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Elucidating SARS-CoV-2 neurotropism: a comprehensive Mendelian randomization study on cerebrospinal fluid biomarkers and their relevance to COVID-19 neurological manifestations

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Abstract

A mendelian randomization (MR) analysis was conducted to investigate whether SARS-CoV-2 invaded the human nervous system. This was confirmed by an increase in biomarkers found in the cerebrospinal fluid (CSF) and plasma of COVID-19 patients. To confirm the neuroinvasive properties of SARS-CoV-2, a series of analyses were conducted utilizing accessible datasets by MR. In addition, external validation was conducted by testing specific proteins in a retrospective cohort study, which included 40 COVID-19 patients with neurological complications and 15 disease controls (DC). Our investigation revealed the hospitalization, severity of COVID-19 increased the area and volume of certain brain regions, but no other significant causal effects were found of brain imaging-derived phenotypes (IDPs) on COVID-19. Notably, the COVID-19 hospitalization significantly increased the area and volume of the left caudal middle frontal gyrus (p_fdr = 0.012; p_fdr = 0.012, respectively). Additionally, COVID-19 severity was linked to the area, volume of the right caudal anterior-cingulate cortex and the volume of the right cuneus cortex (p_fdr = 0.023; p_fdr = 0.025; p_fdr = 0.026, respectively). In the CSF of COVID-19 patients, the median level of CHI3L1 was significantly higher (13677 pg/mL) compared to the DC group (8421 pg/mL, $p < 1.00E-04$). Similar trends were also found in CSF KLK6 and NGF-β. Additionally, the median NRGN level in plasma was significantly higher in the COVID-19 group (1013.00 pg/mL) compared to the control group (360.00 pg/mL, $p = 6.50E-03$). A subgroup analysis demonstrated that COVID-19 patients experiencing moderate to critical symptoms exhibited higher levels of GFAP in their CSF compared to those without. Elevated CSF levels of GFAP and S100B were also found in COVID-19

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patients with decreased consciousness and comorbidities. This MR analysis provided evidence that SARS-CoV-2 may invade the human nervous system, as indicated by the increased levels of CSF biomarkers CHI3L1, NGF-β, and KLK6 in COVID-19 patients. These findings suggested that neuroinflammation could be a potential mechanism underlying the neurological complications seen in COVID-19 patients.

Keywords Mendelian randomization, COVID-19, Neurological complications, CSF, Neuroinflammation.

Introduction

Infection with severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) could lead to coronavirus disease 2019 (COVID-19), which could not only cause respiratory symptoms but could also result in neurological complications [1]. Approximately 30% of the COVID-19 patients experienced neurologic symptoms, including headache, encephalitis, cerebrovascular disorders, smell or taste impairment, skeletal muscle symptoms [2]. However, the pathophysiological mechanism behind these COVID-19-related neurological complications remained unclear.

To address this issue, we conducted a mendelian randomization (MR) analysis to investigate the potential neuroinvasive capacity of SARS-CoV-2 into the human nervous system. It had been demonstrated in previous research that brain inflammation may be a contributing factor to these neurological symptoms. A number of potential explanations for these manifestations had been put forth, including the direct invasion of SARS-CoV-2 into the brain via retrograde neuronal or the bloodstream, as well as cerebral ischemia and neuronal fusion induced by the virus [3, 4].

A considerable number of studies had investigated the distribution of cytokines and other proteins in COVID-19 patients, yet the findings had been inconsistent. In additional, the majority of these studies focused on serum or plasma samples, with a paucity of analysis of conducted on cerebrospinal fluid (CSF) [5–14]. Consequently, we conducted a MR analysis to ascertain whether SARS-CoV-2 invaded the human nervous system. This hypothesis was corroborated by an increase in biomarkers identified in the CSF and plasma of COVID-19 patients during Omicron break in China.

Methods

Data sources

Brain imaging-derived phenotypes (IDPs) data

The design of our study was illustrated in Fig. 1, created by BioGDP.com [15]. Summary statistics for the genome-wide association study (GWAS) of brain imaging-derived phenotypes (IDPs) were sourced from the Oxford Brain Imaging Genetics (BIG40, https://open.win.ox.ac.uk/ukbiobank/big40/), comprising data from 33,224 individuals of European ancestry within the UK Biobank [16]. A total of 587 brain IDPs were retained, as detailed in prior descriptions [17].

COVID-19 data

Summary GWAS statistics for COVID-19 phenotypes (in individuals of European ancestry (EUR)) were retrieved from the COVID-19 Host Genetics Initiative (https://www.covid19hg.org, Release 7). Our study included three COVID-19 phenotypes: susceptibility, hospitalization, and severity.

CSF and plasma protein quantitative trait loci (pQTLs)

Data on CSF protein quantitative trait loci (pQTLs) were obtained from a study by Yang et al., which identified several CSF pQTLs [18]. The plasma pQTLs data were sourced from Zheng et al., who integrated data from five previously published GWAS [19]. For the primary analysis, multiple testing was controlled by Bonferroni correction with a significance threshold of $P < 5.63E-05$(0.05/888, 888 representing the number of analyzed pQTLs). For the further validation, a P value threshold of 0.05 was applied. And the plasma pQTLs data retrieved from deCODE Genetics, which generated whole genome sequencing in 49,708 Icelanders [20]. Additional pQTLs for CSF and plasma were obtained from the Online Neurodegenerative Trait Integrative Multi-Omics Explorer (ONTIME, https://ontime.wustl.edu) for further valid ation. Only pQTLs meeting the following criteria were included: (i) a genome-wide significant association ($p < 5$ E-08); (ii) location outside the major histocompatibility complex (MHC) region; (iii) showed independent association [linkage disequilibrium (LD) clumping $r^2 < 0.001$].

Mendelian randomization analysis

Selection of instrumental variables (IVs)

Genetic variants serving as instrumental variables (IVs) were selected according to three principal assumptions: (1) a robust association with the exposure, (2) independence from confounding variables, and (3) influence on the outcome solely via the exposure. IVs were chosen based on genome-wide significance ($p < 5E-08$) and weak IVs were excluded by calculating the F-statistics ($F < 10$). To ensure independence, LD testing was performed for each IV ($r^2 < 0.001$) using European ancestry samples from the 1000 Genomes Project. In cases, proxy single nucleotide polymorphisms (SNPs) with high LD ($r^2 > 0.8$) were utilized.

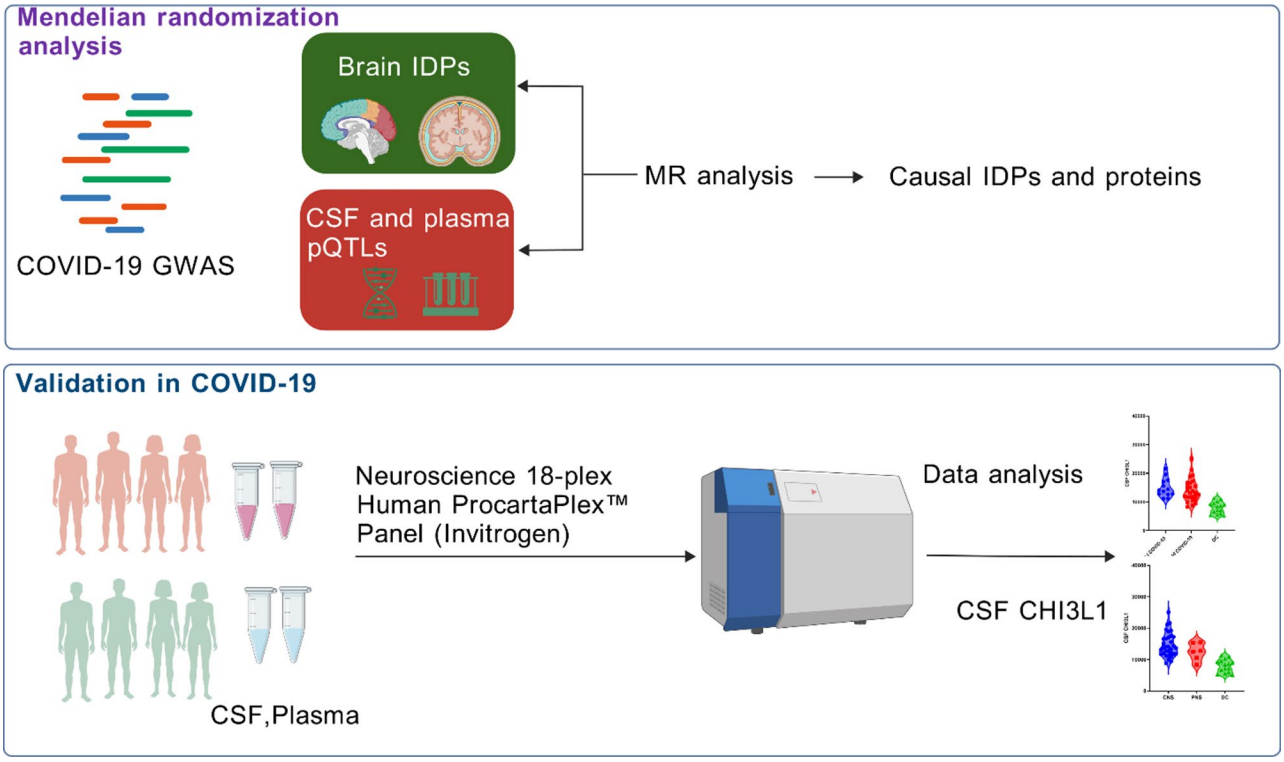

Fig. 1 The overview of our study. The identification of casual IDPs and proteins in COVID-19 by mendelian randomization analysis. The validation of biomarkers in in the CSF and plasma of COVID-19 patients

Main methods

Multiple complementary methods were applied, including inverse variance-weighted (IVW), MR-Egger, weighted median, simple mode, weighted mode, and Wald ratio methods. The IVW model served as the primary method, while the Wald ratio method was employed when only one SNP was available. Cochran's Q statistic ($p < 0.05$ indicating heterogeneity) was used for heterogeneity among IVs. A random-effects model was used in the case of heterogeneity; and a fixed-effects model was used otherwise. Genes exhibiting significant pleiotropy were adjusted, and outlier SNPs were excluded. Leave-one-out tests were performed to evaluate the influence of individual SNPs [21–25]. After applying the false discovery rate (FDR) correction, a significance level of p_fdr < 0.05 was considered statistically significant.

Enrollment of patients and disease controls

We enrolled 40 COVID-19 patients exhibiting neurological symptoms and 15 disease controls (DC) from Peking Union Medical College Hospital between December 2022 and August 2023. All participants in the external validation were ethnically Chinese and unrelated. Additionally, the data used for MR analysis were derived exclusively from individuals of European ancestry. Importantly, the European GWAS dataset and the Chinese validation cohort were entirely separate populations, ensuring no overlap or interference between the two analyses. COVID-19 diagnoses adhered to the "Diagnosis and Treatment Protocol for Novel Coronavirus Pneumonia (Trial Version 10)" [26], with infections confirmed through RT-PCR, serological, or antigen tests. Exclusion criteria included non-neurological COVID-19, cancer, primary neurological diseases, and autoimmune diseases. DCs had cerebral hemorrhage or infarction but no recent SARS-CoV-2 infection. Plasma and cerebrospinal fluid (CSF) samples were collected. However, healthy controls were not included due to the invasive nature of lumbar punctures. The study received approval from the Ethics Committee of Peking Union Medical College Hospital (I-23PJ292), with a waiver for informed consent.

Protein detection in COVID-19 patients and disease controls

Protein levels of Tau, pT181 (a phosphorylated form of tau protein), Amyloid beta 1–42, Neurogranin (NRGN), Neurofilament heavy (NF-H), NCAM-1, Kallikrein-6 (KLK6), TDP-43, FGF-21, Glial Fibrillary Acidic Protein (GFAP), UCHL1, S100B, GDNF, CNTF, BDNF, NGF-β, MIG, and CHI3L1 were quantified using the Neuroscience 18-plex Human ProcartaPlex™ Panel (Invitrogen), based on Luminex xMAP technology, following the manufacturer's protocol.

Statistical analysis

All statistical analyses were performed using R version 4.3.1 (TwoSampleMR packages), GraphPad Prism version 9, MATLAB R2023a, and Adobe Illustrator 2023. Given the non-normal distribution of the data, results were presented as medians with interquartile ranges (IQR). Differences between the COVID-19 and DC groups were assessed using the Mann-Whitney U-test, with statistical significance set at $p < 0.05$.

Results

Bidirectional MR analysis of the effects between IDPs and COVID-19

Figure 2 showed the results of MR analysis investigating the effects of COVID-19 phenotype (susceptibility, hospitalization, and severity) on IDPs. The analysis revealed that COVID-19 hospitalization significantly increased the area and volume of the left caudal middle frontal gyrus (OR = 1.27, 95% CI 1.14–1.42, p_fdr = 0.012; OR = 1.27, 95% CI 1.13–1.42, p_fdr = 0.012, respectively). Additionally, COVID-19 severity was linked to the area, volume of the right caudal anterior-cingulate cortex and the volume of the right cuneus cortex (OR = 1.25, 95% CI 1.12–1.39, p_fdr = 0.023; OR = 1.23, 95% CI 1.11–1.37, p_fdr = 0.025; OR = 1.23, 95% CI 1.10–1.36, p_fdr = 0.026, respectively). No other significant causal effects were found between COVID-19 and IDPs.

Screening the proteome for COVID-19 causal proteins

Decreased plasma ICAM1 increased the risk of COVID-19 hospitalization (OR = 0.96, 95% CI 0.95–0.98, $p = 5.34E-07$), COVID-19 severeness (OR = 0.93, 95% CI 0.91–0.95, $p = 2.67E-10$), respectively. The descend of CSF ICAM1 increased the risk of COVID-19 hospitalization (OR = 0.76, 95% CI 0.68–0.84, $p = 5.34E-07$), COVID-19 severeness (OR = 0.60, 95% CI 0.51–0.70, $p = 2.67E-10$), respectively. ONTIME was additional pQTLs for CSF and plasma obtained from the Online Neurodegenerative Trait Integrative Multi-Omics Explorer. The deCODE was plasma pQTLs data retrieved from deCODE Genetics, which generated whole genome sequencing in 49,708 Icelanders. Similar causal effects of plasma ICAM1 on COVID-19 hospitalization and severeness had been observed in ONTIME, respectively. Also, similar causal effects of ICAM1 on COVID-19 severeness had been observed in deCODE (Fig. 3).

In addition, we also extracted the plasma and CSF of available proteins for MR analysis. CHI3L1, kallikrein-6, NGF-β, NRGN and amyloid beta 1–42 were also included as these five proteins were significantly different in our study with detailed results followed. Increased CSF CHI3L1 increased the risk of COVID-19 (OR = 1.01, 95% CI 1.00–1.02, $p = 0.034$) in ONTIME. And there was causal effect between plasma CHI3L1 and COVID-19 infection ($p = 0.042$). While no other significant causal effects were depicted.

Distribution of special proteins between COVID-19 and DC

We analyzed the levels of 18 proteins in CSF and plasma of COVID-19 patients with neurological symptoms, compared to DC. Significant differences were observed in four proteins in the CSF and one in the plasma (Table 1; Fig. 4). The median level of CHI3L1 in CSF was significantly higher in the COVID-19 (13677 pg/mL, IQR: 11773–16711 pg/mL) compared to DC (8421 pg/mL, IQR: 5960–9510 pg/mL, $p < 1.00E-04$). Similarly, the median KLK6 level in the CSF was significantly higher in the COVID-19 group (46947 pg/mL, IQR: 39243–57080 pg/mL) than in the DC group (12186 pg/mL, IQR: 12186–12186 pg/mL, $p < 1.00E-04$). In addition, the median level of NGF-β in CSF was significantly elevated in the COVID-19 (1.20 pg/mL, IQR: 1.32–1.68 pg/mL) compared to the DC (0.42 pg/mL, IQR: 0.00-1.12 pg/mL, $p < 1.00E-04$). In the plasma, the median NRGN level was also significantly higher in COVID-19 (1013.00 pg/mL, IQR: 243.10–1726.00 pg/mL) than that in the DC (360.00 pg/mL, IQR: 74.94–360.00 pg/mL, $p = 6.50E-03$). However, the median amyloid beta 1–42 level in CSF was

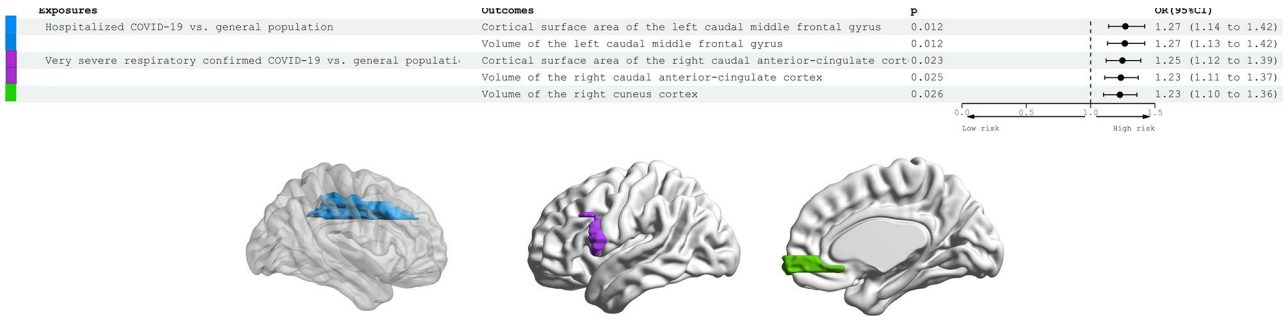

Fig. 2 The results of MR analysis about the effects of COVID-19 phenotype (hospitalization, severity) on IDPs. The COVID-19 hospitalization significantly increased the area and volume of the left caudal middle frontal gyrus (OR = 1.27, 95% CI 1.14–1.42, p_fdr = 0.012; OR = 1.27, 95% CI 1.13–1.42, p_fdr = 0.012, respectively). Additionally, COVID-19 severity was linked to the area, volume of the right caudal anterior-cingulate cortex and the volume of the right cuneus cortex (OR = 1.25, 95% CI 1.12–1.39, p_fdr = 0.023; OR = 1.23, 95% CI 1.11–1.37, p_fdr = 0.025; OR = 1.23, 95% CI 1.10–1.36, p_fdr = 0.026, respectively)

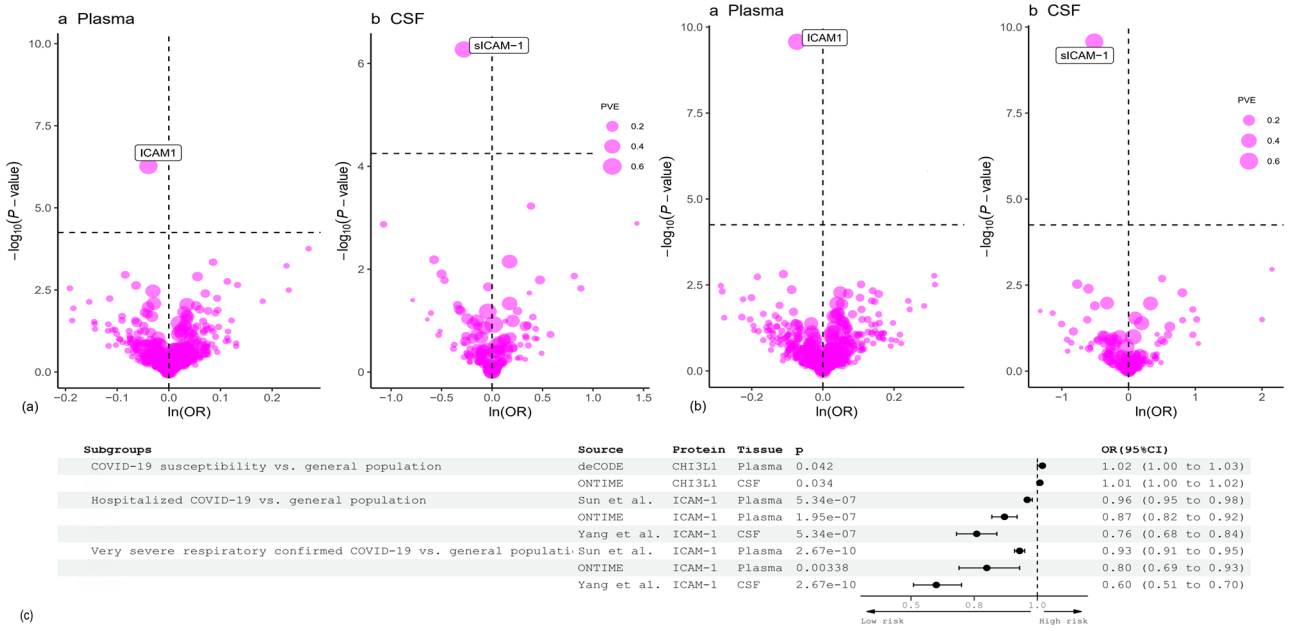

Fig. 3 MR results for plasma and CSF proteins and the risk of COVID-19 phenotype. Volcano plots of the MR results for the effects of plasma and CSF proteins on (**a**) COVID-19 hospitalization and (**b**) COVID-19 severity. Dashed horizontal black line corresponded to $P = 5.63$ E-05 (0.05/888). PVE = proportion of variance explained. (**c**): forest plot of the external validation of the causal relationship between CHI3L1, ICAM-1 and COVID-19 phenotype (susceptibility, hospitalization, and severity)

significantly lower in the COVID-19 (43.89 pg/mL, IQR: 18.66–80.75 pg/mL) compared to the DC (83.36 pg/mL, IQR: 31.48–144.80 pg/mL, $p = 0.033$).

Subgroup analysis of special proteins between COVID-19 and DC

COVID-19 patients in the severe or critical condition group had significantly higher CSF GFAP levels compared to those with milder cases ($p = 0.020$). Higher GFAP levels were also found in COVID-19 patients who experienced decreased consciousness or had comorbidities, compared to those without these conditions ($p = 9.52$E-03 and $p = 4.74$E-03, respectively). Similarly, higher CSF S100B protein levels were found in patients with decreased consciousness or comorbidities ($p = 0.021$ and $p = 0.010$, respectively). Patients with decreased consciousness also showed significantly higher levels of NF-H (neurofilament heavy chain) in the CSF compared to those without ($p = 7.49$E-03). Additionally, COVID-19 patients with comorbidities had higher levels of pT181 in the CSF ($p = 0.014$). For patients with central nervous system (CNS) involvement, CSF Tau protein levels were significantly elevated compared to those without CNS involvement ($p = 1.97$E-03). However, the opposite trend was found in patients with weakness or numbness, who showed lower levels of Tau compared to those without these symptoms ($p = 0.013$ and $p = 1.03$E-03, respectively). COVID-19 patients with numbness also showed decreased levels of NRGN and NGF-β in the CSF compared to those without numbness ($p = 0.015$ and $p = 0.033$, respectively).

Discussion

We analyzed the distribution of several biomarkers in the CSF and plasma of COVID-19 patients with neurological complications during the Omicron wave in China. Additionally, a bio-informational analysis by MR provided evidence that SARS-CoV-2 could invade the human nervous system. The finding suggested that neuroinflammation could be a key mechanism underlying the neurological complications observed in COVID-19 patients.

Our MR results demonstrated a causal relationship between different COVID-19 phenotypes and changes in brain structures. These include alterations in the area and volume of the left caudal middle frontal gyrus, which were associated with cognitive and emotional functions; the area and volume of the right caudal anterior-cingulate cortex, primarily involved in cognitive and emotional processes and the volume of the right cuneus cortex, played a crucial role in visual processing [27]. A multimodal meta-analysis of neuroimaging studies indicated that COVID-19 can lead to both functional and structural changes in the brain, particularly in areas such as the temporal lobe, orbital frontal cortex, and the cerebellum. There were also alterations noted in the insula and the limbic system, which are regions critical for emotional regulation and memory [28]. These findings supported previous research that suggests SARS-CoV-2 could

Table 1 Detailed information of COVID-19 and DC group

Characteristics	COVID-19	DC
Demographic characteristics		
Number	40	15
Mean age ± SD	50.70 ± 23.14	33.87 ± 18.52
Male/Female	1.11	2.00
Clinical syndrome of neurological diseases (%)		
Encephalopathy	18(45.00)	--
Guillain-Barre syndrome	5(12.50)	--
Myelitis	4(10.00)	--
Cerebellar ataxia	3(7.50)	--
Encephalitis	2(5.00)	--
Epileptic seizures	10(25.00)	--
Brainstem encephalitis	2(5.00)	--
Acute ischemic stroke	2(5.00)	--
Intracranial hypertension	1(2.50)	--
Myelopathy	1(2.50)	--
Neuropathy	1(2.50)	--
Conus medullaris syndrome	1(2.50)	--
Clinical manifestation of nervous system (%)		
Decreased level of consciousness	13(32.50)	--
Psychiatric symptoms	8(20.00)	--
Epileptic seizures	10(25.00)	--
Cognitive decline	6(15.00)	--
Ataxia	3(7.50)	--
Limb weakness	10(25.00)	--
Limb numbness	10(25.00)	--
Headache	5(12.50)	--
Hyponatremia	6(15.00)	--
COVID-19 severity status (%)		
Mild	27(67.50)	--
Moderate	6(15.00)	--
Severe	4(10.00)	--
Critical	3(7.50)	--
Comorbidity (%)		
Chronic kidney disease	4(10.00)	--
Chronic liver disease	3(7.50)	--
Coronary heart disease	3(7.5)	--
Diabetes	6(15.00)	--
Hypertension	12(30.00)	--
Lung disease	3(7.5)	--
Miocardial infarction	4(10.00)	--
Symptoms on admission (%)		
Fever (temperature ≥ 37.3 ℃)	32(80.00)	--
Cough or Sputum	4(10.00)	--
Pharyngalgia	12(30.00)	--
Diarrhea	2(5.00)	--
Days of onset neurological symptoms after SARS-COV2 infection, median(IQR)	8(3-23.75)	--
Laboratory findings on admission, median(IQR)		
White blood cell count, 10^9/L	8.91(7.33-12.00)	5.58(5.21–9.75)
Total neutrophil count, 10^9/L	6.30(4.44–9.64)	3.21(2.49–6.02)
Total lymphocyte count, 10^9/L	1.45(0.83–2.47)	1.71(1.48–2.26)
Platelets, 10^9/L	230.50(180.30-282.50)	197.00(160.00-286.00)
Hemoglobin, g/L	137.50(121.30–145.00)	146.00(120.00-155.00)
Prothrombin time, seconds	12.00(11.43–12.80)	11.70(11.30–12.90)

Table 1 (continued)

Characteristics	COVID-19	DC
APTT, seconds	26.45(24.20-29.23)	26.90(25.50–30.70)
TT, seconds	16.50(15.58–17.18)	16.60(16.10–17.30)
Fibrinogen, g/L	3.36(2.73–4.59)	2.66(2.17–4.31)
D-dimer, mg/L FEU	0.97(0.31–2.95)	0.15(0.15–0.86)
High-sensitivity CRP, mg/L^	5.60(0.65–35.65)	0.50(0.50–3.26)
ESR, mm/h*	15.50(6.00-41.25)	2.00(1.00–11.00)
Total cell count in CSF, 10^6/L	2.50(2.00-65.75)	4.00(1.00-1501.00)
White blood cell count in CSF, 10^6/L	5.50(2.00-5.50)	2.00(0.00–5.00)
Mononuclear cell count in CSF, 10^6/L	1.00(0.00-2.75)	2.00(0.00–5.00)
Multiple nuclear cells count in CSF, 10^6/L	0.00(0.00-1.75)	0.00(0.00–2.00)
Glucose in CSF, mmol/L	3.90(3.33–5.23)	3.50(3.30–4.50)
Chlorides in CSF, mmol/L	126.00(123.00-129.30)	125.00(124.00-126.00)
Protein in CSF, g/L	0.42(0.28–0.93)	0.48(0.41–0.72)
IL-6 in CSF, pg/mL[&]	7.55(2.73-35.00)	3.65(2.18–4.83)
IgG synthesis rate[#]	-3.35(-4.85-2.40)	--
Abnormal Alb CSF/Alb Serum(%)[#]	5(26.32)	--
Abnormal IgG CSF/IgG Serum (%)[@]	16(66.67)	--
Positive IgG oligoclonal band in CSF(%)[@]	7(29.17)	--
Positive IgG oligoclonal band in Serum(%)[@]	4(16.67)	--
Positive specific IgG oligoclonal band in CSF(%)[@]	4(16.67)	--
CSF Amyloid beta 1–42 pg/mL	43.89(18.66–80.75)	83.36 (31.48–144.80)
CSF CHI3L1 pg/mL	13,677 (11773–16711)	8421(5960–9510)
CSF KLK6 pg/mL	46,947(39243–57080)	12,186(12186–12186)
CSF NGF-β pg/mL	1.20(1.32–1.68)	0.42(0.00-1.12)
Plasma NRGN pg/mL	1013.00(243.10–1726.00)	360.00(74.94–360.00)
CSF GFAP in mild COVID-19 pg/mL	2872.35(1308.38-3620.52)	525.66(398.80-1546.02)
CSF GFAP in non-mild COVID-19 pg/mL	1064.04(540.20-1805.94)	
CSF S100B in decreased consciousness COVID-19 pg/mL	7.14(3.10-19.28)	3.46(2.69–6.02)
CSF S100B in consciousness COVID-19 pg/mL	3.21(1.90–6.82)	
CSF NF-H in decreased consciousness COVID-19 pg/mL	75.18(0.00-212.27)	0.00(0.00-377.85)
CSF NF-H in consciousness COVID-19 pg/mL	0.00(0.00–0.00)	
CSF Tau in CNS COVID-19 pg/mL	377.41(135.09-717.61)	143.60(40.27-385.79)
CSF Tau in PNS COVID-19 pg/mL	98.18(55.91-130.04)	
CSF pT181 in COVID-19 with comorbidities pg/mL	9.55(4.34–14.18)	10.56(5.32–16.54)
CSF pT181 in COVID-19 without comorbidities pg/mL	5.76(1.19–7.29)	
Treatments (%)		
Antiviral therapy	4(10.00)	--
Corticosteroids	20(50.00)	--
Biological agents	5(12.50)	--
Intravenous immunoglobulin	11(27.50)	--
Noninvasive mechanical ventilation	13(32.50)	--
Invasive mechanical ventilation	7(17.50)	--
ECMO	0(0.00)	--

*Only 26 COVID-19 patients with ESR, [&]20 COVID-19 patients with IL-6 in CSF, [#]19 COVID-19 patients with IgG synthesis rate, Alb CSF/Alb Serum, [@]24 COVID-19 patients with IgG CSF/IgG Serum, IgG oligoclonal band in CSF, IgG oligoclonal band in Serum, specific IgG oligoclonal band in CSF were available. ^Only 11 DC with high-sensitivity CRP, *7 DC with ESR, [&]6 DC with IL-6 in CSF were available.

cause nerve damage. This was consistent with reports of COVID-19 patients experiencing neurological symptoms such as headaches, impaired consciousness, acute cerebrovascular events, and epilepsy. The findings suggested that SARS-CoV-2 may invade the nervous system through mechanisms such as: disrupting normal CNS function via cytokine storms; inducing cerebral ischemia or hypoxia, leading to neurological complications.

The special proteins could be divided into two categories, one group was the biomarkers of neuroinflammatory or neuro-injury, including CHI3L1,NGF-β,KLK6,NRGN, GFAP, S100B and NF-H; the other group was those of

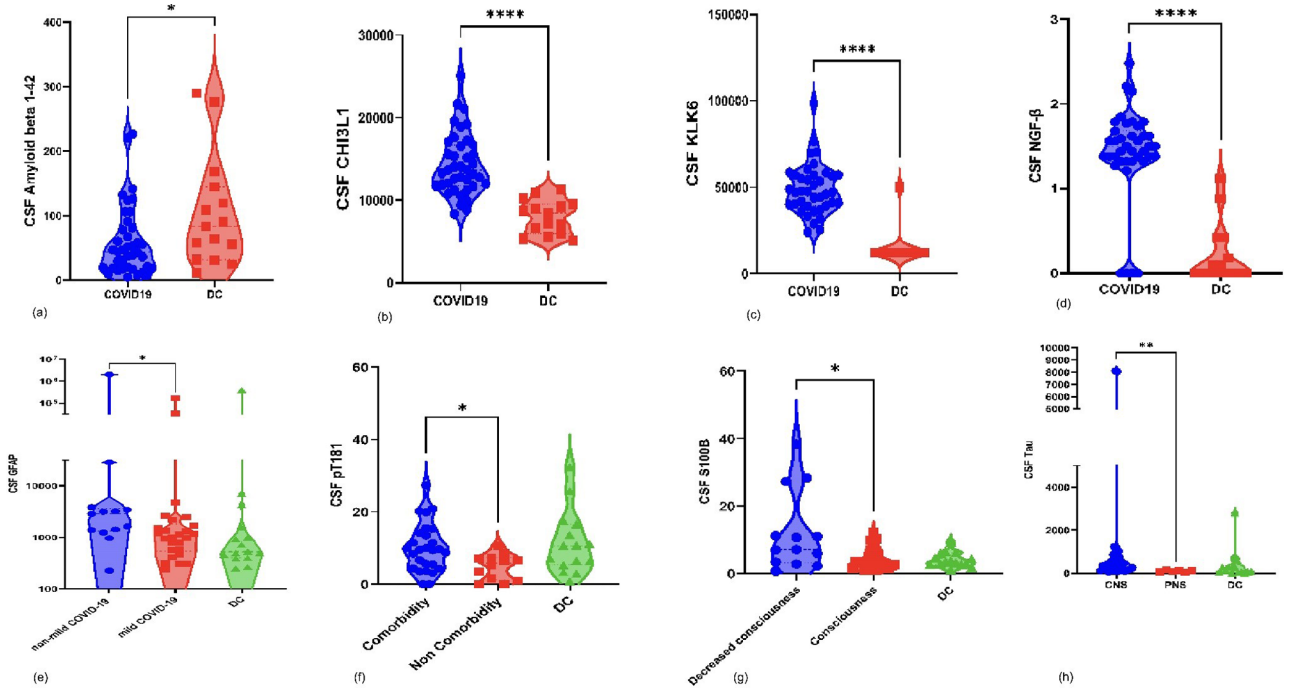

Fig. 4 The distribution of special proteins between COVID-19 and DC. (**a**): the median amyloid beta 1–42 level in CSF was significantly lower in the COVID-19 (43.89 pg/mL) compared to the DC (83.36 pg/mL, $p = 0.033$). (**b**): The median level of CHI3L1 in CSF was significantly higher in the COVID-19 (13677 pg/mL) compared to DC (8421 pg/mL, $p < 1.00E-04$). (**c**): the median KLK6 level in the CSF was significantly higher in the COVID-19 group (46947 pg/mL) than in the DC group (12186 pg/mL, $p < 1.00E-04$). (**d**): the median level of NGF-β in CSF was significantly elevated in the COVID-19 (1.20 pg/mL) compared to the DC (0.42 pg/mL, $p < 1.00E-04$). (**e**): COVID-19 patients in the severe or critical condition group had significantly higher CSF GFAP levels compared to those with milder cases ($p = 0.020$). (**f**): COVID-19 patients with comorbidities had higher levels of pT181 in the CSF ($p = 0.014$). (**g**): higher CSF S100B protein levels were found in patients with decreased consciousness ($p = 0.021$). (**h**): for patients with central nervous system (CNS) involvement, CSF Tau protein levels were significantly elevated compared to those without peripheral nervous system (PNS) involvement ($p = 1.97E-03$). * p:0.01–0.05; ** p:0.001–0.01; ***p: 1.00E-04-0.001, ****$p < 1.00E-04$

neurodegenerative, for instance: Amyloid beta 1–42, Tau. CHI3L1 was a glycoprotein involved in inflammation, endothelial dysfunction, and tissue remodeling. Within the CNS, CHI3L1 was linked to neuroinflammatory processes and reactive gliosis. CHI3L1 levels were found to be higher in COVID-19 patients compared to controls [6, 8]. Additionally, elevated serum CHI3L1 level have been associated with more severe cases of COVID-19 [10, 11]. CHI3L1 played a role in the pathogenesis of COVID-19 by increasing the expression of angiotensin-converting enzyme 2 (ACE2), which facilitated viral entry, and the viral spike protein in pulmonary and vascular cells [29]. However, previous studies primarily focused on serum CHI3L1 in COVID-19 patients, with no research specifically addressing its role in the CSF of COVID-19 patients with neurological complications [5–14]. In our study, we found that CHI3L1 levels in the CSF were significantly higher in COVID-19 patients compared to disease controls (DC). There was also a trend suggesting that CHI3L1 levels were higher in COVID-19 patients with various clinical syndromes, though these differences were not statistically significant. We also observed elevated levels of NGF-β and KLK6 in the CSF of COVID-19 patients. NGF-β was a neuropeptide that contributes to neurogenic inflammation by activating immune cells through proinflammatory cytokines. It had been shown to be effective in inducing both pain and neuroinflammation [30]. KLK6 was a serine protease that was abundantly expressed in the brain and spinal cord, and its expression in CNS diseases was varied [31]. NRGN was a regulatory molecule found in the brain, specifically in dendritic spines, where it played a role in synaptic plasticity by linking protein kinase C and calcium/calmodulin signaling pathways [32]. In our study, NRGN levels were elevated in the plasma of COVID-19 patients compared to DCs. GFAP is a protein released during microglial dysregulation and is involved in tissue healing processes during neuroinflammation and neurodegeneration. Recent studies have suggested that serum GFAP may be a potential biomarker for various neurological complications, particularly during the acute phase of COVID-19 when systemic inflammation was at its peak [13, 33]. Our findings supported this, as we observed higher CSF GFAP levels in COVID-19 patients with moderate to severe disease, as well as in those with decreased consciousness or comorbidities. Similarly, S100B was a

well-known biomarker used to assess brain damage and blood-brain barrier integrity. It was commonly employed for risk assessment in cases of mild brain injury [34]. Our study found higher CSF S100B levels in COVID-19 patients with decreased consciousness and comorbidities compared to those without these conditions. Neurofilaments, including NF-H (the heaviest neurofilament), are structural proteins in neurons, and their levels are often elevated in cases of neurodegeneration or injury [35]. Amyloid beta 1–42, a key protein in the development of Alzheimer's disease, played a critical role in the pathogenesis of the disease. It had been found to bind to various viral proteins, particularly the spike protein of SARS-CoV-2 and the ACE2 receptor. In our study, we observed that the median level of amyloid beta 1–42 in the CSF was significantly lower in COVID-19 patients, consistent with previous research findings [36].

However, our study had several limitations. First, while we applied multiple robust MR methods to assess different types of pleiotropic effects, we could not fully eliminate the influence of potential confounding factors on the MR results. Second, our analysis relied solely on GWAS of European ancestry populations. Despite the large sample size of this dataset, caution was required when extrapolating these findings to other ethnic groups. Third, our MR analysis did not account for certain factors that influenced COVID-19 hospitalization and severity, such as differences in healthcare systems, treatment protocols, or socioeconomic conditions across countries. Fourth, validation of our findings was limited to a single hospital cohort of 40 COVID-19 patients, which lacked follow-up data and broader demographic diversity. Further large-scale studies with multiethnic cohorts and longitudinal designs were needed to confirm and expand upon these results. Additionally, aside from direct CNS invasion by SARS-CoV-2, research had also shown that brain inflammation and cytokine storms may contribute to neurological symptoms [1]. The precise mechanisms linking SARS-CoV-2 infection to neurological complications remained unclear. Future experimental studies were necessary to elucidate these pathways.

Conclusions

In conclusion, MR analysis provided evidence that SARS-CoV-2 may invade the human nervous system. This was validated through the analysis of several biomarkers in the CSF and plasma of COVID-19 patients with neurological complications during the Omicron wave in China. Our findings suggest that neuroinflammation may be a key mechanism underlying these neurological complications.

Author contributions

ZYW and HLX wrote the main manuscript text, SLZ and YZL reviewed the manuscript. Professor. Yongzhe Li had full access to all the data in the study and takes responsibility for the integrity of the data and the accuracy of the data analysis.

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Data availability

No datasets were generated or analysed during the current study.

Declarations

Ethics approval and consent to participate

The study received approval from the Ethics Committee of Peking Union Medical College Hospital (I-23PJ292), with a waiver for informed consent.

Consent for publication

Not applicable.

Competing interests

The authors declare no competing interests.

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