosting in response to viral infection. Recent research indicated cross-links between gut microbiota composition and expression of several inflammation markers such as CCL4, CXCL8, CXCL10, and IL10, in agreement with identified critical genes in our work. COVID-19 individuals have significant variations in the gut microbiota composition regardless of medication treatment.[26] Faecal metabolomics analysis recently uncovered the association between the gut-microbial–host-immune axis and inflammatory status and clinical severity of COVID-19, and SARS-CoV-2 could influence the intestinal microbiome by altering opportunistic pathogens, causing dysbiosis.[57] Collectively, SARS-CoV-2 complications are more likely frequent in people with an unhealthy microbiota status linked to impaired immune response and pro-inflammatory conditions.

## LPS acts as a potential marker for monitoring HF in COVID-19

Our findings on pathogenic linkage potentially suggested that LPS, as a gut leakage marker, could be used to monitor cardiac injury in COVID-19. Based on the high ACE2 expression in enterocytes, SARS-CoV-2 caused intestinal permeability impairment, allowing bacteria systemic spread and microbial product leakage, especially LPS into the systemic circulation, thereby persistently activating the immune system.[58] The role of gut dysbiosis and gut–blood barrier degeneration is also highlighted in HF pathogenesis,[59] and cardiovascular events could be predicted through higher LPS levels.[60] Additionally, Hoel et al. recently found that LPS-binding protein and gut homing marker levels were significantly elevated in COVID-19 and individuals with cardiac involvement had twice as high LPS levels compared with those without, positively correlated with up-regulated inflammasome markers and troponin.[61] Overall, SARS-CoV-2-induced gut leakage of microbial products and subsequent inflammatory cascades could amplify cardiac injury, indicating a potential role of the gut–heart axis in co-morbidity.

## Reduced perception of cardiac pain causes missing diagnosis of COVID-19 complicated with HF

The MCODE modules were binned into sensory perception of pain, raising concerns regarding pain manifestations of SARS-CoV-2. Viral infections in various mechanisms could intensify pain perception. As a characteristic symptom, acute pain prevails during active COVID-19.[62,63] Additionally, it has been hypothesized that pain might be a biomarker for viral burden or virulence.[64] Pain related to COVID-19 would also be a valuable indicator for timely diagnosis and treatment.

Often, less or missing severe pain would result in inaccurate prognosis and later intensive care. An intense systemic stimulation such as respiratory distress or the catecholamine surge may distract nociceptive signals.[65] Some COVID-19 individuals presented with chest pain, even angina pectoris, attributed to HF. However, a noteworthy feature of SARS-CoV-2 is the missing diagnosis of patients with co-morbidity, which could be explained because painful myocardial ischaemia episodes decrease, replaced with silent HF episodes.[66] The modulation of the pain pathway anatomical integrity has been reported to be affected by the pharmaceutical treatment for HF.[67] It is reasonable to suggest that missing emergent assistance of COVID-19 individuals with HF is partly attributed to impaired cardiac pain perception.

## Shared molecular regulatory signature

A strong correlation between COVID-19 and HF was discovered within the regulatory signature. RORA and BCL2, with the highest connectivity in networks, were representative components in regulatory co-expression pattern. NF-κB1, STAT3, FOXC1, miR-181, miR-520, and miR-143 were identified as the most required functional mediators to common DEGs.

Elevating Ang-II levels is linearly associated with virus load that aggravates COVID-19 syndromes and causes cardiac remodelling and dysfunction in HF.[68] As an Ang II-responsive modulator, RORA protects against Ang II-induced HF by suppressing IL-6 and NF-κB pathways and downstream

pro-inflammatory STAT3 Tyr705 phosphorylation. RORA reduction in mice and human HF patients contributes to the deterioration,[69] corroborating our analysis. Heart tissue with cardiomyocyte-specific RORA over-expression shows reduced susceptibility to damage and enhanced capacity to repair vascular endothelium injury.[33,70,71] RORA is found up-regulated in SARS-CoV-2 infected human cell-derived cardiomyocytes, indicating a cardioprotective mechanism under COVID-19.[72] Interestingly, our study found decreased RORA expression in COVID-19 and HF biopsy samples. Given that endothelium dysfunction causes cardiovascular compromise during SARS-CoV-2 infection, it is believed that a pre-existing genetic predisposition for RORA down-regulation makes patients more vulnerable to HF.

As identified predominant regulatory signature, NF-κB1 was released to nuclear translocation induced by SARS-CoV-2 external stimuli, leading to cytokine storm and severe condition.[73] Similarly, NF-κB1 causes cardiac remodelling in HF patients via transcription activation of inflammatory processes.[74] Considering STAT3 essential for NF-κB1 activation, targeting the STAT3–NF-κB1 axis represents a promising strategy for COVID-19.[75] Because SARS-CoV-2 induces a positive feedback loop between STAT3/plasminogen activator inhibitor 1, causing widespread coagulopathy in COVID-19, inhibition of STAT3 therapy has been recommended.[76] A targeting STAT3 inhibitor Celastrol has been reported to inhibit Ang-II-induced cardiac dysfunction in HF by attenuating cellular fibrotic responses.[77] FOXC1, up-regulated in advanced HF cardiomyocytes, is associated with pathological hypertrophy[78] and epigenetic patterns across the viral genome.[79] Our study also established miR-181 as a component of immense value involved in co-regulatory network, which is a biomarker for HF immune state.[80]

## Promising therapeutics: GSH-based antioxidant strategy combined with MDP-based microbe-derived immunostimulatory therapies

Standard treatment for SARS-CoV-2 has yet to be established. Over 200 vaccine candidates are being pursued globally; however, many fail to inhibit cytokine storm.[81] More importantly, frequent mutations in target antigen SARS-CoV-2 S protein compromise early generation vaccine efficacy.[82] Research efforts are being devoted to accelerating pharmacological therapies.[83] Drug candidates are categorized into antiviral and chemotherapeutic agents and alternative strategies through regulating the immune system using anti-inflammatory agents, cell-based therapy, and convalescent plasma therapy.[84]

In COVID-19 with cardiovascular complications, several candidate drugs have shown unsatisfactory clinical outcomes attributed to cardiotoxicity.[85] We identified multiple specific pharmacologic agents targeting potential genetic links between both diseases. The most significant MDP is required for numerous biological reactions and cell survival maintenance, boosting immune responses to exogenous antigens.[86] The microbe-derived immunostimulatory agents have gained popularity against SARS-CoV-2. Particularly, as promising pharmaceutical substances, MDPs can reproduce the nonspecific effects of multicomponent bacterial adjuvants, immunostimulants, and vaccines in preventing risk factors during the pandemic.[87]

Adequate GSH level is critical for antiviral defence and immune system protection through the anti-oxidant mechanism.[88] Endogenous GSH deficiency exacerbates SARS-CoV-2-induced oxidative damage and uncontrolled SARS-CoV-2 reproduction, leading to excessive inflammatory response and further multiple organ failure.[89] Increasing evidence has demonstrated the value of restoring GSH levels in COVID-19 cases.[90] Considering the non-specific antiviral effect, GSH is recommended as supplementary therapy.[91,92] Elevated oxidative stress production and decreased activities together with a high amount of GSH, are correlated with inferior prognosis in HF.[93–95] Endogenous anti-oxidant capacity is a novel and promising target to treat HF, demonstrated to ameliorate heart function.[95] We believe that GSH-based antioxidant approaches would be a promising strategy for treating COVID-19 with HF. Our study identifies promising therapeutics, and experimental verification should be further conducted to translate bioinformatic evidence into clinical interventions.

## Limitations

Information and procedures bias from a computational biology approach are inevitable, limiting the ability to recapitulate the underlying genetic links fully. The challenge in sample collection results in fewer standard-compliant datasets and the incapability of experimental validation for the pathogenic role of identified signature key genes, TFs, and miRNAs, within a short period.

Considering shared transcriptional signature may not comprehensively illustrate increased severity in SARS-CoV-2 patients with heart disease, further study on heart samples from patients with both HF and COVID-19 and data with individual pathologies (COVID-19-induced transcriptional changes and HF-induced transcriptional changes) will be included in our future work.

# Conclusions

We identified the molecular mechanisms underlying connections between COVID-19 and HF as well as shared transcriptomic and corresponding regulatory signatures as emerging

therapeutic targets. We also screened potential pharmacologic agents targeting genetic links. Our study provides further groundwork and insights for the molecular mechanism of COVID-19 with HF and pick up the pace of therapeutic research against the ongoing pandemic.

## Conflict of interest

The authors declare that there are no conflicts of interest.

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## Supporting information

Additional supporting information may be found online in the Supporting Information section at the end of the article.

**Table S1.** The top gene ontology categories enriched with shared genes between COVID-19 and HF
**Table S2.** The top pathways enriched with common genes between COVID-19 and HF from four databases
**Table S3.** Topological analysis for top 10 hub genes where the network density is 0.379, heterogeneity 0.487 and centralization 0.339.
**Table S4.** The top 10 genes rank according to seven cytoHubba algorithms.
**Figure S1.** The expression of the highest-ranked TF which are implicated in shared transcriptional regulation.

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