Genetic association of COVID-19 severe versus non-severe cases by RNA sequencing in patients hospitalised in Hong Kong

Qi Li #, Zigui Chen #, Yexian Zhang #, Renee WY Chan, Marc KC Chong, Benny CY Zee, Lowell Ling, Grace Lui, Paul KS Chan, Maggie H Wang *

ABSTRACT

Introduction: The coronavirus disease 2019 (COVID-19) pandemic has caused extensive disruption of public health worldwide. There were reports of COVID-19 patients having multiple complications. This study investigated COVID-19 from a genetic perspective.

Methods: We conducted RNA sequencing (RNA-Seq) analysis of respiratory tract samples from 24 patients with COVID-19. Eight patients receiving mechanical ventilation or extracorporeal membrane oxygenation were regarded as severe cases; the remaining 16 patients were regarded as non-severe cases. After quality control, statistical analyses were performed by logistic regression and the Kolmogorov–Smirnov test to identify genes associated with disease severity.

Results: Six genes were associated with COVID-19 severity in both statistical tests, namely *RPL15*, *BACE1-AS*, *CEPT1*, *EIF4G1*, *TMEM91*, and *TBCK*. Among these genes, *RPL15* and *EIF4G1* played roles in the regulation of mRNA translation. Gene ontology analysis showed that the differentially expressed genes were mainly involved in nervous system diseases.

Conclusion: RNA sequencing analysis showed that severe acute respiratory syndrome coronavirus 2 infection is associated with the overexpression of genes involved in nervous system disorders.

This article was published on 8 Feb 2024 at www.hkmj.org.

Hong Kong Med J 2024;30:25–31
https://doi.org/10.12809/hkmj2210178

[1,2] **Q Li #,** PhD
[3] **Z Chen #,** PhD
[2] **Y Zhang #,** PhD
[4,5,6,7] **RWY Chan,** PhD
[1,2] **MKC Chong,** PhD
[1,2] **BCY Zee,** PhD
[8] **L Ling,** MD
[8] **G Lui,** MD
[3] **PKS Chan,** MD
[1,2] **MH Wang *,** PhD

[1] *The Jockey Club School of Public Health and Primary Care, The Chinese University of Hong Kong, Hong Kong SAR, China*
[2] *The Chinese University of Hong Kong Shenzhen Research Institute, Shenzhen, China*
[3] *Department of Microbiology, Stanley Ho Centre for Emerging Infectious Diseases, Li Ka Shing Institute of Health Sciences, The Chinese University of Hong Kong, Hong Kong SAR, China*
[4] *Department of Paediatrics, Faculty of Medicine, The Chinese University of Hong Kong, Hong Kong SAR, China*
[5] *Laboratory for Paediatric Respiratory Research, Li Ka Shing Institute of Health Sciences, Faculty of Medicine, The Chinese University of Hong Kong, Hong Kong SAR, China*
[6] *CUHK-UMCU Joint Research Laboratory of Respiratory Virus and Immunobiology, Department of Paediatrics, Faculty of Medicine, The Chinese University of Hong Kong, Hong Kong SAR, China*
[7] *Hong Kong Hub of Paediatric Excellence, The Chinese University of Hong Kong, Hong Kong SAR, China*
[8] *Department of Anaesthesia and Intensive Care, The Chinese University of Hong Kong, Hong Kong SAR, China*

# Equal contribution

* Corresponding author: maggiew@cuhk.edu.hk

New knowledge added by this study

- Differentially expressed genes between patients with severe and non-severe cases of coronavirus disease 2019 (COVID-19) were reported.
- Overexpression of genes involved in cell proliferation, viral binding and replication, and neurological and lung diseases was observed, suggesting a pathophysiological mechanism by which severe acute respiratory syndrome coronavirus 2 induces lung inflammation and neurological complications.

Implications for clinical practice or policy

- Future studies that involve gene expression profiling with larger sample sizes, in vitro infection experiments, and animal models can help to elucidate the mechanisms and corresponding therapeutic approaches for neurological complications of COVID-19.

Introduction

Coronavirus disease 2019 (COVID-19) has spread to >500 million people and caused 6.2 million deaths worldwide as of 22 April 2022.[1] Approximately 20% of patients with COVID-19 develop severe symptoms and 5% of patients require intensive care.[2] A wide range of complications were reported with COVID-19 infection, including nervous system diseases,[3,4] circulatory system diseases,[5-9] urinary system diseases,[10] and digestive system diseases.[11]

通過RNA定序對香港住院患者進行新冠肺炎重症與非重症病例的基因關聯性研究

李奇、陳子桂、張業先、陳韻怡、莊家俊、徐仲錡、凌若崴、雷頌恩、陳基湘、王海天

引言：新冠肺炎大流行對全球公共衛生造成了廣泛破壞。新冠肺炎患者出現多種併發症。本研究由基因角度探究新冠肺炎。

方法：我們對24名新冠肺炎患者進行了呼吸道樣本RNA定序分析，其中8位接受機械通氣或體外膜氧合的患者被視為重症，餘下16名患者則被視為非重症病例。我們在控制樣本品質後，通過邏輯迴歸和柯爾莫哥洛夫—斯米爾諾夫檢定來找出與疾病嚴重程度相關的基因。

結果：有6個基因在上述兩個檢定中均與新冠肺炎的嚴重程度顯著相關，包括*RPL15*、*BACE1-AS*、*CEPT1*、*EIF4G1*、*TMEM91*和*TBCK*，其中*RPL15*和*EIF4G1*調控mRNA的翻譯。基因本體分析顯示差異表現的基因主要涉及神經系統疾病。

結論：RNA定序分析顯示嚴重急性呼吸系統綜合症冠狀病毒2型與神經系統疾病涉及的基因過度表現相關。

Various genetic associations with COVID-19 outcomes have been explored.[12-15] A whole-genome sequencing study of germline mutations revealed a cluster of six genes (*SLC6A20*, *CCR9*, *FYCO1*, *CXCR6*, *XCR1*, and *LZTFL1*) that increased susceptibility to severe COVID-19 with respiratory failure.[16] In a Chinese population, a whole-genome sequencing study of 332 patients with COVID-19 identified loci in the genes *TMEM189* and *UBE2V1* with potential genome-wide implications through the *IL-1* signalling pathway.[17] In an intensive care unit cohort of 15 patients with severe COVID-19, analysis of RNA sequencing (RNA-Seq) data from blood samples showed that the immune-modulating genes *PD-L1* and *PD-L2* were differentially expressed among patients with fatal outcomes.[18]

Thus far, studies of gene expression at initial sites of infection in patients with severe and non-severe COVID-19 remain limited. To investigate COVID-19 from a genetic perspective, we conducted RNA-Seq analysis of respiratory tract samples from patients with COVID-19; we sought to identify genes associated with disease severity.

Methods

Patients

Twenty-four patients were recruited from Prince of Wales Hospital in Hong Kong between 7 February and 10 April 2020. All patients had severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) infection, confirmed by two independent real-time reverse transcription–polymerase chain reaction assays targeting the N gene.[19] Symptoms on admission were recorded, and medical histories were collected from clinical health records. Among the recruited patients, eight who received mechanical ventilation or extracorporeal membrane oxygenation were regarded as severe cases; the remaining 16 patients showed asymptomatic or mild (no pneumonia) to moderate (pneumonia but not requiring oxygen supplementation) disease and were regarded as non-severe cases. RNA sequencing was performed on upper and lower respiratory swab samples collected within 3 days after hospitalisation.

RNA sequencing data

Total RNA was extracted from respiratory swab samples using the QIAamp Viral RNA Mini Kit (Qiagen, Hilden, Germany), pre-treated with DNase I and depleted of human rRNA and globin genes using the QIAseq FastSelect ribosomal RNA and globin mRNA Removal Kit (Qiagen, Hilden, Germany). Illumina libraries for RNA-Seq next-generation sequencing were prepared using the KAPA HyperPrep Kit (Roche, Pleasanton [CA], US) in accordance with the manufacturer's instructions, then sequenced on an Illumina NextSeq 500 system (Illumina, San Diego [CA], US) using 150 bp paired-end reads. The raw data consisted of 58 735 Ensembl-annotated[20] genes. Quality control was performed to remove patients with low numbers of RNA-Seq reads (three samples) and genes with zero reads in >20% of samples (55 571 genes). Thus, 3164 genes remained available for differential expression analysis. Raw read count data were summarised as fragments per million reads mapped[21] and then $\log_2$-transformed.

Statistical analysis

Logistic regression was utilised to identify genes associated with severity outcomes. Subsequent evaluations by the Kolmogorov–Smirnov (KS) test were performed to test for differences in gene expression between groups. The Bonferroni corrected significance threshold was 1.58×10^{-5}.

Functional analysis of genes

According to their disease relevance in the GeneCards[21,22] and MalaCards[23,24] databases, genes were categorised into the following 10 disease groups: nervous system, integumentary system, circulatory system, urinary system, digestive system, respiratory system, musculoskeletal system, endocrine system, reproductive system, and infectious diseases.

Results

Demographics and baseline characteristics

The patients' demographics and baseline characteristics are summarised in Table 1. The mean age of patients in the severe group was 62.13 years (95% confidence interval [CI]=53.34-70.91), which was significantly higher than that in the non-severe

group (29.73 years; 95% CI=22.11-37.36). Compared with the non-severe group, the severe group had higher prevalences of complications including cardiovascular, liver, endocrine, and metabolic disorders, as well as higher rates of respiratory, fever, and diarrhoea symptoms. The COVID-19 World Health Organization score[25] was significantly higher in the severe group than in the non-severe group. Lopinavir, antibiotics, conventional oxygen therapy, and mechanical ventilation were more commonly used for treatment in the severe group than in the non-severe group (Table 1).

TABLE 1. Demographic and baseline clinical characteristics of patients with severe and non-severe cases of coronavirus disease 2019 (COVID-19)*

	Severe group (n=8)†	Non-severe group (n=15)‡	P value§
Male sex	6 (75.0%)	9 (60.0%)	0.657
Age, y	62.13 (53.34-70.91)	29.73 (22.11-37.36)	<0.0001
Complications			
Cardiovascular diseases	6 (75.0%)	0	<0.001
Liver diseases	3 (37.5%)	0	0.032
Endocrine diseases	3 (37.5%)	0	0.032
Bone diseases	2 (25.0%)	0	0.111
Metabolic diseases	3 (37.5%)	0	0.032
Neurological diseases	2 (25.0%)	3 (20.0%)	1.000
Reproductive diseases	0	1 (6.7%)	1.000
None	0	11 (73.3%)	0.001
Symptoms			
Fever	6 (75.0%)	2 (13.3%)	0.006
Diarrhoea	3 (37.5%)	0	0.032
Respiratory symptoms	7 (87.5%)	2 (13.3%)	0.001
Initial viral load, $\log_{10}$ copy/mL	4.92 (3.61-6.22)	4.95 (3.81-6.10)	0.963
COVID-19 WHO score	5.75 (4.88-6.62)	4.00 (4.00-4.00)	0.002
Medicine			
Lopinavir	7 (87.5%)	5 (33.3%)	0.027
Ribavirin	5 (62.5%)	5 (33.3%)	0.221
Interferon	3 (37.5%)	5 (33.3%)	1.000
Steroid	1 (12.5%)	0	0.348
Antibiotic	8 (100%)	1 (6.7%)	<0.001
Oxygen therapy			
Oxygen	8 (100%)	0	<0.001
High-flow oxygen	0	0	
Non-invasive ventilation	0	0	
Ventilation	3 (37.5%)	0	0.032
ECMO	0	0	

Abbreviations: ECMO = extracorporeal membrane oxygenation; WHO = World Health Organization

* Data are shown as No. (%) or mean (95% confidence interval), unless otherwise specified

† One patient in the severe group had no record of initial viral load

‡ One patient in the non-severe group with no record of medical history was excluded

§ Calculated by *t* test for continuous variables and Fisher's exact test for categorical variables

Differentially expressed genes according to RNA sequencing

Six genes, namely *RPL15*, *BACE1-AS*, *CEPT1*, *EIF4G1*, *TMEM91*, and *TBCK*, were differentially expressed between the severe and non-severe groups (all P values <0.05 in both logistic regression and the KS test) [Table 2]. Fold-change and odds ratio results indicated that these genes were consistently highly expressed in the severe group. The complete list of genes with P values <0.05 in KS test is provided in online supplementary Table 1.

Gene ontology and enrichment analysis

The functions of the identified genes were summarised through database and literature searches. Two genes, 60S ribosomal protein L15 (*RPL15*) and eukaryotic translation initiation factor 4 gamma 1 (*EIF4G1*), play roles in host translation of viral mRNA.[26-28] Furthermore, the top genes were mainly involved in neurological disorders. *RPL15* is involved in the life cycle of human immunodeficiency virus,[29,30] and baculovirus infection reportedly disrupts the expression of this gene.[31,32] *EIF4G1* plays a role in viral binding and affects the pathogenicity and virulence of H5N1 influenza A virus, foot-and-mouth disease virus, and vaccinia virus[33-35]; it also contains multiple mutations among patients with familial Parkinson's disease.[36] *TBCK* encodes a conserved protein kinase that regulates cell size and proliferation.[37] *CEPT1* encodes choline/ethanolamine phosphotransferase, which is used in the synthesis of choline- or ethanolamine-containing phospholipids. The function of *TMEM91*, a transmembrane protein, is unclear; however, the results of genome-wide association studies suggest that loci containing this gene are involved in lung diseases.

The non-coding gene *BACE1-AS* regulates the stability of the *BACE1* protein and directly increases the abundance of amyloid beta-peptide ($A\beta_{1-42}$) in Alzheimer's disease.[38] The implications of this gene in severe COVID-19 are unclear. For the top 15 overexpressed genes (P values in KS test <0.05), disease relevance data were retrieved from GeneCards[22]; 14 of the 15 genes (93.3%) have been linked to neurological diseases, followed by eye (80.0%) and psychiatric (73.3%) diseases. Thus, all of the top genes were involved in nervous system disorders (Fig).

Discussion

Nervous system disorders such as encephalopathy, impaired consciousness, seizure, ataxia,

neuropathies, neurodegenerative diseases, and anosmia have been extensively documented in patients with COVID-19.[39-41] The two major potential pathogenesis pathways are direct viral invasion and immune-mediated injury. Direct viral entry to the central nervous system can travel through hematogenous or olfactory routes, or by transneuronal spread from the lungs.[42] Post-mortem analysis of brain tissue from patients with COVID-19 encephalitis reportedly contained SARS-CoV-2 viral particles.[43,44] Furthermore, a series of autopsy studies showed that localised inflammation of the brainstem nuclei, as well as the cytokine storm associated with SARS-CoV-2 infection, could disrupt the blood–brain barrier and cause necrosis in the brains of patients with severe COVID-19.[45,46] In patients with COVID-19, anosmia may be caused by an inflammation-mediated decrease in odorant receptor expression.[47] Several studies have utilised RNA-Seq to characterise the transcriptomic profiles of patients with COVID-19.[48,49] Significant downregulation of genes related to the hypoxia-inducible factor system was observed during periods of infection and oxygen deprivation.[50] Additionally, transcriptomic profiles of peripheral blood mononuclear cells revealed that patients with COVID-19 shared several dysregulated genes with individuals who had bipolar illness, post-traumatic stress disorder, or schizophrenia.[51] The

TABLE 2. Differentially expressed genes between patients with severe and non-severe cases of coronavirus disease 2019 according to RNA sequencing analysis

	Log_2(FC)*	Odds ratio	P value in logistic regression	P value in Kolmogorov–Smirnov test
RPL15	0.33	1.48	0.022	0.005
BACE1-AS	0.27	1.38	0.034	0.013
CEPT1	0.11	1.42	0.022	0.030
EIF4G1	0.57	1.70	0.015	0.044
TMEM91	0.36	1.42	0.027	0.044
TBCK	0.52	1.34	0.036	0.044

Abbreviation: Log_2(FC) = log_2(fold-change)
* Value >0 indicates higher mean expression in the severe group

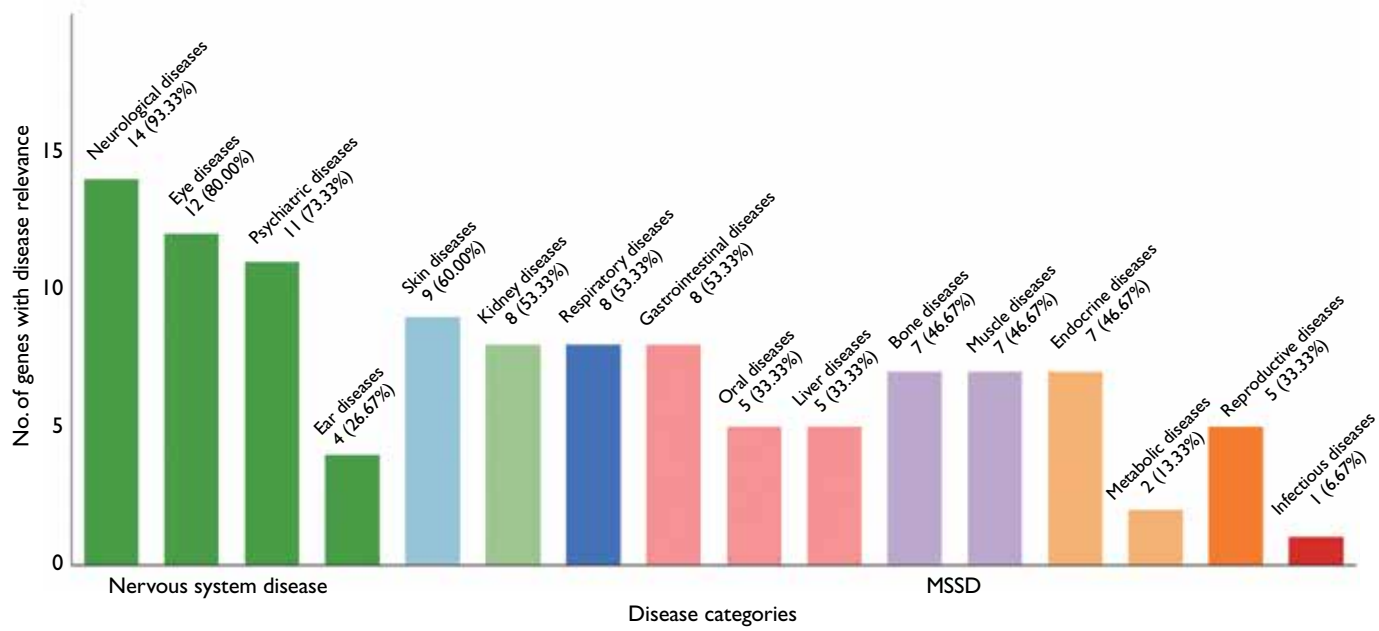

FIG. Frequency of diseases related to the top identified genes (n=15)*
Abbreviation: MSSD = musculoskeletal system disease
* Data are shown as No. (%)

present findings suggest that SARS-CoV-2 infection is associated with differential expression of genes involved in nervous system disorders. Future studies that involve gene expression profiling with larger sample sizes, in vitro infection experiments, and animal models can help to elucidate the mechanisms and corresponding therapeutic approaches for neurological complications of COVID-19.

Limitations

A major limitation of this study was its small sample size. Patient age distributions considerably differed between groups. However, age-stratified analysis showed effects consistent with the directions reported in Table 2, although the statistical significance was hindered by the small sample size (online supplementary Table 2 and online supplementary Fig). Further sequencing of samples collected from respiratory tract sites may provide stronger evidence of protein expression abnormalities at the initial site of SARS-CoV-2 infection.

Conclusion

In this study, we conducted RNA-Seq analysis to identify differentially expressed genes between patients with severe and non-severe cases of COVID-19. We observed overexpression of genes involved in cell proliferation, viral binding and replication, and neurological and lung diseases, suggesting a pathophysiological mechanism by which SARS-CoV-2 induces lung inflammation and neurological complications.

Author contributions

Concept or design: BCY Zee, PKS Chan, MH Wang.
Acquisition of data: Z Chen, PKS Chan.
Analysis or interpretation of data: Q Li, Z Chen, Y Zhang, RWY Chan, MKC Chong, PKS Chan, G Lui, L Ling.
Drafting of the manuscript: Q Li, MH Wang.
Critical revision of the manuscript for important intellectual content: Q Li, Y Zhang, MH Wang.

All authors had full access to the data, contributed to the study, approved the final version for publication, and take responsibility for its accuracy and integrity.

Conflicts of interest

BCY Zee is a shareholder of Health View Bioanalytic Limited. As a statistical adviser of the journal, MKC Chong was not involved in the peer review process. MH Wang is a shareholder of Beth Bioinformatics Co, Ltd. Other authors have disclosed no conflicts of interest.

Funding/support

This research was partially supported by the Health and Medical Research Fund of the former Food and Health Bureau, Hong Kong SAR Government (Ref Nos.: COVID190103, COVID190112 and INF-CUHK-1), The Chinese University of Hong Kong (CUHK) Project Impact Enhancement Fund (Ref No.: CUPIEF/Ph2/COVID/06) and CUHK Direct Grant (Ref No.: 2020.025). The funders had no role in study design, data collection/analysis/interpretation, or manuscript preparation.

Ethics approval

The study protocol of this research was approved by the Joint Chinese University of Hong Kong–New Territories East Cluster Clinical Research Ethics Committee (Ref No.: 2020.076). All patients provided written informed consent for participation in this research.

Supplementary material

The supplementary material was provided by the authors and some information may not have been peer reviewed. Any opinions or recommendations discussed are solely those of the author(s) and are not endorsed by the Hong Kong Academy of Medicine and the Hong Kong Medical Association. The Hong Kong Academy of Medicine and the Hong Kong Medical Association disclaim all liability and responsibility arising from any reliance placed on the content. To view the file, please visit the journal online (https://doi.org/10.12809/hkmj2210178).

References

1. World Health Organization. COVID-19 global data. Available from: https://covid19.who.int/WHO-COVID-19-global-data.csv. Accessed 22 Apr 2022.
2. Wu Z, McGoogan JM. Characteristics of and important lessons from the coronavirus disease 2019 (COVID-19) outbreak in China: summary of a report of 72 314 cases from the Chinese Center for Disease Control and Prevention. JAMA 2020;323:1239-42.
3. Gusev EI, Martynov MY, Boyko AN, et al. The novel coronavirus infection (COVID-19) and nervous system involvement: mechanisms of neurological disorders, clinical manifestations, and the organization of neurological care. Neurosci Behav Physiol 2021;51:147-54.
4. Kubota T, Kuroda N. Exacerbation of neurological symptoms and COVID-19 severity in patients with preexisting neurological disorders and COVID-19: a systematic review. Clin Neurol Neurosurg 2021;200:106349.
5. Zhou F, Yu T, Du R, et al. Clinical course and risk factors for mortality of adult inpatients with COVID-19 in Wuhan, China: a retrospective cohort study. Lancet 2020;395:1054-62.
6. Li X, Xu S, Yu M, et al. Risk factors for severity and mortality in adult COVID-19 inpatients in Wuhan. J Allergy Clin Immunol 2020;146:110-8.
7. Chen R, Liang W, Jiang M, et al. Risk factors of fatal outcome in hospitalized subjects with coronavirus disease 2019 from a nationwide analysis in China. Chest 2020;158:97-105.
8. Docherty AB, Harrison EM, Green CA, et al. Features of 20 133 UK patients in hospital with COVID-19 using the ISARIC WHO Clinical Characterisation Protocol: prospective observational cohort study. BMJ 2020;369:m1985.
9. Richardson S, Hirsch JS, Narasimhan M, et al. Presenting characteristics, comorbidities, and outcomes among 5700 patients hospitalized with COVID-19 in the New York City

area. JAMA 2020;323:2052-9.
10. Cheng Y, Luo R, Wang K, et al. Kidney disease is associated with in-hospital death of patients with COVID-19. Kidney Int 2020;97:829-38.
11. Mao R, Qiu Y, He JS, et al. Manifestations and prognosis of gastrointestinal and liver involvement in patients with COVID-19: a systematic review and meta-analysis. Lancet Gastroenterol Hepatol 2020;5:667-78.
12. Pairo-Castineira E, Clohisey S, Klaric L, et al. Genetic mechanisms of critical illness in COVID-19. Nature 2021;591:92-8.
13. COVID-19 Host Genetics Initiative. The COVID-19 Host Genetics Initiative, a global initiative to elucidate the role of host genetic factors in susceptibility and severity of the SARS-CoV-2 virus pandemic. Eur J Hum Genet 2020;28:715-8.
14. Anastassopoulou C, Gkizarioti Z, Patrinos GP, Tsakris A. Human genetic factors associated with susceptibility to SARS-CoV-2 infection and COVID-19 disease severity. Human Genomics 2020;14:40.
15. Kousathanas A, Pairo-Castineira E, Rawlik K, et al. Whole-genome sequencing reveals host factors underlying critical COVID-19. Nature 2022;607:97-103.
16. Severe Covid-19 GWAS Group; Ellinghaus D, Degenhardt F, et al. Genomewide association study of severe COVID-19 with respiratory failure. N Engl J Med 2020;383:1522-34.
17. Wang F, Huang S, Gao R, et al. Initial whole-genome sequencing and analysis of the host genetic contribution to COVID-19 severity and susceptibility. Cell Discov 2020;6:83.
18. Fredericks AM, Jentzsch MS, Cioffi WG, et al. Deep RNA sequencing of intensive care unit patients with COVID-19. Sci Rep 2022;12:15755.
19. Lui G, Ling L, Lai CK, et al. Viral dynamics of SARS-CoV-2 across a spectrum of disease severity in COVID-19. J Infect 2020;81:318-56.
20. Howe KL, Achuthan P, Allen J, et al. Ensembl 2021. Nucleic Acids Res 2021;49:D884-91.
21. Stelzer G, Rosen N, Plaschkes I, et al. The GeneCards suite: from gene data mining to disease genome sequence analyses. Curr Protoc Bioinformatics 2016;54:1.30.1-33.
22. GeneCards. Available from: www.genecards.org. Accessed 15 Jan 2024.
23. Rappaport N, Twik M, Plaschkes I, et al. MalaCards: an amalgamated human disease compendium with diverse clinical and genetic annotation and structured search. Nucleic Acids Res 2017;45:D877-87.
24. MalaCards. Available from: www.malacards.org. Accessed 15 Jan 2024.
25. WHO Working Group on the Clinical Characterisation and Management of COVID-19 infection. A minimal common outcome measure set for COVID-19 clinical research. Lancet Infect Dis 2020;20:e192-7.
26. Ulyanova V, Shah Mahmud R, Laikov A, et al. Anti-influenza activity of the ribonuclease binase: cellular targets detected by quantitative proteomics. Int J Mol Sci 2020;21:8294.
27. Hanson P, Zhang HM, Hemida MG, Ye X, Qiu Y, Yang D. IRES-dependent translational control during virus-induced endoplasmic reticulum stress and apoptosis. Front Microbiol 2012;3:92.
28. Miller MD, Farnet CM, Bushman FD. Human immunodeficiency virus type 1 preintegration complexes: studies of organization and composition. J Virol 1997;71:5382-90.
29. Frankel AD, Young JA. HIV-1: fifteen proteins and an RNA. Annu Rev Biochem 1998;67:1-25.
30. Kash JC, Goodman AG, Korth MJ, Katze MG. Hijacking of the host-cell response and translational control during influenza virus infection. Virus Res 2006;119:111-20.
31. van Oers MM, Doitsidou M, Thomas AA, de Maagd RA, Vlak JM. Translation of both 5' TOP and non-TOP host mRNAs continues into the late phase of Baculovirus infection. Insect Mol Biol 2003;12:75-84.
32. Zhou H, Zhu J, Tu J, et al. Effect on virulence and pathogenicity of H5N1 influenza A virus through truncations of NS1 eIF4GI binding domain. J Infect Dis 2010;202:1338-46.
33. Foeger N, Kuehnel E, Cencic R, Skern T. The binding of foot-and-mouth disease virus leader proteinase to eIF4GI involves conserved ionic interactions. FEBS J 2005;272:2602-11.
34. Zaborowska I, Kellner K, Henry M, Meleady P, Walsh D. Recruitment of host translation initiation factor eIF4G by the Vaccinia Virus ssDNA-binding protein I3. Virology 2012;425:11-22.
35. Chartier-Harlin MC, Dachsel JC, Vilariño-Güell C, et al. Translation initiator EIF4G1 mutations in familial Parkinson disease. Am J Hum Genet 2011;89:398-406.
36. Bhoj EJ, Li D, Harr M, et al. Mutations in TBCK, encoding TBC1-domain-containing kinase, lead to a recognizable syndrome of intellectual disability and hypotonia. Am J Hum Genet 2016;98:782-8.
37. Chong JX, Caputo V, Phelps IG, et al. Recessive inactivating mutations in TBCK, encoding a Rab GTPase-activating protein, cause severe infantile syndromic encephalopathy. Am J Hum Genet 2016;98:772-81.
38. Khatoon F, Prasad K, Kumar V. COVID-19 associated nervous system manifestations. Sleep Med 2022;91:231-6.
39. Butowt R, von Bartheld CS. Anosmia in COVID-19: underlying mechanisms and assessment of an olfactory route to brain infection. Neuroscientist 2021;27:582-603.
40. Ye M, Ren Y, Lv T. Encephalitis as a clinical manifestation of COVID-19. Brain Behav Immun 2020;88:945-6.
41. Johansson A, Mohamed MS, Moulin TC, Schiöth HB. Neurological manifestations of COVID-19: a comprehensive literature review and discussion of mechanisms. J Neuroimmunol 2021;358:577658.
42. Paniz-Mondolfi A, Bryce C, Grimes Z, et al. Central nervous system involvement by severe acute respiratory syndrome coronavirus-2 (SARS-CoV-2). J Med Virol 2020;92:699-702.
43. Moriguchi T, Harii N, Goto J, et al. A first case of meningitis/encephalitis associated with SARS-coronavirus-2. Int J Infect Dis 2020;94:55-8.
44. von Weyhern CH, Kaufmann I, Neff F, Kremer M. Early evidence of pronounced brain involvement in fatal COVID-19 outcomes. Lancet 2020;395:e109.
45. Virhammar J, Kumlien E, Fällmar D, et al. Acute necrotizing encephalopathy with SARS-CoV-2 RNA confirmed in cerebrospinal fluid. Neurology 2020;95:445-9.
46. Radmanesh A, Derman A, Ishida K. COVID-19–associated delayed posthypoxic necrotizing leukoencephalopathy. J Neurol Sci 2020;415:116945.
47. Daamen AR, Bachali P, Owen KA, et al. Comprehensive

transcriptomic analysis of COVID-19 blood, lung, and airway. Sci Rep 2021;11:7052.
48. Xiong Y, Liu Y, Cao L, et al. Transcriptomic characteristics of bronchoalveolar lavage fluid and peripheral blood mononuclear cells in COVID-19 patients. Emerg Microbes Infect 2020;9:761-70.
49. Gagliardi S, Poloni ET, Pandini C, et al. Detection of SARS-CoV-2 genome and whole transcriptome sequencing in frontal cortex of COVID-19 patients. Brain Behav Immun 2021;97:13-21.
50. Moni MA, Lin PI, Quinn JM, Eapen V. COVID-19 patient transcriptomic and genomic profiling reveals comorbidity interactions with psychiatric disorders. Transl Psychiatry 2021;11:160.
51. MAQC Consortium; Shi L, Reid LH, et al. The MicroArray Quality Control (MAQC) project shows inter- and intraplatform reproducibility of gene expression measurements. Nat Biotechnol 2006;24:1151-61.