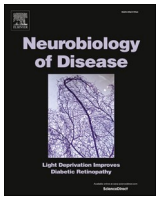
Since January 2020 Elsevier has created a COVID-19 resource centre with free information in English and Mandarin on the novel coronavirus COVID-19. The COVID-19 resource centre is hosted on Elsevier Connect, the company's public news and information website.

Neurobiology of Disease 182 (2023) 106147

Contents lists available at ScienceDirect

Neurobiology of Disease

journal homepage: www.elsevier.com/locate/ynbdi



Uncovering a neurological protein signature for severe COVID-19

Omar El-Agnaf [a,b], Ilham Bensmail [c], Maryam A.Y. Al-Nesf [d,e], James Flynn [f], Mark Taylor [g], Nour K. Majbour [a], Ilham Y. Abdi [a], Nishant N. Vaikath [a], Abdulaziz Farooq [h], Praveen B. Vemulapalli [i], Frank Schmidt [i], Khalid Ouararhni [j], Heba H. Al-Siddiqi [k,b], Abdelilah Arredouani [k,b], Patrick Wijten [k], Mohammed Al-Maadheed [e,l], Vidya Mohamed-Ali [e,l], Julie Decock [m,b], Houari B. Abdesselem [a,b,c,*]

[a] Neurological Disorders Research Center (NDRC), Qatar Biomedical Research Institute (QBRI), Hamad Bin Khalifa University (HBKU), Qatar Foundation, Doha, Qatar
[b] College of Health and Life Sciences (CHLS), Hamad Bin Khalifa University (HBKU), Qatar Foundation, Doha, Qatar
[c] Proteomics Core Facility, Qatar Biomedical Research Institute (QBRI), Hamad Bin Khalifa University (HBKU), Qatar Foundation, Doha, Qatar
[d] Department of Medicine, Hamad General Hospital, Hamad Medical Corporation, Doha, Qatar
[e] Center of Metabolism and Inflammation, Division of Medicine, Royal Free Campus, University College London, Rowland Hill Road, London NW3 2PF, UK
[f] Illumina Corporation, San Diego, CA, USA
[g] Bioinfo Solutions LLC, Parker, CO, USA
[h] Aspetar Hospital, Orthopaedic and Sports Medicine, Hospital, FIFA Medical Centre of Excellence, Doha, Qatar
[i] Proteomics Core, Weill Cornell Medicine - Qatar, Doha, Qatar
[j] Genomics Core Facility, Qatar Biomedical Research Institute (QBRI), Hamad Bin Khalifa University (HBKU), Qatar Foundation, Doha, Qatar
[k] Diabetes Research Center, Qatar Biomedical Research Institute (QBRI), Hamad Bin Khalifa University (HBKU), Qatar Foundation, Doha, Qatar
[l] Anti-Doping Laboratory Qatar, Doha, Qatar
[m] Translational Cancer and Immunity Center (TCIC), Qatar Biomedical Research Institute (QBRI), Hamad Bin Khalifa University (HBKU), Qatar Foundation, Doha, Qatar

ARTICLE INFO

Keywords:
Severe COVID-19
Neurological complications
Olink proteomics
Protein signature

ABSTRACT

Coronavirus disease of 2019 (COVID-19), caused by severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), has sparked a global pandemic with severe complications and high morbidity rate. Neurological symptoms in COVID-19 patients, and neurological sequelae post COVID-19 recovery have been extensively reported. Yet, neurological molecular signature and signaling pathways that are affected in the central nervous system (CNS) of COVID-19 severe patients remain still unknown and need to be identified. Plasma samples from 49 severe COVID-19 patients, 50 mild COVID-19 patients, and 40 healthy controls were subjected to Olink proteomics analysis of 184 CNS-enriched proteins. By using a multi-approach bioinformatics analysis, we identified a 34-neurological protein signature for COVID-19 severity and unveiled dysregulated neurological pathways in severe cases. Here, we identified a new neurological protein signature for severe COVID-19 that was validated in different independent cohorts using blood and postmortem brain samples and shown to correlate with neurological diseases and pharmacological drugs. This protein signature could potentially aid the development of prognostic and diagnostic tools for neurological complications in post-COVID-19 convalescent patients with long term neurological sequelae.

Abbreviations: ACVRL1, Activin A Receptor Like Type 1; ARDS, Acute Respiratory Distress Syndrome; BMI, Body Mass Index; COVID-19, Coronavirus Disease 2019; CE, Correlation Engine; CNS, Central Nervous System; DEPs, Differentially Expressed Proteins; DKK4, Dickkopf WNT Signaling Pathway Inhibitor 4; FDR, False Discovery Rate; GDNFR3, GDNF Family Receptor alpha-3; GDNF, Glial Derived Neurotrophic Factor; GFAP, Glial Fibrillary Acidic Protein; GO, Gene Ontology; ICU, Intensive Care Unit; IPA, Ingenuity Pathway Analysis; KEGG, Kyoto Encyclopedia of Genes and Genomes; LGI1, Leucine Rich Glioma Inactivated 1; MGH, Massachusetts General Hospital; NEFL, Neurofilament Light Chain; NGF, Nerve Growth Factor; NfL, Neurofilament Light Chain Protein; NPX, Normalized Protein eXpression; O_2, Oxygen; PNS, Peripheral Nervous System; PCA, Principal Component Analysis; PHOSPHO1, Phosphoethanolamine/Phosphocholine Phosphatase 1; RGMB, Repulsive Guidance Molecule BMP Co-Receptor B; SARS-CoV-2, Severe Acute Respiratory Syndrome Coronavirus 2; SIGLEC1, Sialic acid-binding ImmunoGlobulin-type Lectins 1; THOP1, Thimet Oligopeptidase 1; TDGF1, Teratocarcinoma-Derived Growth Factor 1; UCH-L1, Ubiquitin Carboxyl-terminal Hydrolase L1; WGCNA, Weighted Gene Co-expression Network Analysis.

* Corresponding author at: Neurological Disorders Research Center (NDRC), Qatar Biomedical Research Institute (QBRI), Hamad Bin Khalifa University (HBKU), Qatar Foundation, Doha, Qatar.
E-mail address: habdesselem@hbku.edu.qa (H.B. Abdesselem).

https://doi.org/10.1016/j.nbd.2023.106147
Received 16 February 2023; Received in revised form 30 April 2023; Accepted 7 May 2023
Available online 11 May 2023

1. Introduction

COVID-19 exhibits a highly heterogeneous clinical profile with no, mild or severe symptoms, the latter associated with the need of life support in intensive care units (ICU) or fatal outcomes. Neurologic manifestations are common features of severe COVID-19 disease including neurological and neuropsychiatric symptoms of infection of the central nervous system (CNS) such as dizziness, headache, impaired consciousness, ataxia, altered mental status, anosmia, chronic fatigue, memory impairment, and depression (Varatharaj et al., 2020; Paterson et al., 2020; Tsivgoulis et al., 2020; Helms et al., 2020; Rogers et al., 2020; Leonardi et al., 2020; Achar and Ghosh, 2020; Mao et al., 2020; Eden et al., 2021a; Oliveira et al., 2021; Sinanovic et al., 2020; Bodnar et al., 2021). Moreover, a variety of CNS disorders; encephalopathies, seizures, ataxia and strokes, have been found particularly in severe COVID-19 patients(Chaumont et al., 2020; Logmin et al., 2020; Princiotta Cariddi et al., 2020; Zachariadis et al., 2020; Virhammar et al., 2020; Virhammar et al., 2021), and perturbations related to neurological/neurodegenerative diseases have been reported in severe COVID-19 brains(Yang et al., 2021; Fullard et al., 2021; Emmi et al., 2023; Schwabenland et al., 2021). Several emerging reports have demonstrated that different mechanisms may contribute to some or all aspects of the neuro-invasive potential of SARS-CoV-2(Achar and Ghosh, 2020; Mao et al., 2020). These include (i) direct viral interaction with neuronal and supportive cells in a retrograde axonal transport manner, primarily in the olfactory mucosa; (ii) indirect effect of the systemic immune response leading to activation of CNS-resident immune cells; and (iii) viral interaction with cells of the vasculature including pericytes and/or endothelial cells and CNS invasion through the blood-brain barrier (BBB)(Eden et al., 2021a). Likely, different combinations of these mechanisms could contribute to the clinical disease complications of individual patients. At the cellular level, neuronal injury and glial activation have been observed in patients with severe COVID-19 using CNS biomarkers of axonal and astrocytic damage[23,] (Hirzel et al., 2022). CNS biomarkers such as Neurofilament light chain (NEFL), Glial fibrillary acidic protein (GFAP), ubiquitin carboxy-terminal hydrolase L1 (UCH-L1), and Tau have been shown to reflect CNS damage in traumatic brain injury(Virhammar et al., 2021; De Lorenzo et al., 2021; Bacioglu et al., 2016; McMahon et al., 2015; Wang et al., 2021). These biomarkers have been extensively used to assess COVID-19 neuropathogenesis in ICU admitted severe patients and post-COVID-19 convalescent patients as well(Hanson et al., 2022; Eden et al., 2021b; Sun et al., 2021). Nevertheless, there is a need to explore other CNS proteins and decipher the molecular pathways underlying neurological/neuropsychiatric dysfunctions in severe COVID-19 cases.

Furthermore, as a significant number of case reports and studies have described different types of neurological complications in severe COVID-19 patients; early predictive and diagnostic tools to classify the risk of developing neurological complications and assess the neurological status of post-COVID-19 convalescent patients are crucial for clinical management. In this study, we hypothesized that alterations in plasma proteins could offer predictive and diagnostic neurological protein signatures that can help to predict the risk of developing complications and diagnose neurological complications in convalescent patients. To address these questions, we used high throughput Olink proteomics analysis of plasma samples from patients with severe complications versus mild symptoms and control subjects and assessed changes in neurological protein profiles and pathways associated with severe COVID-19.

2. Methods

2.1. Study cohorts

We used two cohorts for this study; one cohort of COVID-19 patients and one cohort of healthy subjects(Al-Nesf et al., 2022) (Table 1, Supplementary data 1). All participants (patients and controls) provided written informed consent prior to enrolment in the study (reference MRC-05-003).

2.1.1. Qatar COVID-19 cohort

This cohort included 99 COVID-19 patients from Qatar who were admitted to Hamad Medical Corporation hospitals. All recruited patients had confirmed SARS-CoV-2 positive RT-PCR results of sputum and throat swabs, of which 49 patients had severe COVID-19 disease and were admitted to intensive care unit (ICU) and 50 patients had mild-moderate COVID-19 disease. Peripheral blood was collected within 5–7 days post-admittance and plasma was stored at –80 °C until use. Ethical approvals for this cohort were obtained from the Hamad Medical Corporation Institutional Review Board Research Ethics Committee (reference MRC-05-003), and the Qatar Biomedical Research Institute-Institutional Review Boards (Reference QBRI-IRB 2020-06-19).

2.1.2. Healthy control cohort

Age- and gender- matched healthy subjects ($n = 40$) with no history of prior COVID-19 infection and with normal oxygen saturation and vital signs were recruited by the Anti-Doping Laboratory-Qatar. Individuals with medical history or with cognitive disabilities were excluded.

2.2. Olink proteomic assays

Plasma samples were profiled using the Olink proximity extension assays (PEA), 92-plex immunoassay (Uppsala, Sweden)(Assarsson et al., 2014) following the standard protocol. Quality control and data normalization were carried out using the Normalized Protein eXpression (NPX) software and every run was checked and validated by the Olink support team in Uppsala, Sweden. Two different protein panels focusing on CNS and neurodegenerative diseases were used in our study; the Neurology target panel and the NeuroExploratory target panel. Protein expression values were calculated as log2(NPX), and Olink data that did not pass quality control were excluded from the analyses (Supplementary data 2).

2.3. Simoa assay

Using single molecule array (Simoa) technology (Quanterix), we measured four neurology biomarkers in human plasma samples from mild and severe cases of COVID-19 as well as healthy controls. We used the Human Neurology 4 Plex B (N4PB) kit and the assay was run on the Quanterix HD-X system. The four targets are total tau, neurofilament light (NEFL), glial fibrillary acidic protein (GFAP), and ubiquitin carboxyl-terminal hydrolase L1 (UCH-L1) and measurements were done according to manufacturer's instructions.

2.4. Bioinformatics

R packages for hierarchical clustering (heatmap.2), principal

Table 1
Characteristics of Qatar patients with COVID-19 and healthy controls.

Variables	Controls	Mild	Severe
N	40	50	49
Age (Year)			
Mean (± SD)	37 ± 7	40 ± 11	45
Sex (N)			
F	2	8	1
M	38	42	48
Ethnicity (%)			
Indian subcontinent	86	60	66
Middle East North Africa	10	30	20
Others	4	10	14

component analysis (PCA), differentially expression analysis (Linear Models for Microarray Data (limma)), volcano plots, WGCNA co-expression networks analyses, gene-ontology biological process (GO) and KEGG pathways enrichment analyses were used through the standalone version of Integrated Differential Expression and Pathway analysis (iDEP) iDEP 0.93 and iDEP 0.96 (https://bioinformatics.sdstate.edu/idep/)(Ge et al., 2018). Statistical analyses were also performed using GraphPad Prism 9.0 (GraphPad Software, Inc., Boston, MA). Molecular pathway analysis was conducted using Ingenuity pathway analysis (IPA). Protein–Protein Interaction (PPI) network analysis was conducted using the STRING database v11.5 (https://string-db.org) as described before(Szklarczyk et al., 2019).

2.5. Correlation analysis

Illumina's Basespace™ Correlation Engine (BSCE; Illumina, San Diego, CA, USA) was used to correlate the 34 neurological protein signature of severe COVID-19 to available SARS-CoV-2 host infection transcriptome data. BSCE contains 166,000 Bioset lists of highly curated, statistically significant genes from over 25,100 omic-scale Curated Studies(Kupershmidt et al., 2010) as of 1 Oct 2022. Curated Studies in BSCE are processed in standard, platform-specific pipelines to generate gene sets and measures. For each study, available Test group vs. Control group results plus experimental metadata are generated. Second, BSCE has robust data-driven correlation, aggregation, and machine learning applications to exploit the consistent processing and curation of omic-scale studies from international public repositories. The BSCE Pharmaco Atlas and Disease Atlas applications were used to respectively categorize, and rank compounds or diseases based on signature correlations across all BSCE Curated Studies.

3. Results

3.1. Study design and cohort specific information

In the present work, SARS-CoV-2 infected patients of a local Qatar cohort were evaluated for their proteomics profiling. The cohort was recruited at HMC hospital in Doha, Qatar and included 49 subjects with severe and 50 subjects with mild disease; and 40 healthy control subjects (Table 1, Supplementary data 1). COVID-19 patients were admitted to ICU and classified as severe cases using World Health Organization (WHO) severity scoring for COVID-19. Severity was defined by low oxygen saturation, severe pneumonia, and/or signs of severe respiratory distress (accessory muscle use, inability to complete full sentences, respiratory rate $>$ 30 breaths per minute). All severe cases were neurologically unaffected at the time of enrolment with no prior medical history of neurological conditions. Non-severe COVID-19 group were admitted to the medical floor with no WHO severity criteria and classified as mild-moderate cases. All the 50 mild COVID-19 patients recovered completely without developing a severe form of the disease.

The population of Qatar with SARS-CoV-2 infection was predominantly males and young during the early wave of the COVID-19 pandemic between March and April 2020. Our studied cohort of SARS-CoV-2 infected patients was collected in the early pandemic and in the same time frame as the nationwide cohort of the first consecutive 5000 patients with COVID-19 in Qatar(Al-Nesf et al., 2022; Omrani et al., 2020), thus had similar characteristics of most of the SARS-CoV-2 infected patients (88.7%) being males, with a median age of 35. Hospitalizations were predominately under 65 years of age; 95.7% for non-ICU and 85.2% for ICU patients. ICU patients in Qatar were further enriched for males (92.6%), and only 16 patients (14.8%) in the ICU were above 65 years or older(Dazzo et al., 2016). Our studied cohort has a limitation for being biased toward male and younger subjects with patients aged between 18 and 65 and being mainly males with only 8% females. This cohort selection was based on local COVID-19 demographics during the early first wave of the pandemic(Al-Nesf et al., 2022; Omrani et al., 2020).

3.2. Olink analysis revealed differential neurological plasma protein profile in patients with severe COVID-19 complications

To examine the effects of SARS-CoV-2 infection on neurological protein expression, we first performed a differential expression analysis from 99 COVID-19 patients (49 severe and 50 mild) and 40 healthy control subjects using two different Neurology Olink panels (Olink target 96 NeuroExploratory panel and Olink target 96 Neurology panel) (Wang et al., 2021). For a comprehensive molecular view, we carried out the analysis after combining the data from the two Olink panels in a single dataset (Supplementary data 3). Unsupervised hierarchical clustering revealed that the combined panels could differentiate severe disease from mild disease and controls as illustrated in the heatmap (Fig. 1A). Principal components analysis (PCA) of protein NPX data from the three groups demonstrated a clear separation of COVID-19 severe cases from COVID-19 mild and control samples (Fig. 1A). The number of differentially expressed proteins (DEPs) is summarized in Fig. 1B and Supplementary data 4. A total of 100 (54.3%) and 81 (44%) proteins were differentially expressed in cases with severe disease compared to control subjects or cases with mild disease, respectively. When comparing patients with mild disease to control subjects, 38 (20.6%) proteins were found to be differentially expressed. As shown in the volcano plots 36% of proteins were upregulated in severe cases, while only 8% and 18% were downregulated in Severe vs. Mild and Severe vs. controls, respectively, with a minimum fold change of 1.25 and FDR cutoff of 0.05. For instance, NGF, NEFL, PLXNB1, EFNA4, and others were significantly upregulated in severe cases, while GDNFR3, ADAM23 and others were significantly downregulated in severe cases (Fig. 1C). Interestingly, axonal injury NEFL protein profile of severe cases was validated by Simoa assay. NEFL was significantly highly expressed in severe cases compared to mild and control groups, showing a strong correlation with Olink data ($r = 0.76$, $p < 00.1$) (Sup Fig. 1A and B). Also, other known biomarkers for axonal injury such as GFAP and UCHL-1 proteins were found upregulated in severe cases (Sup Fig. 1A).

3.3. Protein pathways analysis uncovered upregulated and downregulated neurological pathways in severe COVID-19 patients

GO and KEGG pathways analysis were performed to identify the functional contribution of DEPs in biological processes. We found that 13 pathways associated with nervous system functions were significantly enriched in patients with severe disease as compared to healthy subjects (Neurotrophin signaling pathway, regulation of synapse assembly, nervous system development, neuron projection, axon guidance receptor activity, Semaphorin receptor complex and axon guidance) (Fig. 2A-D, Supplementary data 5). Additionally, peripheral nervous system (PNS) functions were affected in severe vs. mild cases with significantly downregulated PNS development pathway (Sup Fig. 2A). Overall, severe vs. mild cases showed 2 downregulated pathways and 3 upregulated pathways with 2 non-overlapping pathways with severe vs. controls (PNS development pathway and semaphoring receptor activity) (Sup Fig. 2A-D, Supplementary data 6). Such enrichment with neurological pathways was absent in mild vs. controls (Sup Fig. 2E, Supplementary data 7), suggesting that mild and control groups might be devoid of neurological complications. Next, we ran IPA pathway analysis for severe cases versus controls, and we found 7 neurological pathways significantly affected in severe COVID-19 patients, such as axonal guidance signaling, synaptogenesis signaling, semaphorin neuronal repulsive signaling, neuroprotective role of THOP1 in Alzheimer's disease, GDNF family ligand receptor interaction, neuroinflammation signaling and Ephrin A signaling (Sup Fig. 3, Supplementary data 8).

3.4. Neurological protein signature of severe COVID-19

To predict the risk of neurological complications of severe COVID-19, we set out to create a blood-based neurological protein signature. For this purpose, we ran WGCNA analysis for 184 tested proteins to identify network modules with co-expressed and interacting proteins. A network of 155 proteins was identified and divided into 4 modules (Fig. 3A, Supplementary data 9). The turquoise module had the highest number of proteins ($n = 54$), and was significantly enriched in neurological processes, functions, and cellular components such as neuron projection development, axon development, synapse, neuron differentiation, ephrin receptor activity, etc. (Fig. 3B), whereas the other modules did not include any neurological pathways. In addition, STRING analysis revealed that the 54 proteins form a dense protein-protein interaction (PPI) network with multiple interactions (Sup Fig. 4A). To define a neurological protein signature for severe COVID-19 patients that comprises only co-expressed and interacting proteins, we assessed which of the severe vs control DEPs ($n = 100$) overlap with the 54-protein network (Fig. 1B). As such, we identified a severe COVID-19 neurological signature of 34 proteins, 26 upregulated and 8 downregulated, that form multiple interactions within PPI networks (Fig. 3C, Sup Fig. 4B).

3.5. Severe COVID-19 neurological protein signature is validated in different independent cohorts at the protein and RNA levels

Next, we validated the 34-neurological protein signature using the Massachusetts General Hospital (MGH cohort) Olink dataset (Filbin et al., 2021) (Supplementary data 10). More specifically, we used the Olink proteomic data from 139 COVID-19 MGH samples that were collected at day 7 after admittance. We considered cases under acuities (death at 28 days, and intubated, ventilated, supplemented oxygen, $n = 71$) as severe COVID-19 cases, and cases under acuities (hospitalized with supplemented O_2 and hospitalized without supplemented O_2, $n = 68$) as mild COVID-19 cases (Supplementary data 11). Using unsupervised hierarchical clustering, we revealed that the 34-neurological protein signature could differentiate the MGH severe COVID-19 from mild COVID-19 cases as illustrated in the heatmap (Fig. 4A), and PCA

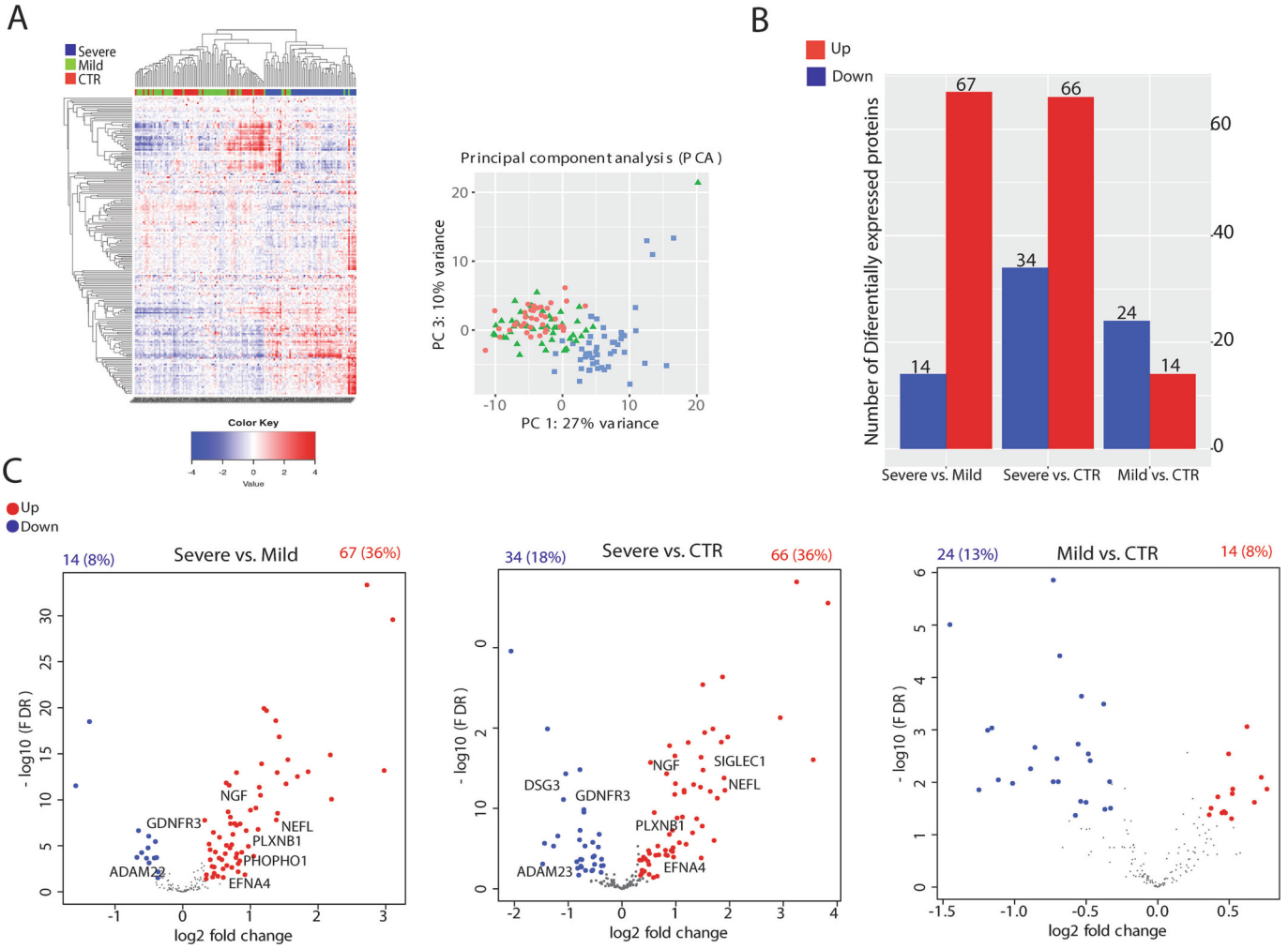


Fig. 1. Differential protein analysis of COVID-19 patients. Differentially expressed proteins (DEPs) were identified from two Olink Neurology panels and the combined dataset (184 unique proteins), which was defined as DEPs with more than 1.25-fold change with a *P*-value <0.05 and FDR < 0.05. A. Hierarchical clustering based on all 184 proteins assayed using the two Olink panels showed a separation between patients with severe complications compared to mild cases and controls. The heatmap shows z-scores with cut-off 4, and principal component analysis (PCA) confirmed the separation of the severe cases based on the expression profiles of all proteins. B. Histogram displaying number of DEPs across the patients' groups. C. Volcano plot summarizing DEPs based on $\log_2$ fold changes across the patient groups. Red and blue circles show significantly differential-expressed proteins $\geq$0.6 $\log_2$ fold change (upregulated) or ≥ -0.6 $\log_2$ fold change (downregulated), and *p* value $\geq$0.05. The number and percentage of the DEPs relevant to all proteins assayed are stated in each panel. Differential expression analysis addressed severity as the main effect and included different factors to correct for interaction with severity, such as obesity, age, sex, nationality, SpO_2, heart rate, and blood pression. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

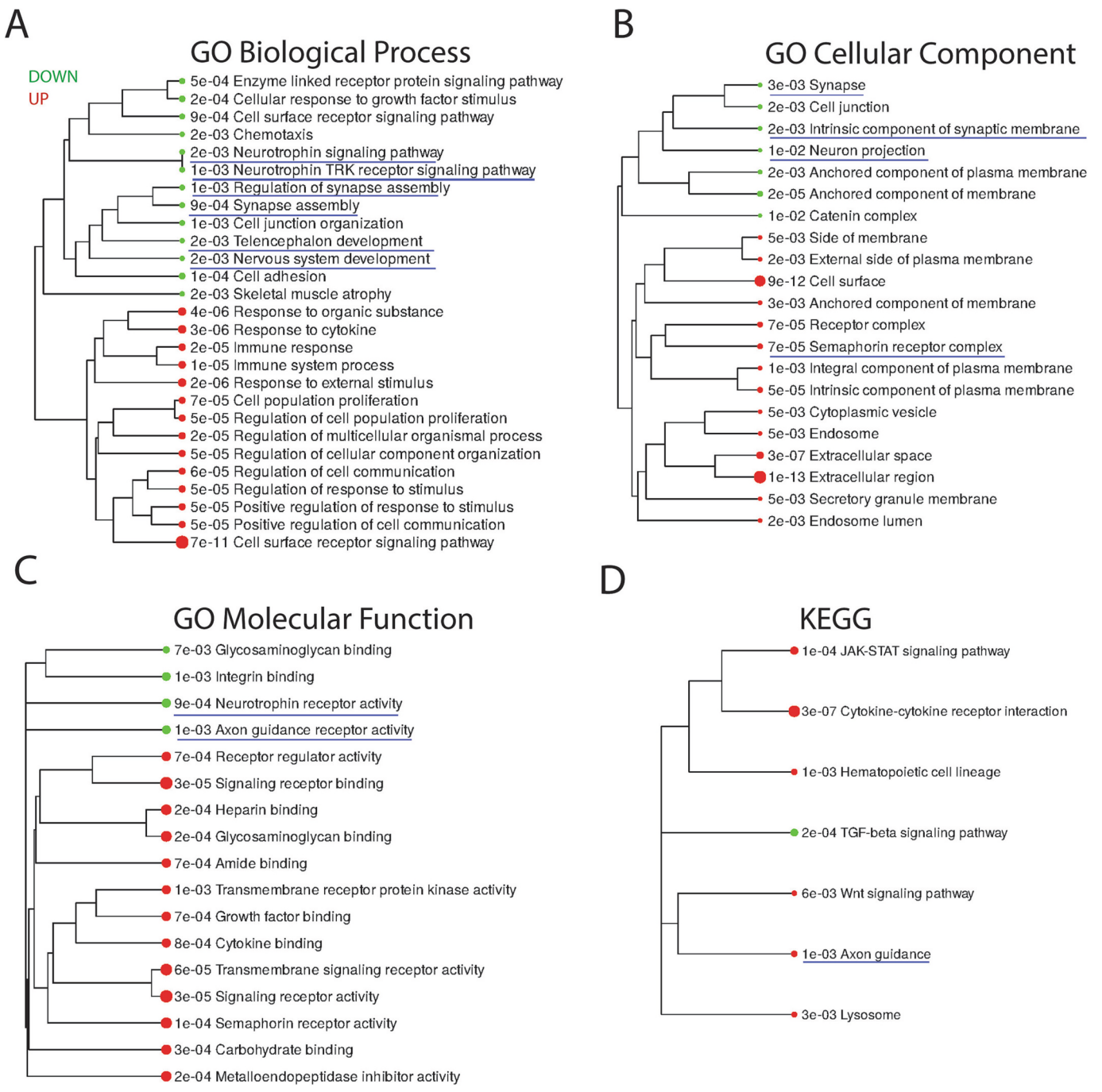
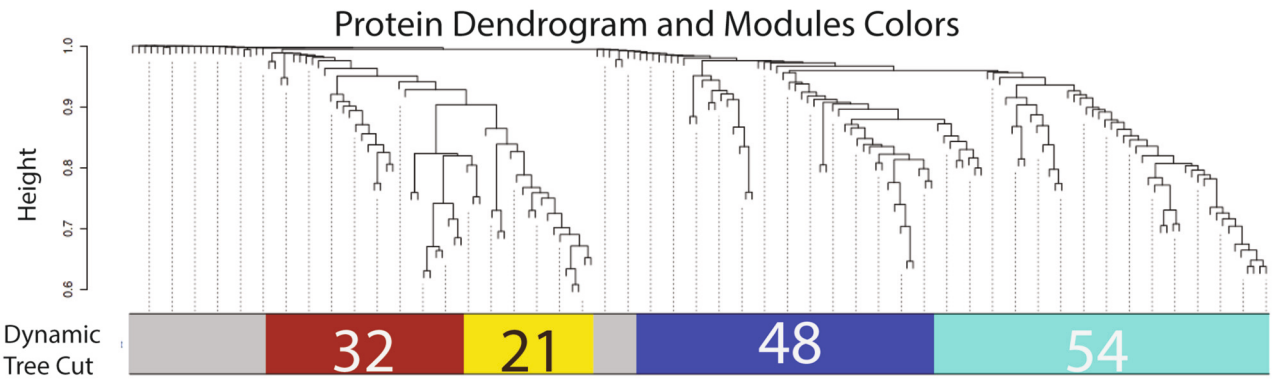


Fig. 2. Pathway analysis of DEPs in severe patients vs. controls. DEPs in patients with severe complications compared to controls were subjected to pathway analysis using the Gene Ontology (GO), and the Kyoto Encyclopedia of Genes and Genomes (KEGG). Thirteen molecular pathways with neurological function were identified deregulated (underlined). A. GO Biological process. B. GO Cellular component. C. GO Molecular Component. D. KEGG pathway analysis. Green and red circles show significantly downregulated and upregulated pathways. Only pathways with *t*-test *p*-value ≤ 0.05 are represented. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

demonstrated a clear separation of COVID-19 severe samples from COVID-19 mild samples (Fig. 4A). Out of the 34 proteins, we found that 21 proteins were significantly upregulated and 1 protein was significantly downregulated in MGH severe COVID-19 patients compared to the mild cases (Fig. 4B and C, Supplementary data 12). Interestingly, all the 22 MGH cohort proteins positively correlated with Qatar cohort severe COVID-19 protein signature profile, with 21 upregulated proteins, and 1 downregulated protein (Fig. 4 D, Supplementary data 13). Interestingly, comparison of protein expression in patients with severe COVID-19 and symptomatic non COVID-19 related respiratory infections confirmed 21 out of the 22 severity-associated proteins to be significantly altered in patients with severe COVID-19 disease only (Sup Fig. 5, Supplementary data 14).

We further correlated the 34 neurological protein signature to transcriptomics data from prefrontal cortex autopsy of COVID-19 patients, including 9525 differentially expressed RNAs(Pujadas et al., 2021). We found 14 genes overlap between the two biosets, 9 genes with positive correlation and 5 genes with negative correlations to the neurological protein signature (Fig. 5A). More interestingly, using RNA data from whole blood of COVID-19 patients critical in ICU with ARDS

A



A network of 155 proteins was divided into 4 modules.

B

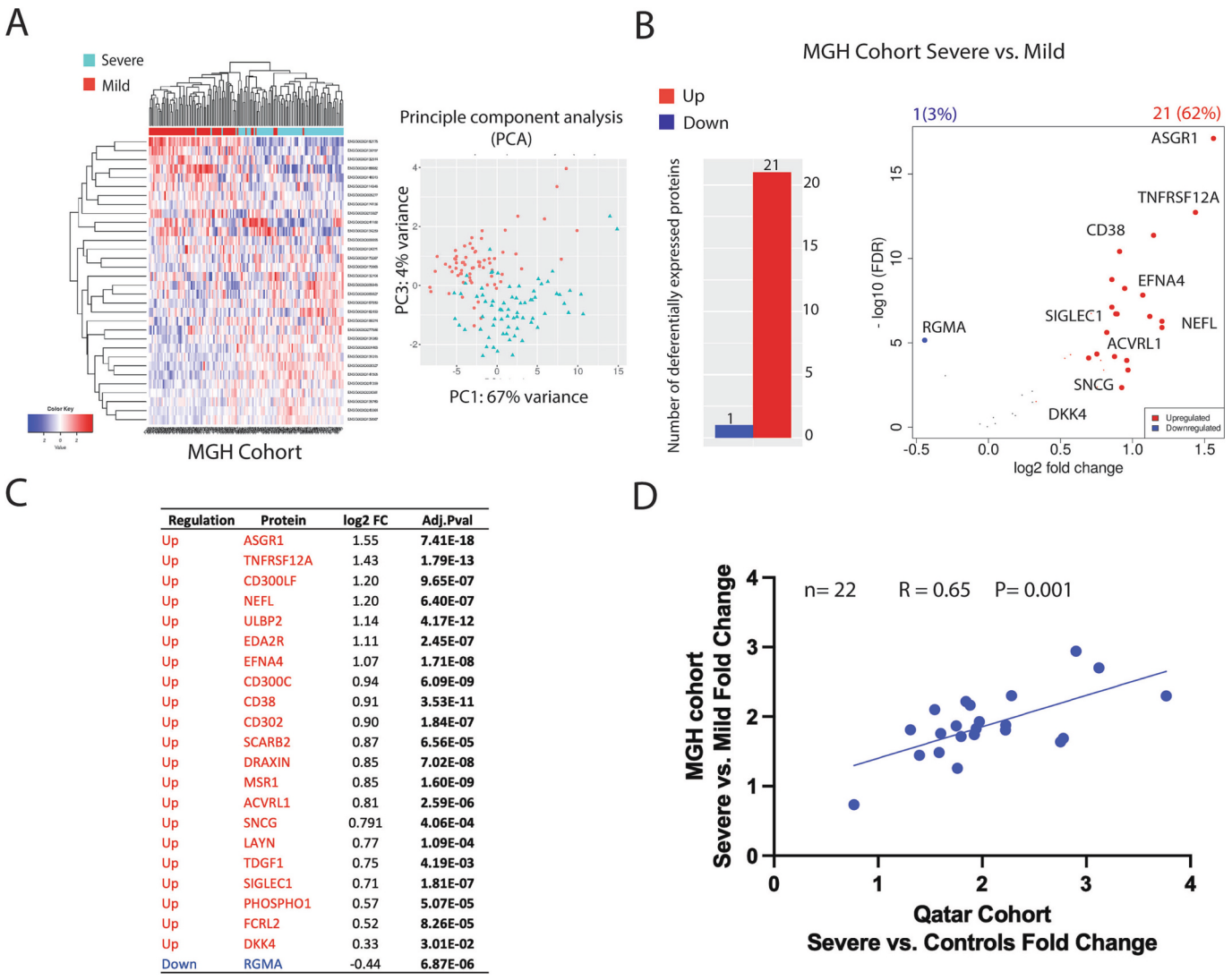
Enriched Neurological pathways among all proteins in turquouse module

Data base	Pathways	n Proteins	adj.Pval
GO Biological Process	Neuron projection development	18	4.40E-09
	Axon development	14	4.40E-09
	Neuron projection morphogenesis	15	4.80E-09
	Axonogenesis	13	7.00E-09
	Cell morphogenesis involved in neuron differentiation	14	8.20E-09
	Neuron development	18	8.30E-09
	Neuron differentiation	19	2.10E-08
GO Cellular Component	Axon	9	3.50E-04
	Neuron projection	12	1.10E-03
	Synapse	11	3.00E-03
	Axolemma	2	3.50E-03
GO Molecular Function	Ephrin receptor activity	2	7.50E-03
KEGG	Axon guidance	4	3.90E-03

C

Regulation	Protein	log2 FC	Adj.Pval
Up	NEFL	1.91	6.07E-13
Up	TNFRSF12A	1.64	7.99E-13
Up	ASGR1	1.53	4.35E-20
Up	TDGF1	1.47	1.45E-04
Up	SIGLEC1	1.46	2.41E-13
Up	CD300LF	1.19	3.02E-06
Up	CD38	1.15	6.07E-13
Up	MSR1	1.15	1.20E-12
Up	CD300C	0.97	2.11E-12
Up	SCARB2	0.95	1.17E-04
Up	SNCG	0.94	8.89E-06
Up	PLXNB1	0.93	5.86E-08
Up	EDA2R	0.91	1.83E-05
Up	ULBP2	0.88	1.77E-07
Up	LAYN	0.84	7.42E-05
Up	NGF	0.82	5.30E-15
Up	DKK4	0.81	6.22E-05
Up	CD302	0.80	1.66E-05
Up	ACVRL1	0.67	6.38E-05
Up	PHOSPHO1	0.66	5.00E-06
Up	EFNA4	0.62	6.84E-05
Up	FCRL2	0.48	3.77E-04
Up	DRAXIN	0.39	7.46E-03
Up	GGT5	0.37	4.96E-03
Up	CCL27	0.36	1.29E-02
Up	UNC5C	0.34	2.10E-02
Down	DSG3	-1.08	9.26E-12
Down	CLEC10A	-0.79	8.20E-07
Down	GDNFR3	-0.70	1.64E-10
Down	ADAM23	-0.68	5.00E-06
Down	RGMA	-0.54	1.88E-06
Down	ADAM22	-0.53	1.17E-04
Down	TMPRSS5	-0.43	7.27E-06
Down	RGMB	-0.38	1.44E-03

Fig. 3. Identifying co-expression network and severity protein signature. Proteins co-expression modules were defined using WGCNA Network analysis. A. Protein dendrogram classifies four different modules of co-expressed proteins. B. Protein interaction network highlighting the turquoise module illustrated enriched-neurological pathways using GO and KEGG data base analysis. C. Severity protein signature of 37 proteins among the turquoise module enriched with neurological pathways. Red and blue show significantly upregulated and downregulated proteins, which were defined as upregulated with protein expression $\geq$1.25 fold change or downregulated with protein expression ≥ -1.25 fold change, *P*-value $<$0.05. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)



Regulation	Protein	log2 FC	Adj.Pval
Up	ASGR1	1.55	7.41E-18
Up	TNFRSF12A	1.43	1.79E-13
Up	CD300LF	1.20	9.65E-07
Up	NEFL	1.20	6.40E-07
Up	ULBP2	1.14	4.17E-12
Up	EDA2R	1.11	2.45E-07
Up	EFNA4	1.07	1.71E-08
Up	CD300C	0.94	6.09E-09
Up	CD38	0.91	3.53E-11
Up	CD302	0.90	1.84E-07
Up	SCARB2	0.87	6.56E-05
Up	DRAXIN	0.85	7.02E-08
Up	MSR1	0.85	1.60E-09
Up	ACVRL1	0.81	2.59E-06
Up	SNCG	0.791	4.06E-04
Up	LAYN	0.77	1.09E-04
Up	TDGF1	0.75	4.19E-03
Up	SIGLEC1	0.71	1.81E-07
Up	PHOSPHO1	0.57	5.07E-05
Up	FCRL2	0.52	8.26E-05
Up	DKK4	0.33	3.01E-02
Down	RGMA	-0.44	6.87E-06

Fig. 4. Validation of severe COVID-19 neurological protein signature in MGH cohort.
22 DEPs were identified from the 34 neurological Protein signature with more than 1.25-fold change with a P-value <0.05 and FDR < 0.05. A. Hierarchical clustering based on all 34 proteins assayed showed a separation between patients with severe complications compared to mild cases. The heatmap shows z-scores with cut-off 4, and principal component analysis (PCA) confirmed the separation of the severe cases based on the expression profiles of all proteins. B. Histogram displaying number of 22 DEPs, and Volcano plot summarizing DEPs based on $\log_2$ fold changes across the patient groups. Red and blue circles show significantly differential-expressed proteins $\geq$0.6 $\log_2$ fold change (upregulated) or ≥ -0.6 $\log_2$ fold change (downregulated), and *p* value $\geq$0.05. The number and percentage of the DEPs relevant to all proteins assayed are stated in each panel. Differential expression analysis addressed severity as the main effect and included different factors to correct for interaction with severity, such as age and BMI. C. MGH severe COVID-19 signature protein list. D. Scatter plot showing positive correlation of severe COVID-19 signature protein between Qatar cohort and MGH cohort. P-value = 0.001.

(Carapito et al., 2022) (Fig. 5B), or peripheral blood mononuclear cells of severe COVID-19 patients(Arunachalam et al., 2020) (Sup Fig. 6A-B), we detected most of the overlapping genes correlated positively to the neurological protein signature, while fewer genes had negative correlations (Supplementary data 15).

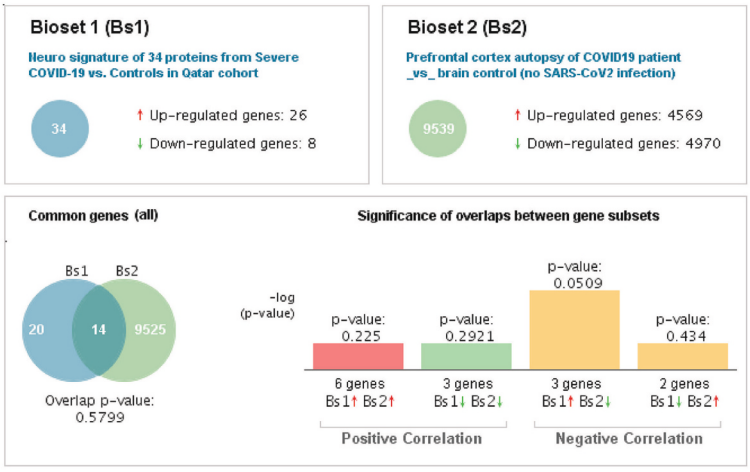
3.6. Neurological diseases and pharmacological drugs correlating with the neurological proteins signature of severe COVID-19

To gain insight into the role of the severe COVID-19 neurological proteins, we conducted correlation analyses for the 34 proteins using the BSCE Disease Atlas. We found 15 brain and nervous system disorders positively correlated with the COVID-19 protein signature, such as Nerve injury, Parkinson's disease, Alzheimer's disease, Schizophrenia, and Amyotrophic Lateral Sclerosis (16, 9, 6, 6 and 3 studies, respectively), and 3 neurological diseases negatively correlated with the proteins signature, such as Meningitis and Epilepsy (Table 2, Supplementary data 16). In addition, using the BSCE Pharmaco Atlas we analyzed protein signature- drug correlations in independent studies and, identified 42 and 58 drugs respectively to be positively and negatively correlated with the 34-protein signature (Supplementary data 17).

4. Discussion

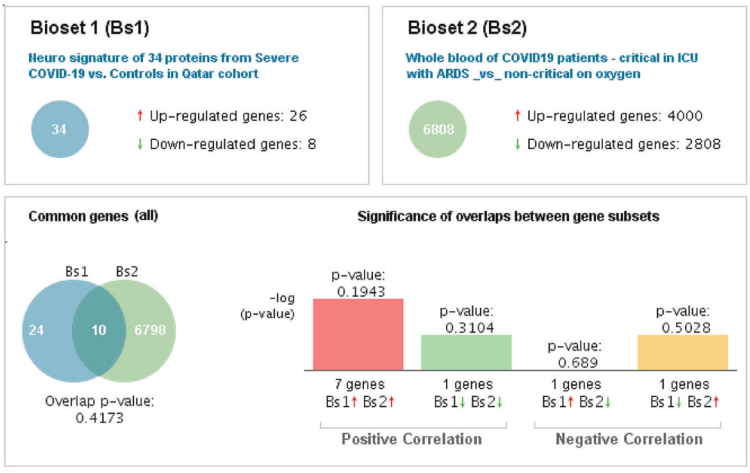
There is an increasing interest in understanding the mechanisms underlying the neurological complications seen in some COVID-19 patients, as well as revealing new health issues arising from COVID-19 neurological complications. Several papers have described different types of neurological complications in severe COVID-19, and have demonstrated the neuro-invasive potential of SARS-CoV-2[1, 2, 4, 7, (Mao et al., 2020). It has been reported that most of the severe COVID-19 patients have developed neurological complications and are among

A



Protein	Description	Fold Change Bs 1	Fold Change Bs2	Correlation
NEFL	neurofilament light	3.76	-2.19	Negative
TDGF1	teratocarcinoma-derived growth factor 1	2.77	2.11	Positive
SIGLEC1	sialic acid binding Ig like lectin 1	2.75	2.12	Positive
CD38	CD38 molecule	2.22	-9.61	Negative
SCARB2	scavenger receptor class B member 2	1.94	-1.63	Negative
PLXNB1	plexin B1	1.91	1.57	Positive
DKK4	dickkopf WNT signaling pathway inhibitor 4	1.76	4.38	Positive
ADAM23	ADAM metallopeptidase domain 23	-1.61	-1.53	Positive
ACVRL1	activin A receptor like type 1	1.60	2.23	Positive
PHOSPHO1	phosphoethanolamine/phosphocholine phosphatase	1.58	1.63	Positive
RGMA	repulsive guidance molecule family member a	-1.45	1.68	Negative
ADAM22	ADAM metallopeptidase domain 22	-1.45	-2.22	Positive
TMPRSS5	transmembrane protease, serine 5	-1.35	1.85	Negative
RGMB	repulsive guidance molecule family member b	-1.30	-1.58	Positive

B



Protein	Description	Fold Change Bs 1	Fold Change Bs2	Correlation
SIGLEC1	sialic acid binding Ig like lectin 1	2.75	1.92	Positive
CD300LF	CD300 molecule like family member f	2.28	1.61	Positive
CD38	CD38 molecule	2.22	-1.52	Negative
SCARB2	scavenger receptor class B member 2	1.94	1.33	Positive
PLXNB1	plexin B1	1.91	1.49	Positive
CLEC10A	C-type lectin domain containing 10A	-1.72	-1.64	Positive
ACVRL1	activin A receptor like type 1	1.60	1.70	Positive
RGMA	repulsive guidance molecule family member a	-1.45	1.79	Negative
DRAXIN	dorsal inhibitory axon guidance protein	1.31	1.41	Positive
GGT5	gamma-glutamyltransferase 5	1.29	1.8	Positive

Fig. 5. BSCE analysis. Correlation of neurological protein signature of severe COVID-19 patients (Qatar Cohort) with transcriptomics data of prefrontal cortex of postmortem COVID-19 patients (A) or whole blood of critical COVID-19 patients (B) (USA Cohort). Identified proteins with positive and negative correlations are listed in each panel. Positive correlations are highlighted in grey.

the most debilitating manifestations(Taquet et al., 2021) With more than 48 million documented infections since the beginning of the pandemic, it is likely that more than 10 million individuals have been or are currently affected by neurological sequelae in the world(Baig, 2021; Nalbandian et al., 2021). Longitudinal studies are needed to evaluate persistent CNS damage in patients with severe COVID-19 neurological sequelae over time and their impact on cognitive functions, neurodegenerative and mental disorders in large populations. Developing blood-based neurological protein signature can serve as a predictive biomarker tool to assess neurological complications in post-severe COVID-19 convalescent individuals.

In this current study, we explored changes in neurological protein expression between severe COVID-19, mild COVID-19, and healthy controls. Severe COVID-19 patients were all neurologically unaffected prior infection and at the time of enrolment. We conducted proteomics profiling of 184 CNS proteins in plasma samples using the Olink neurology and NeuroExploratory panels. Here, we identified a high differential protein expression in plasma patients with severe COVID-19 compared to controls, with 36% DEPs being upregulated and 18% being downregulated. We have observed that most upregulated proteins were related to severe disease but not to mild disease or control subjects.

Together with the Simoa results, our Olink data confirm previously published findings that patients hospitalized with severe COVID-19 have elevated biomarkers of CNS injury in their blood(Kanberg et al., 2020; Ameres et al., 2020; Chung et al., 2021; Geis et al., 2021; Sutter et al., 2021; Kanberg et al., 2021), such as NEFL, GFAP, UCH-L1, reflecting CNS damage and traumatic brain injury(De Lorenzo et al., 2021; Bacioglu et al., 2016; McMahon et al., 2015; Wang et al., 2021). This finding therefore supports the body of literature showing that COVID-19 neurological complications are accompanied by damage to the CNS. Beside the previously reported CNS injury biomarkers, here we identified proteins that have not yet been reported in COVID-19 severity, including NGF, GDNFR3, EFNA4, PLXNB1, and ADAM23, etc. They were found deregulated and may play an important role in neurological complications of severe COVID-19 patients. These proteins are mainly neuronal survival and guidance factors that play a major role in axonal growth, neurogenesis, neuroprotection, neural differentiation, and synaptogenesis(Laussu et al., 2014) (Markus-Koch et al., 2017; Guthrie, 2007; Limoni and Niquille, 2021; Nordvall et al., 2022). Upregulated neuronal survival factors such as NGF is an indication of a healing process taking place in brain tissue(Aloe et al., 2015), as a consequence of brain damage in severe COVID-19 patients. These factors may have long term neurological impact. Although explorative study of blood-based neurological proteins in the blood may add further insight into CNS damage and COVID-19 neurological complications, future studies should use cerebrospinal fluid (CSF) sampling as it reflects alterations occurring more accurately at the level of the CNS.

To identify the functional contribution of DEPs in biological

Table 2
Correlation of neurological protein signature of severe COVID-19 patients with neurological disorders from curated studies.

Phenotypes group	Phenotype	Score	N Studies	Correlation
Brain and Nervous System Disorders	Nerve injury	77.76	16	positive
Brain and Nervous System Disorders	Parkinson's disease	76.85	6	positive
Brain and Nervous System Disorders	Disorder of basal ganglia	69.27	6	positive
Brain and Nervous System Disorders	Neuropathy	68.35	11	positive
Brain and Nervous System Disorders	Movement disorder	66.57	6	positive
Brain and Nervous System Disorders	Alzheimer's disease	58.06	9	positive
Brain and Nervous System Disorders	Hypoxia of brain	55.5	3	positive
Brain and Nervous System Disorders	Amyotrophic lateral sclerosis	55.04	3	positive
Brain and Nervous System Disorders	Spinocerebellar ataxia	54.62	1	positive
Brain and Nervous System Disorders	Motor neuron disease	53.97	4	positive
Brain and Nervous System Disorders	Cerebrovascular disease	53.12	2	positive
Brain and Nervous System Disorders	Leukodystrophy	43.04	1	positive
Brain and Nervous System Disorders	Embolic stroke	42.27	2	positive
Brain and Nervous System Disorders	Encephalomyelopathy	36.69	1	positive
Brain and Nervous System Disorders	Multiple sclerosis	34.14	2	positive
Mental Disorders	Dementia	67	10	positive
Mental Disorders	Alcoholism	59.33	2	positive
Mental Disorders	Schizophrenia	54.69	6	positive
Mental Disorders	Psychotic disorder	54.49	6	positive
Mental Disorders	Autistic disorder	46.51	2	positive
Brain and Nervous System Disorders	Meningitis	57.55	3	negative
Brain and Nervous System Disorders	Epilepsy	50.32	3	negative
Brain and Nervous System Disorders	Disorder of brain	49.33	1	negative
Mental Disorders	Anxiety disorder	52.04	2	negative
Mental Disorders	Mood disorder	51.09	4	negative

processes, we ran GO, KEGG and IPA pathway enrichment analysis and found relative enrichment of neurological signaling pathways in severe COVID-19 disease, including nervous system development, synaptogenesis, neuron projection, axon guidance, Semaphorin receptor complex, Neurotrophin signaling pathway, neuroprotective role of THOP1 in Alzheimer's disease, GDNF family ligand-receptor interaction, neuroinflammation signaling, and others. Using WGCNA analysis, we narrowed down the DEPs number from 100 proteins to 34 co-expressed and co-interacting proteins, establishing a neurological protein signature for severe COVID-19.

To validate our neurological protein signature in an independent cohort, we compared our data to an independent Olink study generated from MGH cohort plasma samples of severe COVID-19. We validated 22 proteins among the 34 neurological protein signature in MGH cohort of severe COVID-19 patients. 21 out of 22 proteins are specific to severe SARS-CoV-2 infection and not to other respiratory infections. Our Qatar cohort is ethnically diverse, which include Middle Eastern, Africans and Asian populations. Here, we provide evidence that our severe COVID-19 neurological protein signature is detected and validated in an independent multi-ethnic cohort and using the same Olink technology.

We then explored other cohorts of severe COVID-19 patients to validate the neurological protein signature in different biological samples and at the RNA level. More importantly, we first sought whether our protein signature can also be detected in brain tissue of severe COVID-19 postmortem patients. Fortunately, 9 genes were found positively correlating with our neurological protein signature (PLXNB1, RGMB, ADAM22, ADAM23, DKK4, SIGLEC1, TDGF1, ACVRL1, and PHOSPHO1), meaning that these molecular changes in expression profile are similarly affected at the protein and RNA levels in both peripheral blood and brain severe COVID-19 samples. These findings suggest that these proteins may have an impact on brain functions, and most probably lead to neurological complications. Some of these proteins are known as neuronal guidance molecules such as PLXNB1 and RGMB. Guidance molecules play an important role during CNS development and are emerging as important factors in neurological diseases and brain injury (Yaron and Zheng, 2007). Deregulation of neuronal guidance molecules might be associated with predisposition to epilepsy, and it was also suggested that guidance molecules play roles in the ability of the adult nervous system to recover and repair after injury(Yaron and Zheng, 2007).

It has been reported that neuronal receptors ADAM22, and ADAM23 play major role in neuronal functions through LGI1 protein and have been associated to epilepsy disorder(Hivert et al., 2019; Dazzo et al., 2016). In our study, we report that both proteins are deregulated in severe COVID-19 patients, suggesting that they may lead to neurological complications in post-severe COVID-19 patients. We also found DKK4 protein deregulated in brain samples of severe COVID-19 patients, which has been shown to be significantly associated with schizophrenia (Zhang et al., 2011).

Glycosylation is vital for brain functions and an alteration in the process may lead to nervous system disorders. One of the types of glycosylation that is abundantly found in the brain is sialylation. SIGLEC proteins (Sialic acid-binding immunoglobulin-type lectins) have been shown to play roles in neuroinflammation and neurodegeneration (Freeze et al., 2015). Thus, having SIGLEC1 protein deregulated in severe COVID-19 patients may indicate a deficiency in brain functions. Also, PHOSPHO1 has been previously found altered in Parkinson's disease patients(Bartl et al., 2022), its protein expression profile is also affected in severe COVID-19 patients.

When exploring the correlation of neurological protein signature in RNA data from other cohorts of severe COVID-19 patients' whole blood or peripheral blood mononuclear cells, most of the common genes correlated positively with the neurological protein signature such as SIGLEC1, SCARB2, PLXNB1, CLEC10A, ACVRL1, DRAXIN, GGT5, TNFRSF12A, ASGR1, RGMB, PHOSPHOS1, CD38 CD300LF, and ADAM23 (Fig. 5, Sup Fig. 6 and Sup Fig. 7). Some of these findings are consistent with the above validations done on brain RNA samples such as PLXNB1, RGMB, ADAM23, SIGLEC1, and ACVRL1. On the other hand, we found NEFL, SCARB2, CD38, RGMA, TMPRSS5 proteins expression negatively correlated with RNA expression from severe COVID-19 brain samples (Fig. 5), meaning that peripheral blood and brain may slightly differ in neurological expression profile of severe COVID-19 patients. This interesting finding needs to be further investigated.

Furthermore, using BSCE Disease Atlas analyses to compare our protein signature to multiple studies, several neurological diseases and disorders were found positively correlating with our protein signature, such as Nerve Injury, Parkinson's disease, Alzheimer's disease, Amyotrophic lateral sclerosis, and Schizophrenia. Thus, it seems there is a risk of developing chronic neurological diseases in post-severe COVID-19 patients. The correlations to 'Nerve Injury' used RNA expression data from rodent induced-nerve injury models. As highlighted by TNFRSF12A, alike severe COVID-19 signature 12 out of 16 biosets had upregulated TNFRSF12A expression (Sup Fig. 8, Supplementary data 16)(Li et al., 2013; Omura et al., 2015; Chen et al., 2013; Wu et al., 2013). We next looked at Parkinson's disease curated studies, we found five out of six were SNP/GWAS: 2 studies had upregulated correlation of CD38, while SCARB2 correlated in 1 Study. TNFRSF12A and LAYN were concordantly upregulated in severe COVID-19 protein signature and

RNA expression from Parkinson's disease iPS derived neurons bioset (Sup Fig. 9, Supplementary data 16)(Satake et al., 2009; Pankratz et al., 2009; Do et al., 2011; Woodard et al., 2014). In superior frontal gyrus, post central gyrus and prefrontal cortex of brain post-mortem Alzheimer's disease patients, PLXNB1 was upregulated while ADAM22 and ADAM 23 were downregulated similar to severe COVID-19 protein signature (Sup Fig. 10, Supplementary data 16)(Wang et al., 2016; Berchtold et al., 2013; Nativio et al., 2018; Stopa et al., 2018; Kant et al., 2018). Further relevance was evident for GGT5, CD300LF and MSR1 being upregulated in severe COVID-19 and in human ALS spinal cord RNA expression, as well as in a FUS1 overexpression mouse ALS model (Sup Fig. 11 and 12, Supplementary data 16)(Durrenberger et al., 2012; Durrenberger et al., 2015; Funikov et al., 2018). Additionally, RGMB, ADAM23 and ADAM22 were downregulated in Schizophrenia hippocampus RNA expression and severe COVID-19 protein signature (Sup Fig. 13)(Lanz et al., 2019; Topol et al., 2015; Topol et al., 2016; Narla et al., 2017). GFRA3 was downregulated in severe COVID-19 protein signature and correlated with Schizophrenia associated SNP biosets (2681 cases, 2653 controls) detecting the presence of a European ancestry intron variant in rs33967909 ($P = 0.001$). DRAXIN was upregulated in severe COVID-19 protein signature as well as in schizophrenia-derived iPSC patients and Schizophrenia associated SNP study (Sup Fig. 13, Supplementary data 16)(Stefansson et al., 2009; Sullivan et al., 2008). Correlating biomarker signatures with neurological diseases can be used as a tool to predict the development of neurological complications in post-severe COVID-19 patients, thus these biomarkers might serve as a clinical prognostic tool. Overall, these analyses support a broad potential impact that could provide context to future studies and for understanding long COVID-19 disease with neurological complications.

Proteomic profiling is widely used for biomarker discovery, but it can also be used to predict potential drugs for intervention. In our study, we analyzed protein signature-drugs correlations in several independent studies, and we found drugs that either correlated positively or negatively with the protein signature. These correlations can be considered for patients' clinical management, as drugs with positive correlation could be dismissed, since they may aggravate the complications, but those with negative correlation may alleviate the complications by reversing the deregulated protein profiles. Drugs that may reverse COVID-19 expression patterns included risperidone, a selective blocker of dopamine D2 and serotonin 5-HT2 receptors that has been shown to improve positive and negative symptoms of schizophrenia(Janssen et al., 1988). Meta-Analysis of 34 proteins in a risperidone RNA expression time course of human SK-N-SH neuroblastoma cells (GSE36678) showed high COVID-19 induced protein levels for LAYN, TNFRSF12A, EFNA2, CD302, EDA2R and NGF that correlated with downregulated RNA levels after 6, 24 and 48 h risperidone treatment (Mas et al., 2013). Furthermore, GFRA3 had an inverse relationship of downregulated protein and upregulated RNA (Sup Fig. 14, Supplementary data 17). There is a need for more comprehensive neurological cell line models of SARS-CoV-2 infection and compound treatment effects at both the protein and RNA levels to better understand the mechanistic potential for repurposing safe and effective drugs.

5. Conclusions

Our study identified deregulated neurological proteins in the plasma of severe COVID-19 patients that may inform therapeutic interventions. Our findings revealed novel neurological protein signature for severe COVID-19, affecting various neurological signaling pathways. This protein signature should play diverse roles though important functions in severe COVID-19 neurological complications and emphasize the value of neurological follow-up in recovered individuals. The neurological protein signature was validated in independent cohorts at both protein and RNA levels, and shown to correlate with neurological diseases and pharmacological drugs. Thus, it may serve as prognostic and diagnostic tools for post-severe COVID-19 patients with neurological sequalae, and prospectively help healthcare management and clinical decision-making to provide adequate drugs to prevent or slow down further neurological complications.

Supplementary data to this article can be found online at https://doi.org/10.1016/j.nbd.2023.106147.

Author contributions

H.B.A. and O.E. designed, conceived, and led the study. M.A.Y.A., V. M.A., and A.M. led the cohort sample collection, processing, and ethical approvals. H.B.A., I.B., O.E., and K.O. optimized the assays. I.B., N.V., N. K.M., I.Y.A. and H.B.A. ran the assays. H.B.A. performed the Olink bioinformatics analyses, A.F performed Simoa statistical analyses, J.F. performed BSCE analyses, and M.T., P.W., and H.B.A. performed MGH Olink analyses. H.B.A., J.D., O.E., I.B., F.S., P.B.V., A.A., H.H.A., J.F., and M.E. interpreted the data. H.B.A. wrote the manuscript. H.B.A., J.D., I.B., J.F., A.A., H.H.A., and K.O. revised the manuscript. All authors reviewed the manuscript and have read and agreed to the published version of the manuscript.

Funding

Research conducted on Qatar cohort was funded by the Infectious Disease Interdisciplinary Research Program (QB-10-ID-IDRP) at QBRI and a grant fund from Hamad Medical Corporation (fund number MRC-05-003).

Institutional review board statement

The study was conducted according to the Ministry of Public Health (MOPH) guidelines and approved by the Institutional Review Board Research Ethics Committee of the Hamad Medical Corporation (reference MRC-05-003). Informed consent was obtained from all participants.

CRediT authorship contribution statement

Omar El-Agnaf: Conceptualization, Methodology, Visualization, Supervision. **Ilham Bensmail:** Investigation, Resources, Writing – review & editing. **Maryam A.Y. Al-Nesf:** Resources, Writing – review & editing. **James Flynn:** Data curation, Writing – review & editing, Formal analysis. **Mark Taylor:** Resources, Formal analysis. **Nour K. Majbour:** Investigation. **Ilham Y. Abdi:** Investigation. **Nishant N. Vaikath:** Investigation. **Abdulaziz Farooq:** Formal analysis. **Praveen B. Vemulapalli:** Resources, Writing – review & editing. **Frank Schmidt:** Resources, Writing – review & editing. **Khalid Ouararhni:** Writing – review & editing. **Heba H. Al-Siddiqi:** Writing – review & editing. **Abdelilah Arredouani:** Writing – review & editing. **Patrick Wijten:** Formal analysis. **Mohammed Al-Maadheed:** Conceptualization. **Vidya Mohamed-Ali:** Conceptualization. **Julie Decock:** Writing – review & editing, Visualization, Conceptualization. **Houari B. Abdesselem:** Conceptualization, Methodology, Visualization, Supervision, Formal analysis, Project administration, Writing – original draft, Writing – review & editing, Validation, Funding acquisition.

Declaration of Competing Interest

None.

Data availability

Data will be made available on request.

Acknowledgments

We would like to thank all the patients, volunteers, and healthcare co-workers from HMC hospital. We also acknowledge the help provided by the Anti-Doping Lab-Qatar (ADLQ) and Qatar Red Crescent (QRC) for recruiting control individuals. We extend our thanks to Dr. Michael Edwards and Dr. Fares Al-Ajeh for advice with data analysis, and Olink Proteomics team, Dr. Cindy Lawley and Mrs. Marrianne Alksnis for their valuable support. This work was also strongly supported by the Proteomics core facility at QBRI.

References

Achar, A., Ghosh, C., 2020. COVID-19-associated neurological disorders: the potential route of CNS invasion and blood-brain relevance. Cells 9.
Al-Nesf, M.A.Y., Abdesselem, H.B., Bensmail, I., et al., 2022. Prognostic tools and candidate drugs based on plasma proteomics of patients with severe COVID-19 complications. Nat. Commun. 13, 946.
Aloe, L., Rocco, M.L., Balzamino, B.O., Micera, A., 2015. Nerve growth factor: a focus on neuroscience and therapy. Curr. Neuropharmacol. 13, 294–303.
Ameres, M., Brandstetter, S., Toncheva, A.A., et al., 2020. Association of neuronal injury blood marker neurofilament light chain with mild-to-moderate COVID-19. J. Neurol. 267, 3476–3478.
Arunachalam, P.S., Wimmers, F., Mok, C.K.P., et al., 2020. Systems biological assessment of immunity to mild versus severe COVID-19 infection in humans. Science 369, 1210–1220.
Assarsson, E., Lundberg, M., Holmquist, G., et al., 2014. Homogenous 96-plex PEA immunoassay exhibiting high sensitivity, specificity, and excellent scalability. PLoS One 9, e95192.
Bacioglu, M., Maia, L.F., Preische, O., et al., 2016. Neurofilament light chain in blood and CSF as marker of disease progression in mouse models and in neurodegenerative diseases. Neuron 91, 494–496.
Baig, A.M., 2021. Chronic COVID syndrome: need for an appropriate medical terminology for long-COVID and COVID long-haulers. J. Med. Virol. 93, 2555–2556.
Bartl, M., Dakna, M., Schade, S., et al., 2022. Blood markers of inflammation, neurodegeneration, and cardiovascular risk in early Parkinson's disease. Mov. Disord 38(1):68-81.
Berchtold, N.C., Coleman, P.D., Cribbs, D.H., Rogers, J., Gillen, D.L., Cotman, C.W., 2013. Synaptic genes are extensively downregulated across multiple brain regions in normal human aging and Alzheimer's disease. Neurobiol. Aging 34, 1653–1661.
Bodnar, B., Patel, K., Ho, W., Luo, J.J., Hu, W., 2021. Cellular mechanisms underlying neurological/neuropsychiatric manifestations of COVID-19. J. Med. Virol. 93, 1983–1998.
Carapito, R., Li, R., Helms, J., et al., 2022. Identification of driver genes for critical forms of COVID-19 in a deeply phenotyped young patient cohort. Sci. Transl. Med. 14 eabj7521.
Chaumont, H., San-Galli, A., Martino, F., et al., 2020. Mixed central and peripheral nervous system disorders in severe SARS-CoV-2 infection. J. Neurol. 267, 3121–3127.
Chen, K., Deng, S., Lu, H., et al., 2013. RNA-seq characterization of spinal cord injury transcriptome in acute/subacute phases: a resource for understanding the pathology at the systems level. PLoS One 8, e72567.
Chung, H.Y., Neu, C., Wickel, J., Kuckertz, S.L., Coldewey, S.M., 2021. Neurofilament light chain in patients with COVID-19 and bacterial pneumonia. Ann. Neurol. 90, 174–175.
Dazzo, E., Leonardi, E., Belluzzi, E., et al., 2016. Secretion-positive LGI1 mutations linked to lateral temporal epilepsy impair binding to ADAM22 and ADAM23 receptors. PLoS Genet. 12, e1006376.
De Lorenzo, R., Lore, N.I., Finardi, A., et al., 2021. Blood neurofilament light chain and total tau levels at admission predict death in COVID-19 patients. J. Neurol. 268, 4436–4442.
Do, C.B., Tung, J.Y., Dorfman, E., et al., 2011. Web-based genome-wide association study identifies two novel loci and a substantial genetic component for Parkinson's disease. PLoS Genet. 7, e1002141.
Durrenberger, P.F., Fernando, F.S., Magliozzi, R., et al., 2012. Selection of novel reference genes for use in the human central nervous system: a BrainNet Europe study. Acta Neuropathol. 124, 893–903.
Durrenberger, P.F., Fernando, F.S., Kashefi, S.N., et al., 2015. Common mechanisms in neurodegeneration and neuroinflammation: a BrainNet Europe gene expression microarray study. J. Neural Transm. (Vienna) 122, 1055–1068.
Eden, A., Simren, J., Price, R.W., Zetterberg, H., Gisslen, M., 2021a. Neurochemical biomarkers to study CNS effects of COVID-19: a narrative review and synthesis. J. Neurochem. 159, 61–77.
Eden, A., Kanberg, N., Gostner, J., et al., 2021b. CSF biomarkers in patients with COVID-19 and neurologic symptoms: a case series. Neurology 96, e294–e300.
Emmi, A., Rizzo, S., Barzon, L., et al., 2023. Detection of SARS-CoV-2 viral proteins and genomic sequences in human brainstem nuclei. NPJ Parkinsons Dis. 9, 25.
Filbin, M.R., Mehta, A., Schneider, A.M., et al., 2021. Longitudinal proteomic analysis of severe COVID-19 reveals survival-associated signatures, tissue-specific cell death, and cell-cell interactions. Cell Rep. Med. 2, 100287.
Freeze, H.H., Eklund, E.A., Ng, B.G., Patterson, M.C., 2015. Neurological aspects of human glycosylation disorders. Annu. Rev. Neurosci. 38, 105–125.
Fullard, J.F., Lee, H.C., Voloudakis, G., et al., 2021. Single-nucleus transcriptome analysis of human brain immune response in patients with severe COVID-19. Genome Med. 13, 118.
Funikov, S.Y., Rezvykh, A.P., Mazin, P.V., et al., 2018. FUS(1-359) transgenic mice as a model of ALS: pathophysiological and molecular aspects of the proteinopathy. Neurogenetics 19, 189–204.
Ge, S.X., Son, E.W., Yao, R., 2018. iDEP: an integrated web application for differential expression and pathway analysis of RNA-Seq data. BMC Bioinforma. 19, 534.
Geis, T., Brandstetter, S., Toncheva, A.A., et al., 2021. Serum neurofilament light chain (sNfL) values in a large cross-sectional population of children with asymptomatic to moderate COVID-19. J. Neurol. 268, 3969–3974.
Guthrie, S., 2007. Neurotrophic factors: are they axon guidance molecules? Adv. Exp. Med. Biol. 621, 81–94.
Hanson, B.A., Visvabharathy, L., Ali, S.T., et al., 2022. Plasma biomarkers of Neuropathogenesis in hospitalized patients with COVID-19 and those with Postacute sequelae of SARS-CoV-2 infection. Neurol. Neuroimmunol. Neuroinflamm. 9.
Helms, J., Kremer, S., Merdji, H., et al., 2020. Neurologic features in severe SARS-CoV-2 infection. N. Engl. J. Med. 382, 2268–2270.
Hirzel, C., Grandgirard, D., Surial, B., et al., 2022. Neuro-axonal injury in COVID-19: the role of systemic inflammation and SARS-CoV-2 specific immune response. Ther. Adv. Neurol. Disord. 15, 17562864221080528.
Hivert, B., Marien, L., Agbam, K.N., Faivre-Sarrailh, C., 2019. ADAM22 and ADAM23 modulate the targeting of the Kv1 channel-associated protein LGI1 to the axon initial segment. J. Cell Sci. 132.
Janssen, P.A., Niemegeers, C.J., Awouters, F., Schellekens, K.H., Megens, A.A., Meert, T. F., 1988. Pharmacology of risperidone (R 64 766), a new antipsychotic with serotonin-S2 and dopamine-D2 antagonistic properties. J. Pharmacol. Exp. Ther. 244, 685–693.
Kanberg, N., Ashton, N.J., Andersson, L.M., et al., 2020. Neurochemical evidence of astrocytic and neuronal injury commonly found in COVID-19. Neurology 95, e1754–e1759.
Kanberg, N., Simren, J., Eden, A., et al., 2021. Neurochemical signs of astrocytic and neuronal injury in acute COVID-19 normalizes during long-term follow-up. EBioMedicine 70, 103512.
Kant, S., Stopa, E.G., Johanson, C.E., Baird, A., Silverberg, G.D., 2018. Choroid plexus genes for CSF production and brain homeostasis are altered in Alzheimer's disease. Fluids Barriers CNS 15, 34.
Kupershmidt, I., Su, Q.J., Grewal, A., et al., 2010. Ontology-based meta-analysis of global collections of high-throughput public data. PLoS One 5.
Lanz, T.A., Reinhart, V., Sheehan, M.J., et al., 2019. Postmortem transcriptional profiling reveals widespread increase in inflammation in schizophrenia: a comparison of prefrontal cortex, striatum, and hippocampus among matched tetrads of controls with subjects diagnosed with schizophrenia, bipolar or major depressive disorder. Transl. Psychiatry 9, 151.
Laussu, J., Khuong, A., Gautrais, J., Davy, A., 2014. Beyond boundaries–Eph:ephrin signaling in neurogenesis. Cell Adhes. Migr. 8, 349–359.
Leonardi, M., Padovani, A., McArthur, J.C., 2020. Neurological manifestations associated with COVID-19: a review and a call for action. J. Neurol. 267, 1573–1576.
Li, S., Liu, Q., Wang, Y., et al., 2013. Differential gene expression profiling and biological process analysis in proximal nerve segments after sciatic nerve transection. PLoS One 8, e57000.
Limoni, G., Niquille, M., 2021. Semaphorins and Plexins in central nervous system patterning: the key to it all? Curr. Opin. Neurobiol. 66, 224–232.
Logmin, K., Karam, M., Schichel, T., Harmel, J., Wojtecki, L., 2020. Non-epileptic seizures in autonomic dysfunction as the initial symptom of COVID-19. J. Neurol. 267, 2490–2491.
Mao, L., Jin, H., Wang, M., et al., 2020. Neurologic manifestations of hospitalized patients with coronavirus disease 2019 in Wuhan, China. JAMA Neurol. 77, 683–690.
Markus-Koch, A., Schmitt, O., Seemann, S., et al., 2017. ADAM23 promotes neuronal differentiation of human neural progenitor cells. Cell. Mol. Biol. Lett. 22, 16.
Mas, S., Gasso, P., Bernardo, M., Lafuente, A., 2013. Functional analysis of gene expression in risperidone treated cells provide new insights in molecular mechanism and new candidate genes for pharmacogenetic studies. Eur. Neuropsychopharmacol. 23, 329–337.
McMahon, P.J., Panczykowski, D.M., Yue, J.K., et al., 2015. Measurement of the glial fibrillary acidic protein and its breakdown products GFAP-BDP biomarker for the detection of traumatic brain injury compared to computed tomography and magnetic resonance imaging. J. Neurotrauma 32, 527–533.
Nalbandian, A., Sehgal, K., Gupta, A., et al., 2021. Post-acute COVID-19 syndrome. Nat. Med. 27, 601–615.
Narla, S.T., Lee, Y.W., Benson, C.A., et al., 2017. Common developmental genome deprogramming in schizophrenia - role of integrative nuclear FGFR1 Signaling (INFS). Schizophr. Res. 185, 17–32.
Nativio, R., Donahue, G., Berson, A., et al., 2018. Dysregulation of the epigenetic landscape of normal aging in Alzheimer's disease. Nat. Neurosci. 21, 497–505.
Nordvall, G., Forsell, P., Sandin, J., 2022. Neurotrophin-targeted therapeutics: a gateway to cognition and more? Drug Discov. Today 27, 103318.
Oliveira, R., Sotero, F.D., Teodoro, T., 2021. NeuroCOVID: critical review of neuropsychiatric manifestations of SARS-CoV-2 infection. Ir. J. Med. Sci. 190, 851–852.
Omrani, A.S., Almaslamani, M.A., Daghfal, J., et al., 2020. The first consecutive 5000 patients with coronavirus disease 2019 from Qatar; a nation-wide cohort study. BMC Infect. Dis. 20, 777.
Omura, T., Omura, K., Tedeschi, A., et al., 2015. Robust axonal regeneration occurs in the injured CAST/Ei mouse CNS. Neuron 86, 1215–1227.

Pankratz, N., Wilk, J.B., Latourelle, J.C., et al., 2009. Genomewide association study for susceptibility genes contributing to familial Parkinson disease. Hum. Genet. 124, 593–605.
Paterson, R.W., Brown, R.L., Benjamin, L., et al., 2020. The emerging spectrum of COVID-19 neurology: clinical, radiological and laboratory findings. Brain 143, 3104–3120.
Princiotta Cariddi, L., Tabaee Damavandi, P., Carimati, F., et al., 2020. Reversible encephalopathy syndrome (PRES) in a COVID-19 patient. J. Neurol. 267, 3157–3160.
Pujadas, E., Beaumont, M., Shah, H., et al., 2021. Molecular profiling of coronavirus disease 2019 (COVID-19) autopsies uncovers novel disease mechanisms. Am. J. Pathol. 191, 2064–2071.
Rogers, J.P., Chesney, E., Oliver, D., et al., 2020. Psychiatric and neuropsychiatric presentations associated with severe coronavirus infections: a systematic review and meta-analysis with comparison to the COVID-19 pandemic. Lancet Psychiatry 7, 611–627.
Satake, W., Nakabayashi, Y., Mizuta, I., et al., 2009. Genome-wide association study identifies common variants at four loci as genetic risk factors for Parkinson's disease. Nat. Genet. 41, 1303–1307.
Schwabenland, M., Salie, H., Tanevski, J., et al., 2021. Deep spatial profiling of human COVID-19 brains reveals neuroinflammation with distinct microanatomical microglia-T-cell interactions. Immunity 54, 1594–1610 e1511.
Sinanovic, O., Muftic, M., Sinanovic, S., 2020. COVID-19 Pandemia: neuropsychiatric comorbidity and consequences. Psychiatr. Danub. 32, 236–244.
Stefansson, H., Ophoff, R.A., Steinberg, S., et al., 2009. Common variants conferring risk of schizophrenia. Nature 460, 744–747.
Stopa, E.G., Tanis, K.Q., Miller, M.C., et al., 2018. Comparative transcriptomics of choroid plexus in Alzheimer's disease, frontotemporal dementia and Huntington's disease: implications for CSF homeostasis. Fluids Barriers CNS 15, 18.
Sullivan, P.F., Lin, D., Tzeng, J.Y., et al., 2008. Genomewide association for schizophrenia in the CATIE study: results of stage 1. Mol. Psychiatry 13, 570–584.
Sun, B., Tang, N., Peluso, M.J., et al., 2021. Characterization and biomarker analyses of post-COVID-19 complications and neurological manifestations. Cells 10.
Sutter, R., Hert, L., De Marchis, G.M., et al., 2021. Serum Neurofilament light chain levels in the intensive care unit: comparison between severely ill patients with and without coronavirus disease 2019. Ann. Neurol. 89, 610–616.
Szklarczyk, D., Gable, A.L., Lyon, D., et al., 2019. STRING v11: protein-protein association networks with increased coverage, supporting functional discovery in genome-wide experimental datasets. Nucleic Acids Res. 47, D607–D613.
Taquet, M., Dercon, Q., Luciano, S., Geddes, J.R., Husain, M., Harrison, P.J., 2021. Incidence, co-occurrence, and evolution of long-COVID features: a 6-month retrospective cohort study of 273,618 survivors of COVID-19. PLoS Med. 18, e1003773.
Topol, A., Zhu, S., Tran, N., Simone, A., Fang, G., Brennand, K.J., 2015. Altered WNT Signaling in human induced pluripotent stem cell neural progenitor cells derived from four schizophrenia patients. Biol. Psychiatry 78, e29–e34.
Topol, A., Zhu, S., Hartley, B.J., et al., 2016. Dysregulation of miRNA-9 in a subset of schizophrenia patient-derived neural progenitor cells. Cell Rep. 15, 1024–1036.
Tsivgoulis, G., Palaiodimou, L., Katsanos, A.H., et al., 2020. Neurological manifestations and implications of COVID-19 pandemic. Ther. Adv. Neurol. Disord. 13, 1756286420932036.
Varatharaj, A., Thomas, N., Ellul, M.A., et al., 2020. Neurological and neuropsychiatric complications of COVID-19 in 153 patients: a UK-wide surveillance study. Lancet Psychiatry 7, 875–882.
Virhammar, J., Kumlien, E., Fallmar, D., et al., 2020. Acute necrotizing encephalopathy with SARS-CoV-2 RNA confirmed in cerebrospinal fluid. Neurology 95, 445–449.
Virhammar, J., Naas, A., Fallmar, D., et al., 2021. Biomarkers for central nervous system injury in cerebrospinal fluid are elevated in COVID-19 and associated with neurological symptoms and disease severity. Eur. J. Neurol. 28, 3324–3331.
Wang, M., Roussos, P., McKenzie, A., et al., 2016. Integrative network analysis of nineteen brain regions identifies molecular signatures and networks underlying selective regional vulnerability to Alzheimer's disease. Genome Med. 8, 104.
Wang, K.K.W., Kobeissy, F.H., Shakkour, Z., Tyndall, J.A., 2021. Thorough overview of ubiquitin C-terminal hydrolase-L1 and glial fibrillary acidic protein as tandem biomarkers recently cleared by US Food and Drug Administration for the evaluation of intracranial injuries among patients with traumatic brain injury. Acute Med. Surg. 8, e622.
Woodard, C.M., Campos, B.A., Kuo, S.H., et al., 2014. iPSC-derived dopamine neurons reveal differences between monozygotic twins discordant for Parkinson's disease. Cell Rep. 9, 1173–1182.
Wu, J., Renn, C.L., Faden, A.I., Dorsey, S.G., 2013. TrkB.T1 contributes to neuropathic pain after spinal cord injury through regulation of cell cycle pathways. J. Neurosci. 33, 12447–12463.
Yang, A.C., Kern, F., Losada, P.M., et al., 2021. Dysregulation of brain and choroid plexus cell types in severe COVID-19. Nature 595, 565–571.
Yaron, A., Zheng, B., 2007. Navigating their way to the clinic: emerging roles for axon guidance molecules in neurological disorders and injury. Dev. Neurobiol. 67, 1216–1231.
Zachariadis, A., Tulbu, A., Strambo, D., Dumoulin, A., Di Virgilio, G., 2020. Transverse myelitis related to COVID-19 infection. J. Neurol. 267, 3459–3461.
Zhang, L.H., Li, M., Shi, X.D., Ren, H., Su, B., Xu, Z., 2011. Association of the schizophrenia susceptible gene DKK4 with brain volume in Chinese populations. Dongwuxue Yanjiu 32, 62–65.