# A Computational-Based Drug Repurposing Method **Targeting SARS-CoV-2 and its Neurological Manifestations Genes and Signaling Pathways**

# Ali Sepehrinezhad<sup>1,2,3</sup>, Fariborz Rezaeitalab<sup>2,4</sup>, Ali Shahbazi<sup>1,3</sup> and Sajad Sahab-Negah<sup>2,5,6,7</sup>

<sup>1</sup>Department of Neuroscience, Faculty of Advanced Technologies in Medicine, Iran University of Medical Sciences, Tehran, Iran. <sup>2</sup>Neuroscience Research Center, Mashhad University of Medical Sciences, Mashhad, Iran. <sup>3</sup>Cellular and Molecular Research Center, Iran University of Medical Sciences, Tehran, Iran. <sup>4</sup>Department of Neurology, School of Medicine, Mashhad University of Medical Sciences, Mashhad, Iran. <sup>5</sup>Department of Neuroscience, Faculty of Medicine, Mashhad University of Medical Sciences, Mashhad, Iran. 6Shefa Neuroscience Research Center, Khatam Alanbia Hospital, Tehran, Iran. 7Society for Brain Mapping and Therapeutics (SBMT), Iranian Chapter, Los Angeles, CA, USA.

Bioinformatics and Biology Insights Volume 15: 1-11 © The Author(s) 2021 Article reuse guidelines: sagepub.com/journals-permissions DOI: 10.1177/11779322211026728



ABSTRACT: Coronavirus disease 2019 (COVID-19) caused by the severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) as a global concern involves infections in multiple organs. Much of the research up to now has been descriptive on neurological manifestations followed by SARS-CoV-2 infection. Despite considerable efforts on effective SARS-CoV-2 vaccine, novel therapeutic options for COVID-19 comorbidities are warranted. One of the fast ways to introduce possible effective drugs for clinical trials is bioinformatics methods. We have conducted a comprehensive enrichment analysis of genes involved in SARS-CoV-2 and neurological disorders associated with COVID-19. For this purpose, gene sets were extracted from the GeneWeaver database. To find out some significant enriched findings for common genes between SARS-CoV-2 and its neurological disorders, several practical databases were used. Finally, to repurpose an efficient drug, DrugBank databases were used. Overall, we detected 139 common genes concerning SARS-CoV-2 and their neurological disorders. Interestingly, our study predicted around 6 existing drugs (ie, carvedilol, andrographolide, 2-methoxyestradiol, etanercept, polaprezinc, and arsenic trioxide) that can be used for repurposing. We found that polaprezinc (zinc L-carnosine) drug is not investigated in the context of COVID-19 till now and it could be used for the treatment of COVID-19 and its neurological manifestations. To summarize, enrichment and network data get us a coherent picture to predict drug repurposing to speed up clinical trials.

KEYWORDS: Bioinformatics approach, COVID-19, neurological disorders, polaprezinc, neurotropism

RECEIVED: March 9, 2021. ACCEPTED: June 1, 2021.

**TYPE:** Original Research

FUNDING: The author(s) received no financial support for the research, authorship, and/or publication of this article

DECLARATION OF CONFLICTING INTERESTS: The author(s) declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this

CORRESPONDING AUTHOR: Sajad Sahab-Negah, Neuroscience Research Center, Mashhad University of Medical Sciences, Pardis Campus, Azadi Square, Kalantari Blvd., Mashhad, Iran. Email: sahabnegahs@mums.ac.ir

# Introduction

As of January 28, 2021, the largest pandemic after the Spanish influenza pandemic, the Coronavirus disease 2019 (COVID-19) pandemic caused by the novel coronavirus severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) has led to more than 102 million confirmed cases and more than 2.19 million deaths.1 Although patients infected with SARS-CoV-2 usually have respiratory symptoms, a wide range of neurological manifestations have been recognized and reports their severity and persistence are growing.<sup>2-8</sup> A wide range of neurological manifestations and disorders, such as headache, dizziness, impaired consciousness, hyposmia, anosmia, hypogeusia, ageusia, ischemic stroke, cerebral vasculitis, meningitis, encephalopathy epilepsy, and other serious neurological complications have been reported in COVID-19 patients.<sup>3,9-13</sup> Also, our previous findings suggested 4 possible routes, such as primary olfactory neurons, infected crossed monocytes, angiotensin-converting enzyme 2 (ACE2) receptors on blood-brain barrier endothelial cells, and peripheral nerves for the invasion of SARS-CoV-2 into the central nervous system.14 This evidence will conduct clinicians, neurologists, neuroscientists, and other researchers to bring up and prove the neuroinvasion properties and neurotropism hypotheses by the SARS-CoV-2.14-17 Therefore, to minimize long-lasting neurological disorders caused by SARS-CoV-2, clinical studies are needed to improve the manifestations. To get this point, a fast way to find out a critical drug that can affect the virus and its manifestation signaling pathways is bioinformatics analyses. Here, we conducted a comprehensive enrichment analysis on biological processes, molecular functions, and cellular components of target organs of SARS-CoV-2, especially nervous tissue. Finally, to identify drug repurposing for common genes involved in SARS-CoV-2 and neurological disorders associated with COVID-19, a computational approach was performed.

# **Materials and Methods**

# Gene set selection

All genes used in this study were extracted from the GeneWeaver database (https://www.geneweaver.org/). GeneWeaver is a web-based tool for integrating and analysis of functional



Creative Commons Non Commercial CC BY-NC: This article is distributed under the terms of the Creative Commons Attribution-NonCommercial 4.0 License (https://creativecommons.org/licenses/by-nc/4.0/) which permits non-commercial use, reproduction and distribution of the work without further permission provided the original work is attributed as specified on the SAGE and Open Access pages (https://us.sagepub.com/en-us/nam/open-access-at-sage).

Table 1	Neurological	disorders-as	ssociated (	nene sets	extracted from	GeneWeaver
	neurological	uisorucis a	source	yone sets	CALLACICU HOIT	acric v caver.

ID.	GENE COUNT	TITLE	DESCRIPTION AND REPORTED IN COVID-19
GS242018	381	Stroke	A group of pathological conditions characterized by sudden, non-convulsive loss of neurological function due to BRAIN ISCHEMIA or INTRACRANIAL HEMORRHAGES. Stroke is classified by the type of tissue NECROSIS, such as the anatomic location, vasculature involved, cause, age of the affected individual, and hemorrhagic vs. non-hemorrhagic nature. PMID: 32343504, PMID: 32354768, PMID: 32568626, PMID: 32453685, PMID: 32822622, PMID: 32362244, PMID: 33101164.
GS237840	380	Epilepsy	A disorder characterized by recurrent episodes of paroxysmal brain dysfunction due to a sudden, disorderly, and excessive neuronal discharge. Epilepsy has been reported as a comorbidity factor in COVID-19 caused by SARS-CoV-2 infection: PMID: 32799337, PMID: 32275288, PMID: 32484990, PMID: 32563170, PMID: 32484418.
GS241032	232	Central Nervous System Infections	Pathogenic infections of the brain, spinal cord, and meninges. Neuroinvasion and neurotropism have been reported in COVID-19 caused by SARS-CoV-2 infection: PMID: 32753756, PMID: 32367205, PMID: 32935108.
GS237037	87	Meningitis	Inflammation of the coverings of the brain and/or spinal cord, which consist of the PIA MATER; ARACHNOID; and DURA MATER. Infections (viral, bacterial, and fungal) are the most common causes of this condition. Meningitis has been reported as a comorbidity factor in COVID-19 caused by SARS-CoV-2 infection: PMID: 32251791, PMID: 32926584, PMID: 32926675, PMID: 33072684, PMID: 32843469.
GS242985	72	Neuralgia	Intense or aching pain that occurs along the course or distribution of a peripheral or cranial nerve. PMID: 32275288, PMID: 32572380, PMID: 32588367, PMID: 32896463.
GS241015	50	Encephalitis, Viral	Inflammation of brain parenchymal tissue as a result of viral infection. Encephalitis has been reported as a comorbidity factor in COVID-19 caused by SARS-CoV-2 infection: PMID: 32251791, PMID: 32283294, PMID: 32611761, PMID: 32636212, PMID: 32661082, PMID: 32387508.
GS245651	16	Vasculitis, Central Nervous System	Inflammation of blood vessels within the central nervous system. Primary vasculitis is usually caused by autoimmune or idiopathic factors, while secondary vasculitis is caused by existing disease process. Vasculitis has been reported as a comorbidity factor in COVID-19 caused by SARS-CoV-2 infection: PMID: 32554425, PMID: 33070922, PMID: 32618041, PMID: 32609196.
GS235625	12	Guillain-Barre Syndrome	An acute inflammatory autoimmune neuritis caused by T cell-mediated cellular immune response directed toward peripheral myelin. Demyelination occurs in peripheral nerves and nerve roots. The process is often preceded by a viral or bacterial infection, surgery, immunization, lymphoma, or exposure to toxins. Common clinical manifestations include progressive weakness, loss of sensation, and loss of deep tendon reflexes. Weakness of respiratory muscles and autonomic dysfunction may occur. PMID: 32312628, PMID: 32302082, PMID: 32350026, PMID: 32540883, PMID: 32840686, PMID: 32399950.

genomics data among cross-species.<sup>18</sup> To find out gene sets associated with main intended neurological disorders, the following MESH terms in GeneWeaver were selected: Stroke, Epilepsy, Central Nervous System Infections, Meningitis, Neuralgia, Encephalitis-Viral, Vasculitis-Central Nervous System, and Guillain-Barre Syndrome (Table 1). We considered these gene sets as the main neurological disorders which are related to COVID-19 according to previous studies.<sup>10,11,19,20</sup> Afterward, we also selected and extracted gene sets that were directly associated with COVID-19 in GeneWeaver (Table 2). All genes into the COVID-19 gene sets are directly taken from current studies on patients with SARS-CoV-2 infection.<sup>21,22</sup> Subsequently, we exported all gene sets into an excel file to identify shared genes between our separated gene sets (Figure 1). All subsequent analyses were performed on shared genes between neurological disorders and SARS-CoV-2 associated genes (Figure 2).

# Genetic network reconstruction

To predict gene-gene interactions, we submitted our target shared genes into the STRING (https://string-db.org/). STRING generally is a free access database that provides functional

interactions between proteins/genes as comprehensive networks for different species.<sup>23,24</sup> The obtained network was then uploaded into the Cytoscape version 3.7.0 to interpret the physical and functional interactions between the desired genes. Cytoscape is a free and practical software for visualizing, integrating, and analyzing molecular connections and genetic interaction networks.<sup>25</sup> We finally reconstructed our networks according to main topological features, such as degree, closeness centrality, and betweenness centrality. In a network, these parameters determine which genes are more powerful than the other genes. Most connections nodes (degree), shorter path lengths to reach the other nodes (closeness), and the most central position (betweenness) are 3 advantages for desired gene/genes in a network.26 To classify the involvement of each COVID-19-related genes in each neurological disorders, we submitted our desired shared genes in GraphPad PRISM version 8 and demonstrated them through a heat map.

# Gene enrichment analysis and drug repurposing

To evaluate and visualize all related phenotypes for our intended genes, we used an integrative enrichment analysis

#### Table 2. SARS-CoV-2 associated gene sets extracted from GeneWeaver.

ID.	GENE COUNT	TITLE	DESCRIPTION AND REPORTED IN COVID-19
GS380581	35	Upregulated genes in host transcriptional response to SARS-CoV-2 in Human adenocarcinomic alveolar basal epithelial (A549) cells	This gene set describes genes that are upregulated by the host transcriptional response to SARS-CoV-2 infection in human adenocarcinomic alveolar basal epithelial (A549) cells. COVID-19 is a disease caused by the SARS-CoV-2 virus. We define upregulated as those genes that show a (log 2 fold change) of $\geq$ 1.5. These data are from the supplementary materials associated with a publication that, as of 5/5/2020, has not yet been peerreviewed: https://www.biorxiv.org/content/10.1101/2020.03.24.004655v1
GS380583	22	Upregulated genes in host transcriptional response to SARS-CoV-2 in Normal human bronchial epithelial (NHBE) cells	This gene set describes genes that are upregulated by the host transcriptional response to SARS-CoV-2 infection in normal human bronchial epithelial (NHBE) cells. COVID-19 is a disease caused by the SARS-CoV-2 virus. We define upregulated as those genes that show a (log 2 fold change) of $\geq$ 1.5. These data are from the supplementary materials associated with a publication that, as of 5/5/2020, has not yet been peer-reviewed: https://www.biorxiv.org/content/10.1101/2020.03.24.004655v1
GS398287	4	Genes that are overexpressed in severe compared to mild cases of COVID-19	People with severe cases of COVID-19 express these proteins at significantly higher levels than people with mild cases of COVID-19. Data from Figure 2 of the paper: plasma cytokine levels in patients with COVID-19. PMID: 32217835
GS398321	5	Apoptosis-related pathway in peripheral blood, autophagy (- animal species) signal pathway overexpressed genes from peripheral blood of healthy volunteers and COVID-19 patients	RNA-seq data were analyzed from peripheral blood of 3 healthy volunteers and 2 COVID-19 patients. Apoptosis-related pathway genes were overexpressed in COVID-19 patients vs healthy volunteers. PMID: 32228226
GS398334	587	Upregulated genes in post- mortem lung samples from COVID-19-positive patients	This gene set describes genes that are upregulated in post-mortem lung samples from COVID-19-positive patients relative to biopsied healthy lung tissue from uninfected individuals. COVID-19 is a disease caused by the SARS-CoV-2 virus. We define upregulated as those genes that show a (log 2 fold change) of $\geq$ 2. These data are from the supplementary materials associated with the publication. Note: the following HGNC id is part of this data set but was not recognized HGNC:13378. PMID: 32416070
GS398533	14	Upregulated genes in host transcriptional response to SARS-CoV-2 in normal human bronchial epithelium (NHBE) cells	This gene set describes genes that are upregulated by the host transcriptional response to SARS-CoV-2 infection in normal human bronchial epithelial cells (NHBE). COVID-19 is a disease caused by the SARS-CoV-2 virus. We define upregulated as those genes that show a (log 2 fold change) of $\geq$ 2. These data are from the supplementary materials associated with the publication. PMID: 32416070
GS398534	333	Upregulated genes in host transcriptional response to SARS-CoV-2 in Human lung adenocarcinoma epithelial (Calu3) cells	This gene set describes genes that are upregulated by the host transcriptional response to SARS-CoV-2 infection in human lung adenocarcinoma epithelial cells derived from a pleural effusion (Calu3). COVID-19 is a disease caused by the SARS-CoV-2 virus. We define upregulated as those genes that show a (log 2 fold change) of $\geq$ 2. These data are from the supplementary materials associated with the publication. PMID: 32416070
GS398539	102	Upregulated genes in host transcriptional response to SARS-CoV-2 in Human adenocarcinomic alveolar basal epithelial	This gene set describes genes that are upregulated by the host transcriptional response to SARS-CoV-2 infection in human adenocarcinomic alveolar basal epithelial (A549) cells. COVID-19 is a disease caused by the SARS-CoV-2 virus. We define upregulated as those genes that show a (log 2 fold change) of $\geq$ 2. These data are from the supplementary materials associated with the publication. PMID: 32416070
GS398329	119	Upregulated angiogenesis and inflammation genes in lungs from patients who died from COVID-19	This gene set describes genes that are upregulated in lungs from patients who died from COVID-19. COVID-19 is a disease caused by the SARS-CoV-2 virus. Note that this expression analysis includes only the angiogenesis-associated and inflammation-associated genes available on NanoString panels. The authors define upregulated as those genes that show an (FDR) of $\leq 0.05$ . These data are from the publication (angiogenesis) and supplementary (inflammation) materials associated with the publication. PMID: 32437596

through multiple sources. Using WebGestalt (WEB-based GEne SeT AnaLysis Toolkit), we conducted an over-representation analysis to identify biological processes, cellular components, and molecular functions (Gene Ontology) for our targeted shared genes. Finally, the significant level of false discovery rate (FDR) < 0.05 was considered. To predict cell type/ cell marker-genetic interaction, we submitted our identified genes in cell types—Human Gene Atlas panel through



Figure 1. The computational analysis flowchart for conducting over-representation analysis and repurposing potential therapeutic options for SARS-CoV-2-induced neurological disorders.



Figure 2. Schematic overview of proposed work. All genes involved in SARS-CoV-2 and neurological disorders associated with COVID-19 were extracted from GeneWeaver. Then, common genes in both categories were isolated and proceeded for further computational analysis. Finally, cellular and molecular basis, and signaling pathways were enriched to repurpose the drug and predict possible mechanisms of neurotropism by SARS-CoV-2.

Enricht.<sup>27</sup> To reveal the phenotype and pathway category, we uploaded the intended genes into the Reactome pathway database. Our analysis was proceeded in a suggested functional database (ie, Drug Bank) through WebGestalt to predict genedrug interactions and potential therapeutic options for candidate genes. To predict the main significant drugs for our target genes, we selected drug and DrugBank as the functional database and enrichment category, respectively. We considered our analysis as the significance level of FDR < 0.05.

# Results

# Genetic networks reconstruction and analysis

According to extracted data from GeneWeaver, 846 genes from a total of 9 gene sets associated with COVID-19-related

neurological disorders were identified (Table 2). Furthermore, 1011 genes associated with SARS-CoV-2 were detected (in total 9 gene sets; Table 1). To determine the most significant genes in SARS-CoV-2, genetic network analysis showed interleukin-6 (IL-6), tumor necrosis factor (TNF), and RAC-alpha serine/threonine-protein kinase (AKT1) as the highest degree and maximum betweenness centrality (Figure 3). However, genes with the highest degree and maximum betweenness centrality, such as IL-6, AKT1, TNF, tumor protein P53 (TP53), and amyloid-beta precursor protein (APP) were detected in neurological disorders associated genes (Figure 4). After obtaining the gene networks separately, a common network containing 139 genes between COVID-19 and its neurological disorders was constructed (Figure 5). Among the shared genes, TNF and interleukin-10 (IL-10) were involved in all

АКТІ	Materia	$\bigcirc$	111111111111111	$\bigcirc$	111111111111	$\bigcirc$			$\bigcirc$	$\bigcirc$		$\bigcirc$	$\bigcirc$	$\bigcirc$	$\bigcirc$	0		$\bigcirc$	$\bigcirc$		$\bigcirc$		-	$\bigcirc$				$\bigcirc$
X	SLCIEAG	THY	PARLI	VEGFC	POZDI	CXCLI6	MCIP2	NUPRI	CSARI	DDX60	ECM2	PLAU	FGLS	FFAIG	BMP7	EIG.	CACNAIN	LILRB2	CISD	HSJSTJAI	CLECAD	HSH2D	GABRES	OLRI	HGF	PKEII	CDIet	CLECAR
ПСАМ	Thoi	CIQTNFI	RVASES	ANKBD22	ISTI	IFI30	НСК	suuri	Claef192	SRPK2	COLIAN	CCLIS	NCL.	LIGNS	EAP	NFKRID	Tama2	RSAD2	(VIII.)	TME30140	SPIIO	THEC	П.6	WEDCS	HPGDS	MMP13	TMPI	IGSP6
ARESE	KPBP	EMRI	GPX2	RASGRPJ	CULXI	BCL3	GNLY	SRB01	МАРЗКТ	SAMD9L	USPIS	CORI	FID.3	APOL3	CD38	RASGRP2	EGR2	DCUNID3	ARRDC4	SLCBA3	STEAP4	RNDI	XUICUS	RUNX2	CCLI9	ABCC3	NPBI	OASJ
RAMPI	(TTI)	OASI	EÇMI	CASPS	TEP92	MAP2KS	CSTA	CION2	ACTB	SEMALL	RG).4	ACTNS	MSMO1	DDIT3	RAFTIL	YPEL3	SUSD3	EREG	POUZAFI	CLEC12B	CLEEIA	GECBI	FANKSR	ILIORA	лровесза	MRQH2B	LIB	ME
ADRBE	CCIADOR	AZA SERØN	R9 NOP10	RASGRF2	\$100A12	ALDHAAI	SGPP2	HSPBI	HESNI	VCAMI	SLC39A8	COLLAZ	ULBPI	TRDIM	COLANE	EPHA?	PDCD1LG2	IRSZ	SELL	C40/13	SDR16C5	TESIS	(NFX)	ANKRD33B	CXCL6	KIR	IFIHI	SL(3).14
KDM7A	PPER4	ILIRA	PIOP2	N4BP3	RIIOÙ	GBPS	INIIBA	IL10	BASPI	RNASE	PGL/YRP4	504	MXI	SIXII	DOITH4	SLC3A7	BIRC3	FNI	BIN2	TMEM7	MEDI	SAMIIDI	FABP6	œ	CFLI	(1)	HERCE	SERPINB7
BBC3	(L2)	SURPB1	SEZ6L2	GADDAKA	PHLOB2	GPR65	СТЅВ	EGRI	GCNIJ	UBEZLO	RP\$6KA2	GPR:56	PLANGT	AIF3	SERPINEZ	PKLR	CIĘCA7	NDCEN4L2	MISCA	РІКЗСА	TNESF14	PNEDCI	MCISI	KR172	BCLILA	101	GLRX	ZBÍBIO
PARP12	SLC35F1	FCGRIA	APBBIIR	(TRIM22)	(0)7	METJ1.7B	RG54	n'n	(CXCL9)	KI/K10	(FCGR2A)	ng	CDA	(KLF4)	DNAH17	DUSPS	FYB	TRPC4	FGR	HRA2	WST7B	CC0C114	COLIAI	K(§117	PORDC3	NTNG2	TREMI	PGLSRP2
RAFI	ZNE267	XAFI	HGNC:9982	PTERE	CD53	TIMD123	noor	нр	CISE	N(4A3	AGLI	(CXCLII)	PAQR9	GCA	CETNI	PGLYRPI	(RNF213)	CLECAN	PKP	P1012	HDX	ATPO/IC2	LŷN	RECS	CYPBI	HELZ	PCK	СМРК2
NOD2	TYROBP	LICRAS	(IL3RA)	HCAR3	(IFII6)	SIONAS	EXILA	ANKROI	(TIIBS2)	KLFS	PCSK	SPAC6	MAP2K3	SERPINFI	(IFITMB)	LR/BN3	PARPII	CJARI	ZCHIAVI	FGG	(AQP9)	RASCRPI	C60158	(ccr2)	DTUDI	(CDII2)	ECSCR	LBP
(MMP2)	104	GOII	TM68F1	PTEN	GOBI	ALOXSAP	CIRCPI	(MMP14)	ADRAZA	(CSF1)	лімі	STOP	GEX2	HBEGF	TAGAP	WER16	ADAMS	CCBEI	PS(@1P2	СУТНА	(GNG2)	OPIN	(ILIA)	(US)	REPSI	TYMP	DUXIOL	TNERSEIZA
TUTT	CCRL2	PROK2	SCALA	IRAKS	(IFNL2)	PERI	TMEM156	DRASEI	SE3BA	I.GALS9	PLACE	CYSLTRI	( CFP	IRF7	(IFTTI)	(CTGF)	SPARC	MGAM	CRYAB	SI0411	GISS4	CTSL	EV12B	SH3BRERL3	GNG5	(STAT2)	IFNLS	LILRAI
TNF	OASL	F077	( STATI	(\$100.49)	1.5102	TIPARP	GEACAM	EXPH5	CXCLD	(NERSELA	(NGFR)	( CCL4	DEEDC7	COHIS	GFBR2	CRI	ZFP36	CASPI	(TRIM21)	RNF19B	CCDC113	GAPT	NIDH	(cclii)	(FPRI)	FSOIL	SELE	EIFZAK
ABCAL	DBE4B	SF3A2	Porse	ILIRN	ZMATI	SPRRZE	CCDC60	PDCL3	(LY96)	NFKBLA	TREML4	VEGFA	маркі	ALDHIL2	ANGPTLA	NIAP2	(BP)	GMPR	BCIQL14	SAMENI	PLB01	CSEMPI	LAMC2	INFAIP	NRIDI	RAB)9B	PTGS2	TTGBI
FCERIG	(1.8012)	FUTI	<b>T</b>	09	C (QQ2	RAB8B	TNEADP2	I RRK2	RPT	MMP28	ALDOA	Chen wills	P2RV14	ZNERSA	RPB3A	HSPR6	HCARI	N43/2	CARD16	FCN	P(F)1	TNESES	CORNeT.	SI.COMS	DUSP1	OLICI	SFRPINR4	LVESSC
LNINBI	CREB5	(cxcl2)	HIGLS	TMEMUSA	PARP8	STRN4	AMPD3	SEG5	PLA2G4C	STI	OBBP2	( IRFI )	MNDA	PLEK	ADM2	PEPIRISA	(A)2	ROCK2	PARP14	VWA7	FOSHI	HIVEP2	PARPIO	DTX3L	<b>M</b>	SERPINE	AMP	CIQC
PTAFR	RTPA	LYGE	COLAN	TXDP3	TDBD7	(NCF4)	SCOBAI	RGS18	TREAT	FGF2	SP340	GF	COLSA	(CCRI)	ANK?	GPSM3	METAP2	M004	LTF	(сувв)	NRP2	PCGRUB	DOK3	AKRIBIO	(ASP)	TRIMIN	PLAT	(15113)
LILRB	STN	HSBI7BI4	TANP	DUONA2	•	CNTN2	HD3C9	(MX2)	SMAP2	SPRY2	FALLOF	CXCL17	TRIMIS	EPSTI	KIQ1B	Claims9	(IIIFIA)	VNN3	LIFR	BACH2	Z.(311)2A	TLR3	SEMATA	ILAMP	(G6PD)	(HERCS)	(FNA6	TRANKI
AREDC3	LCN2	SERPINI2	HSDIDBI	MXBA5	INFSF138	(GPR84)	SLC3A5	(15112)	SIGLEC7	UBQLNL	(SNAII)	( GBP1 )	AGT	HUSRAP	СГВ	(IGFI)	TRIMS	THEMIS2	(BST2)	RISP	NCOA7	ALPL	TNERSEIIB	RBP	NRPI	TRAFI	(ISG20)	SP(LC2
P2RY13	CKLF	ADAMTS	PIX3	NR112	(GBP3)	TLRI	PROM	SERPINES	EthB	BCL2AI	ADAMIS	PMAIPI	BEX2	( cc12	casm	1114	KIEC2	PIGER2	MTHFD2	ACKR4	ILISRA	(CD69	AKAP12	BCAII	PPP3RI	RASGEFIB	MARCKS	IFTTM2
PDGFA	RADBA	(cxcl3)	CCDC38	KD+	BATF2	FPR2	DRD2	PAD12	RRAS	MAP3K8	ADAMDECI	CVP2A1	( cas	5105	DNAAF3	CISS	DDO	CEACAND	FCRLA	( 5P11 )	COLISA	ASVS	UBASHIA	PSMP3	P@14	TOBIB		7.C3812C
FCGRIB	(ccus)	ITGAS	erne	KRAS	WIPFI	SPRIR2D	PDCD10	(MMP8)	(GBP4)	XKR9	(NOS3)	TRPMS	(CSF3)	VKORCILI	SRGN	CSF2	MIERI	CSF2RB	(OAS2)	CD48	NRCAM	LCP2	EIF2AK2	SAMD9	NK30-1	DHRS9	BAPPI	PSPII
SIGLECI4	CYPISAL	(ILIRG)	CNTN4	TADKJ	K(3)2	KIJ10	PAKI	P14828	NESN	SERPINAIO	TENI	(BMP2)	CDSS	C8orl4	DDX58	GEDN	OTODI	ATEIFI	IRF2	CACNAIE	(CDC42)	DYSETI	USBI	PCK)	TCBS	FLTRIG	(AIM2)	N0/3
ко»	(ITGA2)	LRP2	(CCL20)	ATTA	HCAR2	(CXCR2)	(IFI6	XRNI	DMBTI	CMOM2	DNAAFI	(MTZA)	HCST	HLA-DRHS	BESTI	16283	PIGR	(CD274)	ARHGDIB	MAP2KI	WDB78	11.124	PCDHI	(IFTT5)	KR123	BDKRB2	PR\$\$16	SCI2D1
MMPIE	LRENI	EIFAAI	IFIAL	CXCLS	LYSS	RNEI75	IRGI	JUN	ALEKI	MEV17	EDN:	CODRS	SP100	РРАНК	GREMI	IIRAS	(JAK3)	LILBAS	STEAPI	RBMII	CDL942	TLRO	TIBSI	LIN	RTN	11.360	STOPI	CVBIF3
CLECTA	SYAPI	PDZKUPI	RAC2	his	TCN	(CP)	(PN)	CERSA	CHIESH	sûs	FREM2	(11.23A)	LOIC	(EB)	SEDDA	INFSF10	THOLG	FCAR	ILIR2	KIT	GMPG	TLR7	ANPRA	FCER	LOIP	BLOREISI	RUNXI	PILBA
SATI	CBX4	SURAA	PTGERA	EKBPIA	CCL2	GPR97	UINLI	ARRB2	MMPI	PCDFS	CARRAI	NLRP3	CTNNBI	PIKSAPI	PLENHAN	CISârfia	(074)	SERPINAL	IFITMU	ACR	NEL	ICAMI	PARPE	NMO	ROCKI	ON	APOBECIG	CXICLI
SAAL	PIGIA	CHIT	III OX2	NO	CICA	ATPSIZ	SPRRZA	SOCSI	(1)32	IENRI	ILIB	AMOTI	TSC22D3	HMOXI	(INN2)	THSD7B	CARDIZ	TOPI	DHX58	KOFI	SLADEZ	DIE	CIOR	ISCIS	ORATA	STOPR	CSEIR	502
CAMP	11.120	NIRC	PM	CLERKS	(D)	(N)	cifici	CNCLIN	NIPPI	BAPPER	Small	ATETR	SIGM	KONVI	ECCPLA	TYPE	11641	-	ENEALPA	(WAS)	IDER	N. IN	orr	Chenzi	EDCSP	PARA	CON	4600
PERMIT	and a	and a	Veri	Chi	TIT	(ICH)	PINCEP	Creat	USPIS	PI 4302-	Kipites	FOR	THEALON	PTKP	CARIA	KON		TRPI	PENE	CNC	ABOL	INIT	CIPPI	SEPPINC.	1 00001	0		and a
TO SEN	USH	Cas	AXI	Car	(and	(United States)	PDGFRL	GISFI	USE15	FLARGIA	N 10 81	CHIG	1316411948	CTR2B	COMBI	1000	Cuper	Capi	Sale	Cacis	AndL6	(Inter	STER	Searing				

**Figure 3.** SARS-CoV-2 associated genetic network. The large nodes indicate a higher degree. The color of each node is adjusted based on its betweenness centrality. Red, orange, yellow, and purple nodes represent a spectrum from greater to lesser betweenness centrality (red nodes have greater betweenness centrality). Unconnected nodes have been excluded.

$\bigcirc$	0	$\bigcirc$		$\bigcirc$	$\frown$		0	$\bigcirc$	$\frown$			$\frown$	$\bigcirc$	$\bigcirc$		$\frown$		$\frown$			$\bigcirc$	$\bigcirc$	0	~	$\bigcirc$		
SYP	KCNA	VWF	NPPB	STAT4	CDKN2A	GERAI	GLRA	ESRI	HTR2C	MOR	FI	SVTI	(IL6R	TGFBI	GABRA4	EDNRB	TBP	(CD4	RPS	A003	SERPINCI	PDCDI	FOXGI	CDIE	CYBB	DBH	HYOUI
CTSB	(C)SI	AGT	PPPIRMC	(ccus)	ADORAZA	GRMS	SLC12A2	GRINI	(ISPB)	(ITGA2	PRL	DCX	NEUROD6	ITGB2	• <b>1</b> 32	(B2M)	hors	GRMJ	EMINI	004	SLC12AS	JAK2	(TLR9)	CREBI	STAT2	AGER	CAVI
GFAP	IFNAI	PIRN2	CON8	CHRNAA	GHR	OLRI	HSCAIB	CACN2D1	T8N12	m	SLC6AI	GASS	B4G.Q.NTI	( CD40 )	PODG	KL	( 11.2	P2RYI	( NPY )	(HP)	LTBR	FGA	CDIA	Inte	MSEA2	IMPA2	TREM2
APOH	11.18	GHRL	CACNG3	SCNIOA	PRNP	KLRKI	TEK	CATNAP2	HLA-DOBI	PADD	ABCO2	DEN1	EQN	GRIAA	SELE	(SSTR2)	ITPRI	IFNARI	PTPRZI	CASP3	APOE	ATRN	EGRI	CC1.2	MARKE	FOLM	SLCIAI
PRKCQ	(NTRK2)	CD70	GRIK\$	(ABP)	CYP2J2	KCSA6	ПСВЭ	RACI	(CD27)	PLPI	\$1.024	(III.A-B	PTCS	(CXCL12)	CYPI9AI	PRR12	LRPI	PTGS2	PCOTI	LGars2	KLRG)	(LC)2	REIN	PROCR	11.10	PREMAZ	EMBI
AKAPS	MPDZ	BIRC5	SCNILA	YWILLO	(NFKBI)	KCNQ2	CIND	PONI	GRINI	GJAI	NRIIZ	DMD	HAXI	SLC30A3	HSPAIL	ADAMTSIJ	CCLS	LUCL2	( MPO )	ATN	SLCI7A6	(DKL)	UFNG )	(CD163)	100	GCCX	ARAF
AIFI	SEZ6	FMRI	CIITA		STATI	KCNK17	BDNF	CHRNA7	CFH	KIR2DL3	(HSPA4)	(GNB3)	R051	KCNV2	GRMI	нмаві	SCN2A	(AVP)	IGB	( HGF )	(IRF9)	C(P4F2	MUIA	(IRF7	SPBN	CFP	FGB
GAL	ITGERI	(SNCA)	(CNRI)	(11)	(S100B)	GABRE	KCNJID	<b>F8</b>	(F2R)	OPRMI	QK)	BACEI	GCKR	GRIK	PTGIS	ABCAI	(NFRSF9	C01.942	GRIN2A	SLOAJ	ILIRI	NCR	AGTRI	in the second se	TP53	(SNAP25)	INFSFA
CACNG2	TGFBR2	ATP6AP2	(11.15)	(CASP9)	ADORAL	CLICION	CALBI		(CD40LG)	PARKT	ESR2	CASPO	KCNK2	TRPVI	SPHK	ALG9	CIQA	( CD34	BCIQL12	FISAL	MAG	SCN84	DUCK7	STUBI	SELPLG	ATPIA2	RAGI
MBP	HEE	11.128	(APN)	GPG	CPB2	HSPD	Co	GPXI	VDR	HTT	1.G12	П.6	CXCL16	NPV5R	RB CD15	)	EPO	PVALB	N@R3	( LEP )	(CD274)	RAG?	MAG12	CHINE	GZMB	AMB	( SST )
YWHAB	GLUI	5V2.9	<b>5V2B</b>	MAAB	P())2	( 11.4	SCRGI	NYODI	Сува	PRFI	NGFR	PROZ	CAMK2B	TRPI	AHR	IGFIR	HIFIA	GERA2	RACBPI	LTA	BCL.	CVP2C19	ALOXSAP	T	CEOSRI	TSPO	CURSRI
(cs)	UAGI	KNII	(LCP2)	( FGF2 )	GUDI	FGG	(TGA2B	HLA-DQAI	ZIQN3	CCR2	IFNGRI	CXCLII	steral	PRND	HPRT	PLAT	ADDI	MK167	TNERSF12A	SLC614	CHRYAG	(11.21)	NEP)	CYPAN	CP12	HOMERI	MANF
NTPI	( CD28 )	RTN	RSLS	PARPI	PLA2G2A	SECTION	ATXNJ	(11.9)	8938	LICAS	SLC6AS	CAMK2	(YP2C)	( a )	IC@14	(MMPI)	HTRIA	SCN9A	т82	SOCS3	(NLRP3)	AKTI	ADCYAPI	SCNIA	ene	<b>C()</b> 4	(FLTI)
CXCL8	RELA	(RPA)	NOXI	(PLG)	APENI	VEGFA	)( SPPI )	(CST3)	NUCB2	COPIAS	ACE	MC4B	(IL4R)	ITGAM	XE2	(PF4)	DCEK2	EP(12A	NAMPT	BCL2LI	NOTCHI	REBXI	CB1B	MOP	TLR4	YAMP2	GRIKA
Chinest	POGED	PON2	SLCRAI	GRMA	ADM	Spanicel	PSME2	TLR7	ARO	TNESE14	EIF2AK2	MTZA	SYT.	GABRG2	CFB	CCLII	PDYN	(OLIG2)	GPN3	(MMP9)	PPARG	PIKICG	œ	CACNAIH	Nost	GABRR2	DLG4
TEX4	SL.	GABRB2	CCNDI	HMOXI	NDP	CX3CRI	COLAN	AHSG	MBL2	HONI	BCL2LI	GDNF	AKTS	OPHNI	TRENI	TIMPI	UCP2	GABRD	(NFRSF1)	SRPX2	GRIAI	ILEST	LEDI	(MT)	ERBB4	PTPRC	(SYN1)
MAVS	Cuca	TNESF13	(GRIN2B)	NIAPIB	busp	ADK	CSPG4	ILIRN	KIRDI.2	ADIPOQ	GABRAI	(MAP2)	VM	bisci	MXRA8	ANGPT	СТВ	POMC	CR2	(LDNS	BAIP5	-	an 1990	TROISI	THBD	( NES )	CACNAIG
C(1)2	ILIB	APOAL	ARICEFS	KCNAI	NRP2	MTOR	107	NCAMI	MYOSA	CACNALE	JUNB	TACRI	MTBR	EPM2AIP1	2002	(CTLA4)	(SSTR4)	BDKRBI	GDFIS	PVBLI	KIRIDLI	T <b>C3</b> 2	BILA	ILIR	XRCCI	TNR	PANNI
(SOD2)	APOAS	PSMA6	OASI	(IGFBP3)	FRACD	DLX2	EPHNI	TNU	CRH	PLAUR	CSARI	( 11.13 )	(VCAMI)	K(R2DL1	DGKZ	INFRSFIB	SLOA6	RELN	DLGAP2	ARHGEF10	(CXCL9)	CD8A	( 11.17A )	GRM7	(LPL)	RINIR	NINJ2
NRGI	CHRMI	NOTCHS	NCAY	PRKG	FABP2	PERI	HTR7	DEN5	OVP1A2	SERPINAL	CACNALA	TICAMI	FT	SCARB2	(ILIA)	UGT1A6	NPYIR	PTEN	(ммрз)	COMT	CRI	SERPINII	SLCIAS	Ø	LIPO	(CNR2)	HMOX2
DRDI	MIF	( 13 )	ABCA4	(MAPK14)	ATPIZA	NIVEN	PGF	IGFBPI	AD(A)122	CALL	CRIM	\$TXBP	P(T)2	II.SRA	CHAT	HIA-DPBI	UGT2B7	EEHCI	ABCCS	C01.241	КСЕМВА	NGD	CHRNA2	(ILTR	(SIPR2)	NR4A2	HESI
PIGER2	SODI	TIRAP	PNOC	(HLA-A)	FOS	MYIHIS	MMPIO	AGTR2	TNERSEILB	1.836	HLA-DRBI	АВСВІ	P2RX3		P2RV12	(ADRB2)	ACC 2	PSMDI	DEVSL2	SPTAN	FAS	CSF2	FASLG	(TBX21)	TESMI	NHERCI	NEUROD2
н	RANI	INFSFI	ІКВКВ	MOD	MECP2	(тсві)	SCNIA	BECNI	SC BAI	SIRPA	011	(ST)	HCN2	TAMPS	***	HRH	(T)	KIR2DL4	TAR	EU	PLCGI	APP	HTRAI	ISPALA	TOP	ACADM	SPP.
SMNI	(LPA)	PYCARD	СЕВРВ	CHRYAS	GABRG	CACNA2D2	(CXCR3)	SELP	TBRI	ASPM	PLAU	PARAIIBI	PRICELEI	ELAVE4	REST	(GRIA2)	REN	TNESE9	( FNI )	ALDISAT	(ANXA5)	ALDITA1	(CALB2)	HBAI		TYR	LDLR
CVPITAL	CLON7	(IFNBI)	CVP2C8	(GAD2)	PIRXT	BUNT	B(1.2).2	MMP2	( ТАСІ )	CDKN2B	SLCIA2	TBXA2R	PGR	BBC)3	CXP11B2	HNENPU	tsc	EN	PLA267	(FYN)	SPAST	HLA-G	GPHN	AQP	(TSC2)	PRDMI	CACNES
маркя	RGMA	AP3D1	VKORCI	GRKI	(1001)	CX3CLI	KALRN	(MXI)	RUVBL2	PPARA	ALDOC	(HRH3)	OCLN	(CCLA)	ADRAZA	(AQP4)	NGPT?	( CCR5 )	KIF6	KENKI	ENO2	PROC	TRP14	GRIDI	PTGES	BID	PSMB9
KCNOI	NPY2R	ADRBI	HTR2A	GADI	SLC30AI	CLECKA	(CND)	(PIBA	SYN2	(CXCL10)	RX	SPIBNS	PLCBI	( F2 )	BAN	FEGRAB	(CD14)	KCNQ)	49	GPRSS	(13)	TNF	PROS	(IRF3)	маркз	NEUROG2	GC
SENZB	CYPINI	AVPR2	UNCOBI	MYDSS	SH2DI A	PNKP	NOS2	11.23A	TFP)2	CBS	MTHER	KHDRB53	CHRNB2	P2RN4	0	PSMBS	UNG	PSENI	ALPL		KIRIDL3	DDX 55	CXCLIS	SERPINE	GRIAN	FCGR2A	MEF2D
ICAMI	GHI		BRD2	BACE2	GSTMI	ENG	(KDR)	MAPT	ксурз	(NOD2)	LIF	RASBP2	GABRBI	(DRD2)	(UBC)	KCNH2	BCKDK	(NOX4)	CEBPD	CCR3	CD36	PCSK9	CABRES	PRKCH	HMGCR	SCNIB	FIO
TRESE 12	APOB	SAMILDI	LGI	DNRA	(TLRJ)	( IGFI )	GABRAS	SREBP2	ABOCI	GALRI	GABBRI	P(DII)9	UBESA	LGI4	J®K	TNFRSF4	A (NI	PDE40		$\bigcirc$	-		$\sim$			1000	

Figure 4. Neurological disorders genetic network. The large nodes indicate a high degree. The color of each node is adjusted based on its betweenness centrality. Red, orange, yellow, and purple nodes represent a spectrum from greater to lesser betweenness centrality (red nodes have greater betweenness centrality). Unconnected nodes have been excluded.

predetermined neurological disorders. Besides that, the IL-6 had effective nodes with 7 neurological disorders. Moreover, interleukin-1 beta (IL-1 $\beta$ ), chemokine (C-C motif) ligand 2 (CCL2), and tumor necrosis factor receptor superfamily member 1A (TNFRSF1A) were involved in 5 neurological

disorders (Figure 6). Furthermore, network analysis using Cytoscape indicated that TNF, IL-6, C-X-C motif chemokine ligand 8 (CXCL8), IL-10, vascular endothelial growth factor A (VEGFA), AKT1, CCL2, IL-1 $\beta$ , and intercellular adhesion molecule 1 (ICAM1) were the most significant nodes in terms



**Figure 5.** Reconstructed genetic network for shared genes between neurological disorders and SARS-CoV-2 associated genes. The current network comprised 139 nodes (genes) and 451 edges (interactions). Dark red nodes involve in all predetermined neurological disorders. The light red, dark orange, light orange, green, and yellow nodes involve 7, 5, 4, 3, and 2 neurological disorders, respectively. Purple nodes involve in one neurological disorder. The size of all nodes adjusted based on their degrees into the network (larger nodes indicate higher degree). Nine peripheral nodes have a higher degree and greater closeness and betweenness centrality in the network.



Figure 6. Heat map of differential expression of genes. The involvement of each SARS-CoV-2-related gene in each neurological disorder is adjusted with a color map. The columns represent SARS-CoV-2 associated genes, while rows represent the most important reported neurological disorders in COVID-19 patients. The dark red color indicates positive enrichment and light red indicates negative enrichment.

of closeness centrality and degree (Figure 7; refer to supplementary Table 1 to see topological features of other genes into the network). Following this step, topological parameters, such as degree, betweenness centrality, and closeness centrality for significant genes were included. Among them, IL-6, degree (D) = 99; betweenness centrality (B) = 0.10586358, closeness centrality (C) = 0.75141243, TNF (D = 96; B = 0.14707984; C = 0.75568182), CXCL8 (D = 80, B = 0.03480363, C = 0.6751269), IL-10 (D = 77; B = 0.0311957; C = 0.665), VEGFA (D = 71; B = 0.02983814; C = 0.64563107), AKT1 (D = 68; B = 0.05121735; C = 0.63636364), CCL2 (D = 68; B = 0.01949683; C = 0.63033175), and ICAM1 (D = 66;

B = 0.02154069; C = 0.63033175) were detected as the highest topological parameters

#### Over-representation analysis

In this step, we analyzed biological processes, cell components, molecular functions, and signaling pathways associated with SARS-CoV-2, and its neurological disorders. Our results indicated that cytokine-mediated signaling pathway, FDR = 5.45E-45, cellular response to cytokine (FDR = 2.10E-42) and chemical stimulus (FDR = 5.79E-51), immune (FDR = 1.33E-39) and inflammatory response (FDR = 7.86E-33), and a response to another organism (FDR = 1.35E-40) are



Figure 7. Highest topological features of 9 shared genes between COVID-19 and its neurological manifestations. Quantitative data represented 3 main topological features including degree, closeness centrality, and betweenness centrality.

remarkable processes that are involved in SARS-CoV-2induced neurological disorders (Figure 8A). Gene Ontology (GO) enrichment analysis also displayed the most pathological processes. Among all, cell surface (related genes to this process include CX3CL1, CD4, TNFRSF1A, NGFR, PLAT, ICAM1, VCAM1, ITGA2, NOD2, PCSK9, CXCL10, TNFRSF12A, FGG, C5AR1, TGFBR2, CXCL9, CR1, ABCC2, PLAU, ABCA1, CD274, KCNQ3, ITGB1, IL1R1, ACE2, TNF, ITGAM, VEGFA, TNFRSF9), extracellular space (predicted genes to this microenvironment include CX3CL1, TNFRSF1A, HMOX1, TIMP1, MMP2, PLAT, HGF, SERPINE1, CCL2, IL23A, CTSD, CFP, CHI3L1, IL1RN, IL1A, IL-1B, FGF2, ICAM1, PLA2G7, SCGB1A1, MMP10, FLT1, CXCL13, CXCL16, VCAM1, CSF2, TNFRSF11B, CCL11, IGF1, PCSK9, CXCL10, CXCL8, CXCL11, HBA1, SELE, FGG, CTSB, FN1, HP, IL27, CXCL9, AGT, F5, CTSS, PLAU, IFNB1, CD274, IL-6, ACE2, TNF, LTB, IL-10, SERPINA1, ITGAM, TNFSF14, CCL5, CCL3, VEGFA, TNFRSF9, CCL4), membrane raft (related genes to this structure include CD4, LCP2, TNFRSF1A, MAPK1, HMOX1, CTSD, ICAM1, NOS3, OLR1, SELE, FYN, TGFBR2, PTGS2, ABCA1, ITGB1, ACE2, TNF, ITGAM), dendritic growth cone (related genes to this structure include RTN4, MAP2), axon initial segment (related genes to this phenomenon include MAP2, KCNQ3), and glial cell projection (related genes to this process include FYN, ITGB1) were detected as the most important dysfunctional cellular components in COVID-19-induced neurological disorders (Figure 8B).

Besides that, most important immunological and neural responses including neutrophil degranulation (FDR = 1.35E-05), collagen degradation (FDR = 2.34E-05), degradation of

the extracellular matrix (FDR = 3.31E-05), platelet activation and aggregation (FDR = 2.81E-06), activation of matrix metalloproteinases (FDR = 0.0022), metabolism of angiotensinogen to angiotensins (FDR = 0.0048), axonal growth inhibition (FDR = 0.0198), presynaptic depolarization (FDR = 0.0251), deubiquitination (FDR = 0.0251), and axon guidance (FDR = 0.0298) were detected in COVID19-induced neurological disorders (Figure 8C).

Furthermore, some dominant signaling pathways, such as interferon-alpha/beta signaling (FDR = 9.21E-09), signaling by interleukins (FDR = 2.61E-28), toll-like receptors (FDR = 5.78E-05), tumor necrosis factor receptor 2 (TNFR2) noncanonical nuclear factor kappa B (NF-kB) pathway (FDR = 0.0002), mitogen-activated protein kinase 1 (MAPK1)/mitogen-activated protein kinase 3 (MAPK3; FDR = 0.0044), death receptor (FDR = 0.0231), and tumor necrosis factor receptor 1 (TNFR1)-induced proapoptotic signaling (FDR = 0.0275) were enriched in our target shared genes (Figure 8C). Finally, to target cellular lines, our cell type/specific marker analysis revealed CD14<sup>+</sup> monocytes (P = .0003518), CD33<sup>+</sup> myeloid (P = .0009167), BDCA4<sup>+</sup> dendritic cells (P =.02248), and CD56<sup>+</sup> NK cells (P = .01594) as the most affected cell types by SARS-CoV-2.

# Drug repurposing

At the final step, gene-drug predicted analysis targeted 6 existing drugs, such as carvedilol (P = 9.6150e-7, FDR = 0.00096261), andrographolide (P = .000092022, FDR = 0.026985), 2-methoxyestradiol (P = .00018122, FDR = 0.037239), etanercept (P = .00022223,



**Figure 8.** Biological process, cellular component, Reactome pathway, and molecular function enrichment analysis of our target genes of SARS-CoV-2 and its neurological disorders. (A) The most significant biological processes that may involve in SARS-CoV-2-induced neurological disorders. (B) The important cellular components which can be interrupted by SARS-CoV-2. (C) The Reactome pathway enrichment analysis of shared genes between SARS-CoV-2 and its neurological manifestations. (D) The molecular function enrichment analysis of our target genes. All parameters were sorted according to the enrichment FDR from GO analysis.

FDR = 0.037239), polaprezinc (zinc L-carnosine; P = .00022223, FDR = 0.037239), and arsenic trioxide (P = .00029155, FDR = 0.040699) for SARS-CoV-2 and neurological disorders followed by COVID-19. Among our desired

shared genes, carvedilol was repurposed by 7 genes (ie, VEGFA, adrenoceptor alpha 2A [ADRA2A], vascular cell adhesion protein 1 (VCAM1), selectin E (SELE), hypoxia-inducible factor 1-alpha (HIF1A), cytochrome P450 family 1 subfamily



**Figure 9.** The gene-drug network and the schematic diagram of the possible effect of Polaprezinc on signaling pathways involved in SARS-CoV-2 and its neurological manifestation. (A) The relation between genes and their target drugs has been shown as a network. The size of each drug (orange diamond nodes) was adjusted based on its FDR significance level (biggest nodes indicate smallest FDR levels). (B) Based on drug repurposing and Reactome pathway analysis, 4 target genes, such as Fms related receptor tyrosine kinase 1 (FLT1), tumor necrosis factor (TNF), interleukin-6 (IL-6), and heme oxygenase 1 (HMOX1), as well as their mechanisms, were predicted by Polaprezinc in the context of COVID-19.

A member 1 (CYP1A1), and beta-2 adrenergic receptor (ADRB2)). Based on our results, 3 out of 6 drugs were repurposed by 4 genes. For example, polaprezinc was predicted by TNF, IL-6, fms-related receptor tyrosine kinase 1 (FLT1), and heme oxygenase 1 (HMOX1) genes. Also, the MAPK1, ATP binding cassette subfamily C member 2 (ABCC2), AKT1, and CYP1A1 genes targeted Arsenic trioxide. Four genes including TNF, prostaglandin-endoperoxide synthase 2 (PTGS2), Fc fragment of IgG receptor IIIb (FCGR3B), and low-affinity immunoglobulin gamma Fc region receptor II-a (FCGR2A) represented Etanercept. Andrographolide (eg, TNF, IL-6, and IL-1β genes) and 2-Methoxyestradiol (eg, CYP1A1, HIF1A, and cytochrome P450 family 19 subfamilies A member 1 [CYP19A1] genes) were repurposed by 3 genes (Figure 9A). Among shared genes, TNF and IL-6 genes that are involved in COVID-19 pathogenesis and neurological manifestations pathology were targeted by polaprezinc and andrographolide in our analysis. To find out genes related to polaprezinc, further analyses were performed. Our results showed that several common genes, such as TNF, IL-6, FLT1, and HMOX1, can be targeted by polaprezinc (Figure 9B). To continue, the possible signaling pathways related to the above-mentioned genes followed by polaprezinc were predicted. As shown in Figure 9B, several signaling cascades, such as interleukin-4 (IL-4), interleukin-13 (IL-13), IL-6, VEGF, TNFR2 non-canonical NF-kB pathway, and TNFR1-induced proapoptotic signaling were targeted by polaprezinc in the context of COVID-19 and its neurological manifestations.

#### Discussion

Our findings showed that 139 genes were shared between SARS-CoV-2 and neurological disorders, which appeared after the first COVID-19 symptoms. In this study, common neurological manifestations of SARS-CoV-2, such as stroke, epilepsy, meningitis, neuralgia, encephalitis, Guillain-Barre Syndrome,

vasculitis, and CNS infections were included. To repurpose a drug, sequential computational steps from shared genes between SARS-CoV-2 and neurological to signaling pathways were performed. To our study, high closeness centrality and degree were observed in genes related to cytokines (eg, TNFa, IL-6, IL-10, and  $IL-1\beta$ ), chemokines (eg, CXCL8 and CCL2), growth factor (eg, VEGFA), cell-cell interaction (eg, ICAM1), and signal transduction (eg, AKT1). Based on our enrichment analysis, different impairments, such as extracellular matrix degradation, axonal and synaptic dysfunction, and metabolism destruction were seen in SARS-CoV-2 and neurological disorders associated with COVID-19. Prominent signaling pathways behind the above-mentioned tissue impairments were inflammatory signaling pathways (ie, Interferon alpha/beta signaling, signaling by interleukins, toll-like receptor, and TNFR2 non-canonical NF-kB pathway) and intracellular signaling pathways, ie, MAPK1/MAPK3 and protein tyrosine kinase 2 (PTK2). To dig deep insight, transcription and epigenetic factors played role in shared genes were analyzed by miRNA-predicted and TRANSFAC analysis. Concerning these data, 6 miRNAs and 8 transcription factors were predicted. In the main part of our analyses, 7 potential drugs, such as carvedilol, andrographolide, 2-methoxyestradiol, etanercept, polaprezinc, arsenic trioxide, and clenbuterol were repurposed for SARS-CoV-2 and neurological disorders associated with COVID-19. We found that polaprezinc is not investigated in the context of COVID-19 and its neurological manifestations. Therefore, polaprezinc can be a potential candidate for further clinical studies.

Since SARS-CoV-2 was reported in December 2019, neurological manifestations followed by COVID-19 and the neuroinvasive potential of SARS-CoV-2 have been attracting a lot of interest.<sup>28-30</sup> Most clinical studies have been only conducted in a cross-sectional design to describe neurological manifestations infected with COVID-19.<sup>3,7</sup> Several attempts have been made to explain the neurotropic characteristics of SARS-CoV-2

in post-mortem samples and cerebrospinal fluid analyses.<sup>31-33</sup> However, much of the research up to now has been descriptive in nature and SARS-CoV-2-associated neuropathogenesis to identify novel therapeutic targets very little is known. This study seeks to obtain genetic data that are common between SARS-CoV-2 and neurological disorders associated with COVID-19 which will help to address these research gaps.

As shown by previous data in the literature, infected patients with COVID-19 display high levels of pro-inflammatory cytokines (IFN $\alpha$ , IFN $\gamma$ , IL-1 $\beta$ , IL-6, IL-12, IL-18, IL-33, TNF $\alpha$ , TGF $\beta$ ), anti-inflammatory cytokines (IL-4 and IL-10), and chemokines (CXCL10, CXCL8, CXCL9, CCL2, CCL3, CCL5).<sup>34,35</sup> Our bioinformatics analyses confirmed previous clinical results that the cytokine storm triggers and maintains the abnormal systemic inflammatory response. This phenomenon causes Acute Respiratory Distress Syndrome (ARDS) and multiple organ failure and participates in death in the most severe cases of SARS-CoV-2 infection.<sup>36</sup> These similarities between clinical data and our bioinformatics results encouraged us to continue further analyses on the signaling process and cellular dysfunction in COVID-19 and neurological manifestations.

As we all know, it takes more than 10 years to bring a drug from the trial stages to market availability; therefore, repurpose various approved drugs against COVID-19 to speed up clinical trial are warranted. Recently, several FDA-approved drugs were repurposed by computational studies for COVID-19.37-39 Among all, Atazanavir and Amprenavir can be repurposed for COVID-19 with high inhibitory potency against SARS-CoV-2.<sup>40,41</sup> Our study hypothesizes that the prediction of existing drugs based on genetic and epigenetic interactions data between SARS-COV-2 and neurological disorders associated with COVID-19 may be a potential candidate to treat virus neurological comorbidities. Our computational analysis repurposed different drug categories, including the anti-inflammatory agents (eg, etanercept, andrographolide, polaprezinc, and carvedilol) and anti-tumoral effects (eg, arsenic trioxide and 2-methoxyestradiol). Among all, we found that polaprezinc is not used in COVID-19 till now and it could be used for the treatment of COVID-19 and its neurological manifestations.

Among different types of treatment strategies, there are 3 major views on the effects of existing drugs on the clinical course of severe cases of COVID-19. The first and main strategy is to inhibit the development of overwhelming inflammatory responses (ie, inhibition of cytokines storm). The second strategy is to control hypoxemia in patients hospitalized with COVID-19. The third plan is to manage coagulopathy in severe cases of COVID-19. Among predicted existing drugs, polaprezinc has anti-inflammatory, antioxidant effects, and scavenges free radicals (ROS; reactive oxygen species).<sup>42-45</sup> Also, this drug reduces the activity of the transcription factor NF-kB and diminishes the expression of several pro-inflammatory cytokines, such as 1L-1 $\beta$ , IL-6, IL-8, and TNF $\alpha$ .<sup>45,46</sup> Moreover, several studies have been reported that the beneficial effects of polaprezinc in the course of gastric mucosal injury may be ascribed to its anti-oxidative

Bioinformatics and Biology Insights

(ie inhibition of ROS generation) and anti-inflammatory properties (ie suppression of IL-6 and TNF).<sup>43,45,47,48</sup>

Based on our analysis, several key genes, such as FLT1, TNF, HMOX1, and IL-6 involved in SARS-COV-2 and its neurological manifestations can be targeted by polaprezinc. As stated above, SARS-CoV-2 infection can be associated with cytokine storms, especially in its severe form. The most surprising aspect of our data indicated that polaprezinc can inhibit different inflammatory signaling pathways. Besides that, we found that VEGF, IGF, and MAPK signaling pathways may play important roles in the course of SARS-COV-2 with its neurological manifestations. Furthermore, it has been reported that the HMOX1 pathway can reduce platelet aggregation and can have anti-thrombotic and anti-inflammatory properties.<sup>49</sup> It would be interesting to note potential molecular therapeutics that could modulate the HMOX1 pathway to enhance therapeutic intervention and control the cytokine cascade usually observed in SARS-CoV-2 patients. Data from our computational results indicated that polaprezinc can modulate the expression of HMOX1 gene; therefore, the outcome of COVID-19 patients may be improved by polaprezinc.

Interestingly, our computational results predicted the effect of polaprezinc on these growth factors and intracellular signaling pathways. Therefore, we speculate that polaprezinc may be effective in COVID-19 and its neurological manifestations through different mechanisms. However, it is unfortunate that the study did not include downregulated genes of SARS-CoV-2. Therefore, more information on downregulated genes would help us to establish a greater degree of accuracy on this matter. Furthermore, it should be noted that our results were taken from a computational approach; therefore, to prove the efficacy of polaprezinc in the course of SARS-Cov-2 and its neurological manifestations, clinical trials must be designed.

# Conclusions

Most studies provide evidence for the neurotropism and neuroinvasion of SARAS-CoV-2. Furthermore, numerous reports are describing the neurological manifestations of SARS-CoV-2 in patients with COVID-19. At the current stage, due to the lack of efficient cure strategies for COVID-19, state-of-the-art methods to speed up the clinical trials need to be considered. Our findings repurposed polaprezinc drug as an effective drug for the treatment of COVID-19 and neurological manifestations followed by COVID-19 based on bioinformatics data and their mechanism of actions; however, to prove the beneficial effects on human subjects, clinical trials would be conducted.

# **Author Contributions**

AS contributed to the conception of the analysis, designed the tables and graphs, conceived the study, and contributed to the writing of the manuscript. FR contributed to the interpretation of the neurological results. ASH contributed to the analysis. SSN conceived the study, contributed to the interpretation of the results, wrote the manuscript, and supervised the work.

All authors provided critical feedback and helped shape the manuscript.

#### Availability of Data and Materials

All data sets generated/analyzed for this study are included in the manuscript.

# **ORCID** iD

Sajad Sahab-Negah (D) https://orcid.org/0000-0002-2242-9794

# Supplemental material

Supplemental material for this article is available online.

#### REFERENCES

- Guan W-J, Ni Z, Hu Y, et al. Clinical characteristics of coronavirus disease 2019 in China. N Engl J Med. 2020;382:1708-1720.
- Pan F, Ye T, Sun P, et al. Time course of lung changes at chest CT during recovery from Coronavirus Disease 2019 (COVID-19). *Radiology*. 2020;295:715-721.
- Varatharaj A, Thomas N, Ellul MA, et al. Neurological and neuropsychiatric complications of COVID-19 in 153 patients: a UK-wide surveillance study. *Lancet Psychiatr.* 2020;7:875-882.
- Mao L, Jin H, Wang M, et al. Neurologic manifestations of hospitalized patients with coronavirus disease 2019 in Wuhan, China. *JAMA Neurol*. 2020;77:683-690.
- Kremer S, Lersy F, Anheim M, et al. Neurologic and neuroimaging findings in patients with COVID-19: a retrospective multicenter study. 2020;95: e1868-e1882.
- Xiong W, Mu J, Guo J, et al. New onset neurologic events in people with COVID-19 in 3 regions in China. *Neurology*. 2020;95:e1479-e1487.
- Nepal G, Rehrig JH, Shrestha GS, et al. Neurological manifestations of COVID-19: a systematic review. *Critical Care*. 2020;24:421.
- Corrêa DG, Hygino da Cruz LC Jr, Lopes FCR, et al. Magnetic resonance imaging features of COVID-19-related cranial nerve lesions. J Neurovirol. 2021;27:171-177.
- Zayet S, Klopfenstein T, Kovåcs R, Stancescu S, Hagenkötter B. Acute Cerebral Stroke with Multiple Infarctions and COVID-19, France, 2020. *Emerg Infect Dis*. 2020;26:2258-2260.
- Fonseca E, Quintana M, Lallana S, et al. Epilepsy in time of COVID-19: a survey-based study. *Acta Neurol Scand*. 2020;142:545-554.
- 11. Moriguchi T, Harii N, Goto J, et al. A first case of meningitis/encephalitis associated with SARS-Coronavirus-2. *Int J Infect Dis.* 2020;94:55-58.
- Mahalaxmi I, Kaavya J, Mohana Devi S, Balachandar V. COVID-19 and olfactory dysfunction: a possible associative approach towards neurodegenerative diseases. J Cell Physiol. 2021;236:763-770.
- Prasad K, Al Omar SY, Alqahtani SAM, Malik MZ, Kumar V. Brain disease network analysis to elucidate the neurological manifestations of COVID-19. *Mol Neurobiol.* 2021;58:1875–1893.
- Sepehrinezhad A, Shahbazi A, Negah SS. COVID-19 virus may have neuroinvasive potential and cause neurological complications: a perspective review. J Neurovirol. 2020;26:324-329.
- Bougakov D, Podell K, Goldberg E. Multiple neuroinvasive pathways in COVID-19. *Molec Neurobiol*. 2020;58:564-575.
- Li Y-C, Bai W-Z, Hashikawa T. The neuroinvasive potential of SARS-CoV2 may play a role in the respiratory failure of COVID-19 patients. J Med Virol. 2020;92:552-555.
- Kumari P, Rothan HA, Natekar JP, et al. Neuroinvasion and encephalitis following intranasal inoculation of SARS-CoV-2 in K18-hACE2 Mice. *Viruses*. 2021;13:132.
- Baker EJ, Jay JJ, Bubier JA, Langston MA, Chesler EJ. GeneWeaver: a webbased system for integrative functional genomics. *Nucleic Acids Res.* 2011;40:D1067-D1076.
- Zhang BZ, Chu H, Han S, et al. SARS-CoV-2 infects human neural progenitor cells and brain organoids. *Cell Res.* 2020;30:928-931.
- Hanafi R, Roger PA, Perin B, et al. COVID-19 neurologic complication with CNS vasculitis-like pattern. *AJNR*. 2020;41:1384-1387.
- Blanco-Melo D, Nilsson-Payant BE, Liu W-C, et al. SARS-CoV-2 launches a unique transcriptional signature from in vitro, ex vivo, and in vivo systems [published online ahead of print March 24, 2020]. *Biorxiv.* doi:10.1101/2020.03.24. 004655.
- Chua RL, Lukassen S, Trump S, et al. COVID-19 severity correlates with airway epithelium-immune cell interactions identified by single-cell analysis. *Nat Biotechnol.* 2020;38:970-979.

- Szklarczyk D, Morris JH, Cook H, et al. The STRING database in 2017: quality-controlled protein-protein association networks, made broadly accessible. *Nucleic Acids Res.* 2016;45:D362-D368.
- Hillenmeyer S, Davis LK, Gamazon ER, et al. STAMS: STRING-assisted module search for genome wide association studies and application to autism. *Bioinformatics*. 2016;32:3815-3822.
- 25. Li Y, Goldenberg A, Wong K-C, Zhang Z. A probabilistic approach to explore human miRNA targetome by integrating miRNA-overexpression data and sequence information. *Bioinformatics*. 2013;30:621-628.
- Hanneman RA, Riddle M. Centrality and Power. Introduction to social network methods (pp. 60-76). University of California Riverside, 2005.
- Hillje R, Pelicci PG, Luzi L. Cerebro: interactive visualization of scRNA-seq data. *Bioinformatics*. 2019;36:2311-2313.
- Yashavantha Rao HC, Jayabaskaran C. The emergence of a novel coronavirus (SARS-CoV-2) disease and their neuroinvasive propensity may affect in COVID-19 patients. *J Med Virol.* 2020;92:786-790.
- Xu J, Lazartigues E. Expression of ACE2 in human neurons supports the neuroinvasive potential of COVID-19 virus [published online ahead of print July 4, 2020]. *Cell Molec Neurobiol.* doi:10.1007/s10571-020-00915-1.
- Freni F, Meduri A, Gazia F, et al. Symptomatology in head and neck district in coronavirus disease (COVID-19): a possible neuroinvasive action of SARS-CoV-2. *Am J Otolaryngol.* 2020;41:102612.
- Mukerji SS, Solomon IH. What can we learn from brain autopsies in COVID-19? Neurosci Lett. 2021;742:135528.
- Matschke J, Lütgehetmann M, Hagel C, et al. Neuropathology of patients with COVID-19 in Germany: a post-mortem case series. *Lancet Neurol.* 2020;19: 919-929.
- 33. Lee M-H, Perl DP, Nair G, et al. Microvascular injury in the brains of patients with Covid-19. *N Engl J Med*. 2020;384:481-483.
- Huang C, Wang Y, Li X, et al. Clinical features of patients infected with 2019 novel coronavirus in Wuhan, China. *Lancet.* 2020;395:497-506.
- Zhang C, Wu Z, Li J-W, Zhao H, Wang G-Q. The cytokine release syndrome (CRS) of severe COVID-19 and Interleukin-6 receptor (IL-6R) antagonist Tocilizumab may be the key to reduce the mortality. *Int J Antimicrob Agents*. 2020;2020:105954.
- 36. Xu Z, Shi L, Wang Y, et al. Pathological findings of COVID-19 associated with acute respiratory distress syndrome. *Lancet Respir Med*. 2020;8:420-422.
- Beck BR, Shin B, Choi Y, Park S, Kang K. Predicting commercially available antiviral drugs that may act on the novel coronavirus (SARS-CoV-2) through a drug-target interaction deep learning model. *Comput Struct Biotechnol J.* 2020;18:784-790.
- Ekins S, Mottin M, Ramos PRPS, et al. Déjà vu: stimulating open drug discovery for SARS-CoV-2. Drug Discov Today. 2020;25:928-941.
- Arshad U, Pertinez H, Box H, et al. Prioritisation of potential anti-sars-cov-2 drug repurposing opportunities based on ability to achieve adequate target site concentrations derived from their established human pharmacokinetics [published online ahead of print April 22, 2020]. *medRxiv*. doi:10.1101/2020.04.16.20068379.
- Fintelman-Rodrigues N, Sacromento CQ, Lima CR, et al. Atazanavir inhibits SARS-CoV-2 replication and pro-inflammatory cytokine production. *Biorxiv*. 2020;64:e00825-20.
- Mohapatra S, Nath P, Chatterjee M, et al. Repurposing therapeutics for COVID-19: rapid prediction of commercially available drugs through machine learning and docking. *PLoS ONE*. 2020;15:e0241543.
- 42. Watari I, Oka S, Tanaka S, et al. Effectiveness of polaprezinc for low-dose aspirin-induced small-bowel mucosal injuries as evaluated by capsule endoscopy: a pilot randomized controlled study. *BMC Gastroenterol.* 2013;13:108.
- Yoshikawa T, Naito Y, Tanigawa T, Yoneta T, Kondo M. The antioxidant properties of a novel zinc-carnosine chelate compound, N-(3-aminopropionyl)-Lhistidinato zinc. *Biochim Biophys Acta*. 1991;1115:15-22.
- Naito Y, Yoshikawa T. Molecular and cellular mechanisms involved in Helicobacter pylori-induced inflammation and oxidative stress. *Free Radical Biol Med.* 2002;33:323-336.
- Omatsu T, Naito Y, Handa O, et al. Reactive oxygen species-quenching and anti-apoptotic effect of polaprezinc on indomethacin-induced small intestinal epithelial cell injury. *J Gastroenterol.* 2010;45:692-702.
- Choi HS, Lim J-Y, Chun JH, et al. The effect of polaprezinc on gastric mucosal protection in rats with ethanol-induced gastric mucosal damage: comparison study with rebamipide. *Life Sci.* 2013;93:69-77.
- Ueda K, Ueyama T, Oka M, Ito T, Tsuruo Y, Ichinose M. Polaprezinc (Zinc L-Carnosine) is a potent inducer of anti-oxidative stress enzyme, Heme Oxygenase (HO)-1 — a new mechanism of gastric mucosal protection. *J Pharmacol Sci.* 2009;110:285-294.
- Naito Y, Yoshikawa T, Yagi N, et al. Effects of polaprezinc on lipid peroxidation, neutrophil accumulation, and TNF-alpha expression in rats with aspirininduced gastric mucosal injury. *Dig Dis Sci.* 2001;46:845-851.
- Batra N, De Souza C, Batra J, Raetz AG, Yu AM. The HMOX1 pathway as a promising target for the treatment and prevention of SARS-CoV-2 of 2019 (COVID-19). *Int J Molec Sci.* 2020;21:6412.