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Potential core genes associated with COVID-19 identified via weighted gene co-expression network analysis

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Summary

AIMS: Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) is a novel virus belonging to the Coronaviridae family that causes coronavirus disease (COVID-19). This disease rapidly reached pandemic status, presenting a serious threat to global health. However, the detailed molecular mechanism contributing to COVID-19 has not yet been elucidated.

METHODS: The expression profiles, including the mRNA levels, of samples from patients infected with SARS-CoV-2 along with clinical data were obtained from the GSE152075 dataset in the Gene Expression Omnibus (GEO) database. Weighted gene co-expression network analysis (WGCNA) was used to identify co-expression modules, which were then implemented to evaluate the relationships between fundamental modules and clinical traits. The differentially expressed genes (DEGs), gene ontology (GO) functional enrichment, and Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway were evaluated using R software packages.

RESULTS: A total of 377 SARS-CoV-2-infected samples and 54 normal samples with available clinical and genetic data were obtained from the GEO database. There were 1444 DEGs identified between the sample types, which were used to screen out 11 co-expression modules in the WGCNA. Six co-expression modules were significantly associated with three clinical traits (SARS-CoV-2 positivity, age, and sex). Among the DEGs in two modules significantly correlated with SARS-CoV-2 positivity, enrichment was observed in the biological process of viral infection strategies (viral translation) in the GO analysis. The KEGG signalling pathway analysis demonstrated that the DEGs in the two modules were commonly enriched in oxidative phosphorylation, ribosome, and thermogenesis pathways. Moreover, a five-core gene set (RPL35A, RPL7A, RPS15, RPS20, and RPL17) with top connectivity with other genes was identified in the SARS-CoV-2 infection modules, suggesting that these genes may be indispensable in viral transcription after infection.

CONCLUSION: The identified core genes and signalling pathways associated with SARS-CoV-2 infection can significantly supplement the current understanding of COVID-19. The five core genes encoding ribosomal proteins may be indispensable in viral protein biosynthesis after SARS-CoV-2 infection and serve as therapeutic targets for COVID-19 treatment. These findings can be used as a basis for creating a hypothetical model for future experimental studies regarding associations of SARS-CoV-2 infection with ribosomal protein function.

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Introduction

Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) is the latest member of the Coronaviridae family, which caused a pandemic of coronavirus disease (COVID-19), endangering global health [1–5]. The genome of SARS-CoV-2 is most closely related to that of SARS-CoV, which emerged in 2003 [3, 6, 7]. Although numerous coronaviruses cause pneumonia, SARS-CoV-2 causes the most devastating form of pneumonia that has been noted globally to date. This virus mainly infects lower respiratory tract cells, leading to the development of SARS that could progress to pneumonia and death in severe cases [8]. SARS-CoV-2 consists of 20 different proteins containing four main structural proteins (S: spike; E: envelope; M: membrane; N: nucleocapsid), and several non-structural proteins such as RNA-dependent RNA polymerase, coronavirus main protease, and papain-like protease (PL-pro) may serve as viable antiviral drug targets [9–11]. Although lopinavir and ritonavir have been used as treatment in preliminary clinical studies, there is still no consensus on the effectiveness of these drugs in treating patients with COVID-19 [12]. Therefore, gaining a better understanding of the molecular mechanism by which SARS-CoV-2 interacts with human proteins will play a critical role in identifying appropriate antiviral drug targets [13]. Towards this goal, we adopted a bioinformatics approach using publicly available clinical and genetic data. Weighted gene co-expression network analysis (WGCNA) is a common method for investigating gene–phenotype relationships, including in the context of diseases [14, 15]. The main advantage of WGCNA is that gene expression data are converted to a co-expression module, which is conducive for providing insights into phenotypic traits of interest [16, 17]. A comprehensive R package collection has been developed to interpret various aspects of WGCNA [18–20].

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The increasing morbidity and mortality of COVID-19 cause adverse consequences. However, the performance of vaccine or drug treatment in combating infection with the causative SARS-CoV-2 is not satisfactory. To further understand SARS-CoV-2 infection-induced alterations of host gene expressions and pathways, we aimed to construct co-expression modules from publicly available data of patients with COVID-19. In particular, we screened the modules of co-expressed genes among differentially expressed genes (DEGs) between SARS-CoV-2-infected and non-infected (normal) samples. Gene ontology (GO) and Kyoto Encyclopedia of Genes and Genomes (KEGG) enrichment analyses were further performed to identify the principal functions of core genes in each module related to SARS-CoV-2 infection. The identified core genes and signalling pathways associated with SARS-CoV-2 infection are expected to significantly supplement the current understanding of COVID-19. The findings are also anticipated to serve as a basis for determining a new approach and creating a hypothetical model for future experimental studies regarding SARS-CoV-2 pathogenesis.

Material and methods

Expression analysis of high-throughput sequencing data from patients with COVID-19

The expression profiles from the GSE152075 dataset, including the mRNA levels from nasopharyngeal swabs and clinical data from 430 and 54 patients positive and negative for SARS-CoV-2 infection, respectively, were downloaded from the Gene Expression Omnibus (GEO) database (https://www.ncbi.nlm.nih.gov/geo) [21]. The baseline information of patients is presented in table 1. We excluded data from patients without available information on age or sex. Finally, data from 377 SARS-CoV-2-infected samples and 54 normal samples with available associated clinical information and mRNA level were used for further analysis. Normalisation, batch effect correction, and differential expression were performed using the R package "DEseq2".

Identification of aberrantly expressed mRNAs

The raw microarray data files between the SARS-CoV-2-infected and normal samples were subsequently analysed using the R and Bioconductor package "edgeR". We used a classical t test to identify the DEGs, with P values of <0.05 and fold change values of ≥ 2 being statistically significant. Aberrantly expressed genes between the SARS-CoV-2-infected and normal samples were selected for subsequent analyses using the WGCNA algorithm to calculate the gene expression levels [22].

Construction of the SARS-CoV-2 co-expression module

The WGCNA algorithm was utilised to discern the power value in the construction of modules related to SARS-CoV-2 infection [22]. The independence and average connectivity degree of the power value were assessed using the gradient method. The applicable power value was determined as a degree of independence above 0.8. The WGCNA algorithm was then utilised to construct the modules after the power value was determined, and gene information was extracted from each module. The genes that were not differently expressed following the WGCNA were not used for further analysis because they revealed no or very low discrepancy in expression between the researched samples.

Construction of the co-expression modules and analysis of the module-trait relationships related to SARS-CoV-2 infection

The WGCNA algorithm was applied to screen out the co-expression modules using the R software. The module-trait interactions were assessed on the basis of the relationship between the module eigengenes and phenotypes (clinical traits) to screen out gene expression sets strongly associated with the phenotype. Gene significance was estimated as the outright value of the relationship between the expression profile and each clinical trait; the module membership was identified on the basis of the relationship between the expression profile and each module eigengene [23, 24].

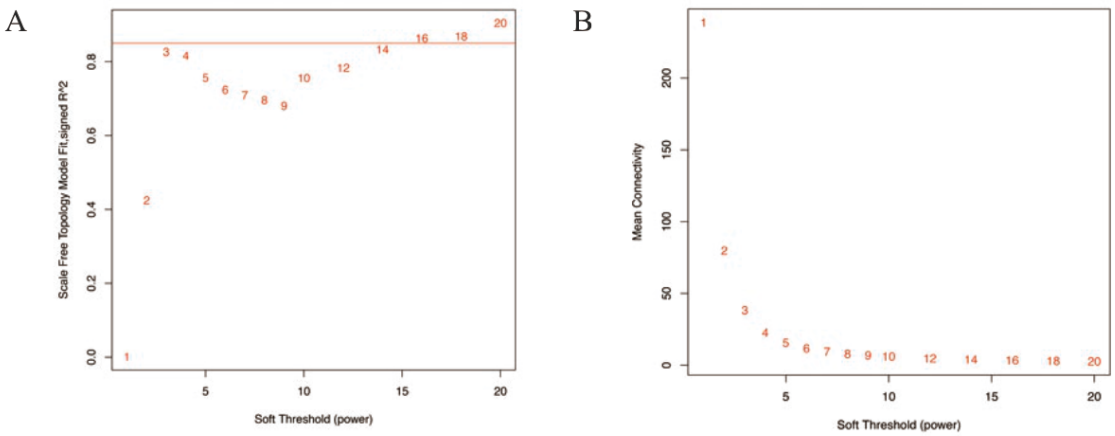
Functional enrichment analysis of the co-expression modules associated with SARS-CoV-2 infection

GO term enrichment and KEGG pathway analyses were implemented to explore a full-scale set of functionally annotated core genes. Thereby, GO functional and KEGG pathway enrichment analyses were utilised to assess the modules significantly associated with SARS-CoV-2 infection using the R packages ‘cluster Profiler’ and ‘ggplot2’ [25, 26]. GO annotation outcomes can generally be sorted into three main categories: molecular function, biological process, and cellular component [27, 28]. Following multiple test calibration, a false discovery rate of ≤ 0.05 was

Table 1:
The baseline characteristics data.

Variables		SARS-CoV-2 positive	SARS-CoV-2 negative
Sex	Male	176	24
	Female	201	30
Age (years)	≤60	223	39
	>60	155	14

Figure 1: Network topology for different soft-thresholding powers. (A) Numbers in the plots indicate the corresponding soft-thresholding powers. (B) The approximate scale-free topology can be attained at a soft-thresholding power of 16.



set as the threshold for significance [28]. The core genes in the modules were then screened out on the basis of the protein–protein interaction (PPI) network constructed using the Cytoscape software.

Ethics statement

The GEO database is freely available to all investigators worldwide. Thus, supplementary approval by an ethics committee was not necessary for this study.

Results

Construction of the co-expression modules for patients with COVID-19

A total of 1444 DEGs were identified between the 430 SARS-CoV-2-infected samples and 54 normal samples, which were utilised to build the co-expression modules using the WGCNA package tools. One of the most vital factors in the WGCNA is the power value, which has a substantial impact on the independence and average connectivity degree of co-expression modules. Therefore, the power value was identified using the gradient method herein (figure 1).

The power value of our module was calculated to be 16; the independence degree was >0.8; and the average connectivity degree was higher. Accordingly, the power value was used to construct the co-expression modules. Eleven co-expression modules related to SARS-CoV-2 positivity were screened out in the WGCNA; these modules are visualised in distinctive colours in figure 2.

Gene co-expression modules correlated with the clinical traits

The gene co-expression modules correlated with SARS-CoV-2 positivity, age, and sex were screened out to evaluate the module-trait associations (figure 3). The yellow and grey modules had the greatest correlation with SARS-CoV-2 positivity. Similarly, these two modules were greatly correlated with age. Therefore, the yellow and grey modules were selected for further analysis.

Functional enrichment analysis of the DEGs in the SARS-CoV-2 infection modules

The DEGs in the yellow and grey modules associated with SARS-COV-2 positivity were subjected to GO enrichment analysis. The top three enrichment results of the GO biological process terms were viral gene expression, viral transcription, and protein targeting the membrane. The top three enrichment results of the GO cellular component terms were mitochondrial inner membrane, ribosome, and cytosol, while those of the GO molecular function terms were structural constituent of the ribosome, electron transfer activity, and oxidoreductase activity, acting on NAD(P)H. The overall top five enrichment results of the GO analysis for the DEGs in the yellow and grey modules are shown in figure 4.

Signalling pathway enrichment analysis of the DEGs in the SARS-CoV-2 infection modules

The most significantly enriched pathways of the DEGs in the yellow and grey modules are shown in figure 5. The

Figure 3: Relationships between gene co-expression modules and different clinical data.

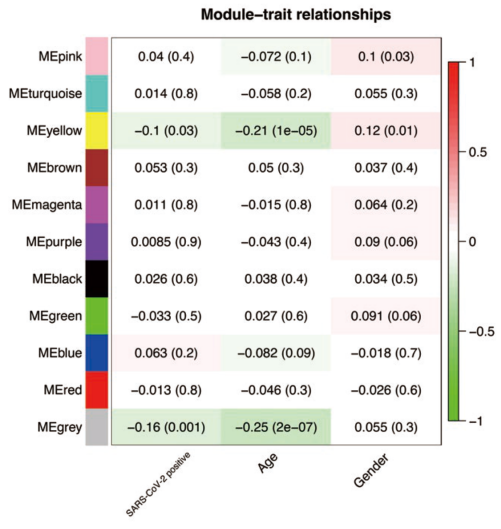


Figure 2: Gene modules identified in the WGCNA. (A) Gene dendrogram obtained by clustering the dissimilarity based on consensus Topological Overlap. The colour rows indicate the corresponding modules. (B) Visualisation of the gene network using a heatmap plot. The heatmap depicts the TOM DEGs in the yellow and grey modules. Abbreviations: WGCNA, weighted gene co-expression network analysis; DEG, differentially expressed gene; TOM, Topological Overlap Matrix.

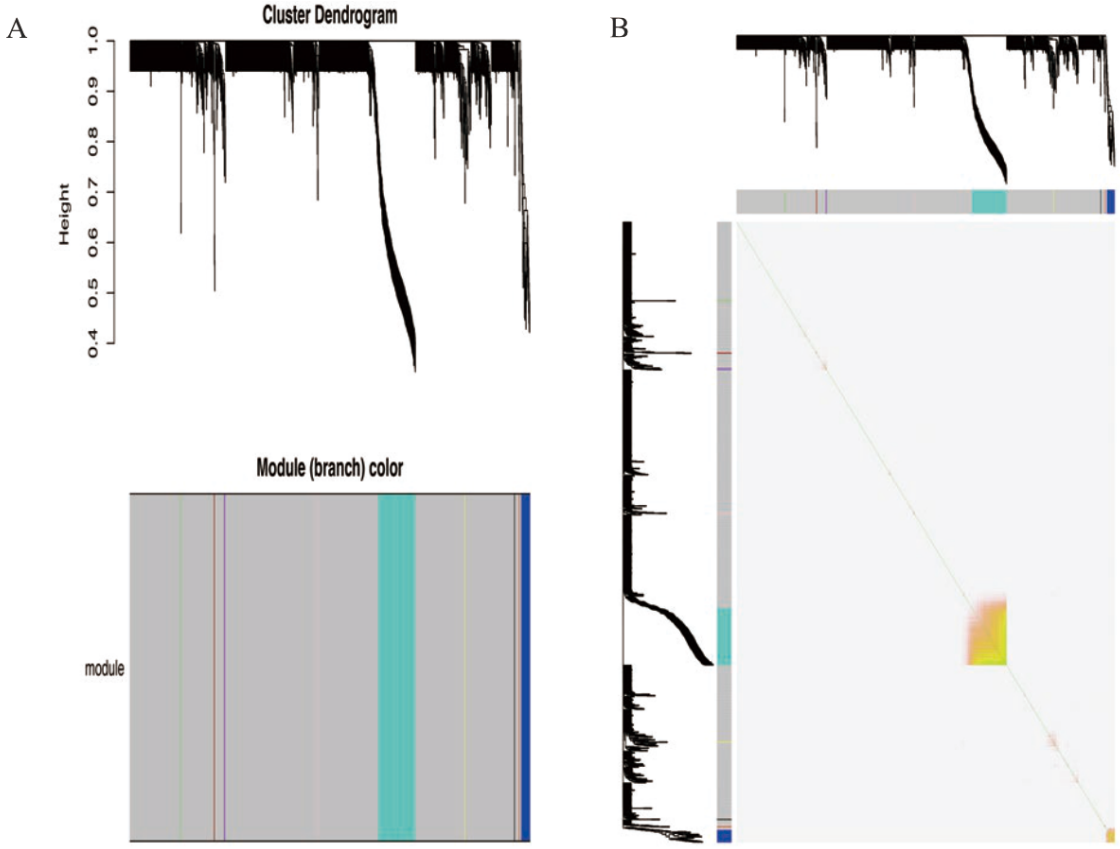
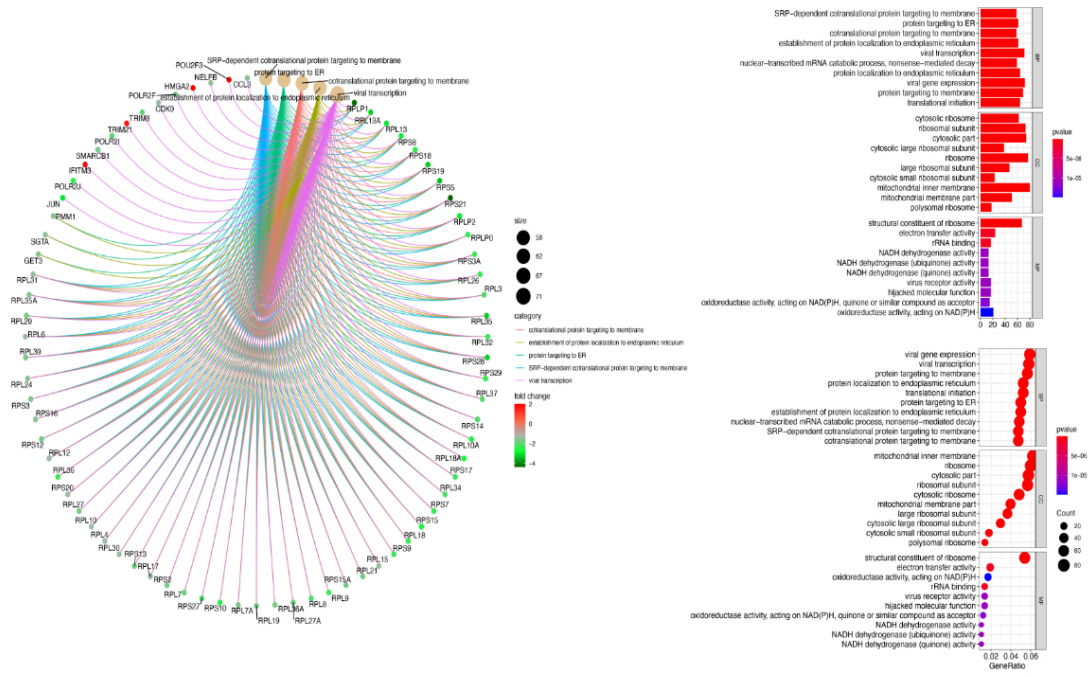


Figure 4: Top five enrichment results of the GO analysis of the DEGs in the yellow and grey modules. Abbreviations: GO, gene ontology; DEG, differentially expressed gene.



KEGG analysis revealed that the upregulated DEGs in the two modules were commonly enriched in oxidative phosphorylation, ribosome, and thermogenesis pathways.

Module visualisation and core genes

The PPI network is shown in figure 6. The intramodular connectivity was analysed for all DEGs in the yellow and grey SARS-CoV-2 infection modules. Among the DEGs, the core genes in these two modules showed a high intramodular connectivity. The five core host response genes were RPL35A, RPL7A, RPS15, RPS20, and RPL17, which all encode ribosomal proteins.

Discussion

Given the rapid spread of SARS-CoV-2 infection, jeopardising global health with no established effective treatment, it is indispensable to identify the molecular mechanisms associated with this infection and determine appropriate treatment and prevention methods. To this end, we employed WGCNA and other systems biology methods to explore the expression profiles from the GSE152075 dataset, including SARS-CoV-2-infected and normal samples. The WGCNA identified several modules, two of which (yellow and grey modules) were significantly correlated with SARS-CoV-2 positivity. Since SARS-CoV-2 consists of PLpro, RNA polymerase, 3CL protease, helicase protein, membrane protein, spike protein, envelope protein, and nucleocapsid protein, we identified RPL35A, RPL7A, RPS15, RPS20, and RPL17 as the five core genes with top connectivity with other genes in these modules. This finding indicates that the transcription of these genes encoding ribosomal proteins may be indispensable in viral protein biosynthesis after SARS-CoV-2 infection.

Ribosomal proteins and viral mRNA are well known to be involved in viral protein biosynthesis [29, 30]. Reduction in the transcription of ribosomal proteins has been associated with a robust antiviral response to SARS-CoV-2 infection [31]. Ribosomal proteins associate with rRNA to regulate the cellular translation cycle and have been shown to be involved in two key antiviral mechanisms. First, these proteins can serve as immune elements to trigger signalling pathways for antiviral defence. For instance, RPS20 has been shown to inhibit viral reproduction in cells by regulating Toll-like receptor 3 (TLR3), which can trigger the immune response [32, 33]. Totura and colleagues further disclosed that TLR3 signalling via TIR domain-containing adapter-inducing interferon-beta serves as a defensive innate immune response to SARS-CoV infection [33]. In the present study, we found that RPS20 expression was significantly downregulated in the patients positive for SARS-CoV-2 infection. This indicates that a reduction in RPS20 levels may regulate TLR3 expression to suppress the immune response after SARS-CoV-2 infection. Second, ribosomal proteins directly interact with viral proteins to inhibit the transcription or translation of viral genes. For example, RPL9 binds to phosphoprotein P to inhibit the transcription of viral genes [29, 34, 35]. Similarly, RPS10 has been shown to integrate HIV-1 to Nef protein, thereby weakening the production of viral proteins [36]. Accordingly, these proteins can be utilised to inhibit viral copying by integrating definite phosphoproteins or can serve as activators for host immune elements. Although RPL9 and RPS10 were not identified as core genes in the SARS-CoV-2 infection modules in the present study, these genes showed relatively low expression levels in the SARS-CoV-2-infected samples compared with those in the normal samples (figure 6). Robledo and colleagues disclosed that RPS15 is indispensable to the late maturation step of the 40S subunit, the large subunit correlated with rRNA processing and ribosome production [37]. Human RPL7a contains eight exons and seven introns spread over 3179

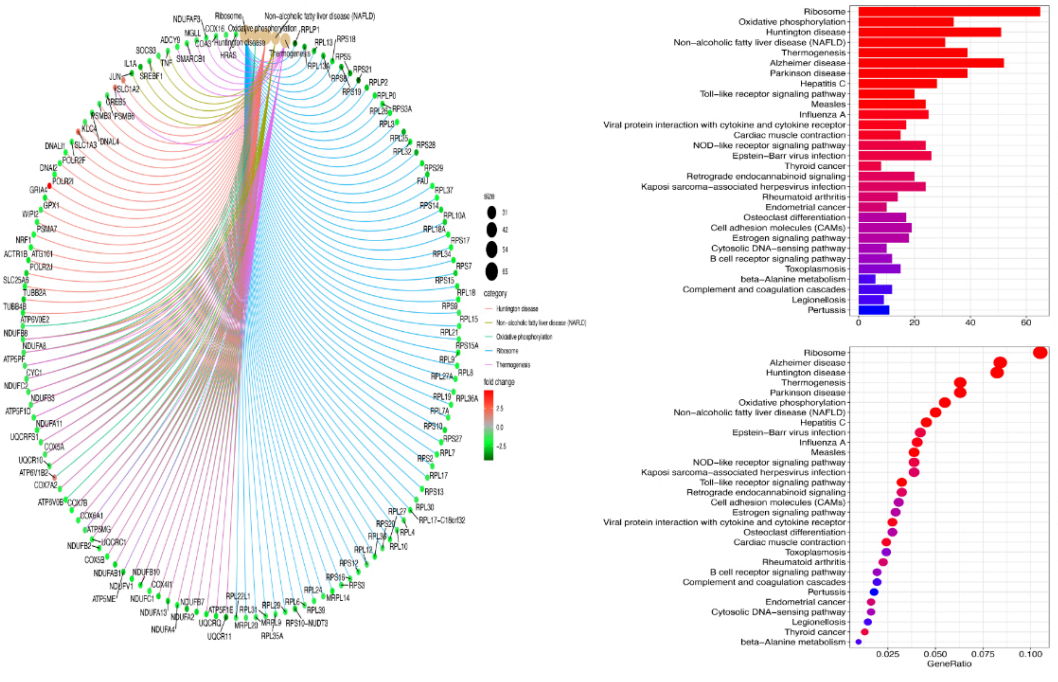


Figure 5: Top five enrichment results of the canonical pathway analysis of the DEGs in the yellow and grey modules. Abbreviations: DEG, differentially expressed gene.

bp [38]. Fumagilli and colleagues revealed that knock off RPL7a leads to a G1 block and a decreased percentage of cells in the S phase [39].

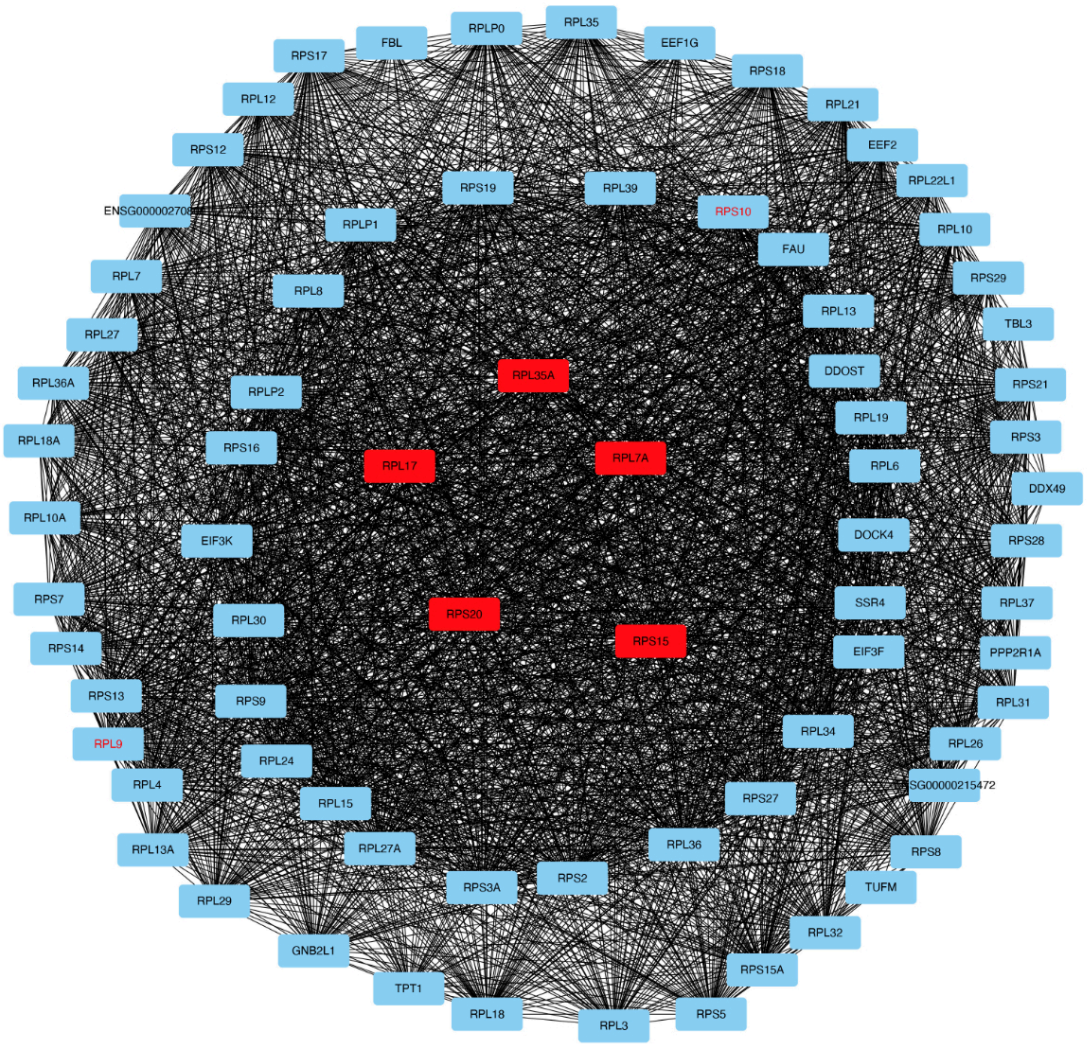
Downregulating RPL17 modifies the diversity of mature ribosomes by upregulating 5.8 S rRNA in mice [40]. RPL17 is a tunnel wall in eukaryotes and archaebacteria and regulates the outcome of a nascent polypeptide [41]. Consequently, these genes can be potential targets for developing vaccines or drugs targeting SARS-CoV-2 in further studies.

The GO analysis demonstrated viral infection strategies (viral translation) as activated biological processes in the yellow and grey SARS-CoV-2 infection modules. Li et al. [42] and Kuba et al. [43] respectively reported that angiotensin-converting enzyme 2 (ACE2) is an efficient SARS-CoV receptor in vitro and in vivo. In addition, SARS-CoV-2 and SARS-CoV have nearly identical three-dimensional structures of spike proteins [44, 45]. This suggests that binding to ACE2 is a key step for entry of the virus to human cells, leading to SARS-CoV-2 infection. Indeed, some studies have shown that upregulated ACE2 expression was associated with augmented disease severity and was a critical factor for viral entry to cells in establishing SARS-CoV-2 infection [46, 47]. These studies provide important insights into the critical step of SARS-CoV-2 infection, suggesting blocking ACE2 receptors as a potential therapeutic approach.

Some limitations of our study are as follows: First, while we performed a general analysis using the GSE152075 dataset containing clinical information and mRNA expression levels, we could not obtain complete clinical data (e.g. illness severity and ethnicity). This study only discusses the difference in the abnormal gene expression between the SARS-CoV-2-infected and normal samples. The correlation between other modules and the remaining clinical traits was not evaluated. Second, although we utilised WGCNA to identify the difference in the abnormal gene expression between the SARS-CoV-2-infected and normal samples, the role of the identified five-core gene set in SARS-CoV-2 infection needs further validation through an external set or a series of experiments.

In conclusion, the core genes and signalling pathways identified in the SARS-CoV-2 infection modules can significantly supplement the current understanding of COVID-19. In particular, the study findings can serve as a basis for creating a hypothetical model for future experimental work with respect to the association of SARS-CoV-2 infection with ribosomal protein function. More-

Figure 6: Protein–protein interaction network of the DEGs in the yellow and grey modules. Abbreviations: DEG, differentially expressed gene.



over, the core genes and signalling pathways may be used as therapeutic targets for COVID-19 treatment.

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Potential competing interests
All authors have completed and submitted the International Committee of Medical Journal Editors form for disclosure of potential conflicts of interest. No potential conflict of interest was disclosed.

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