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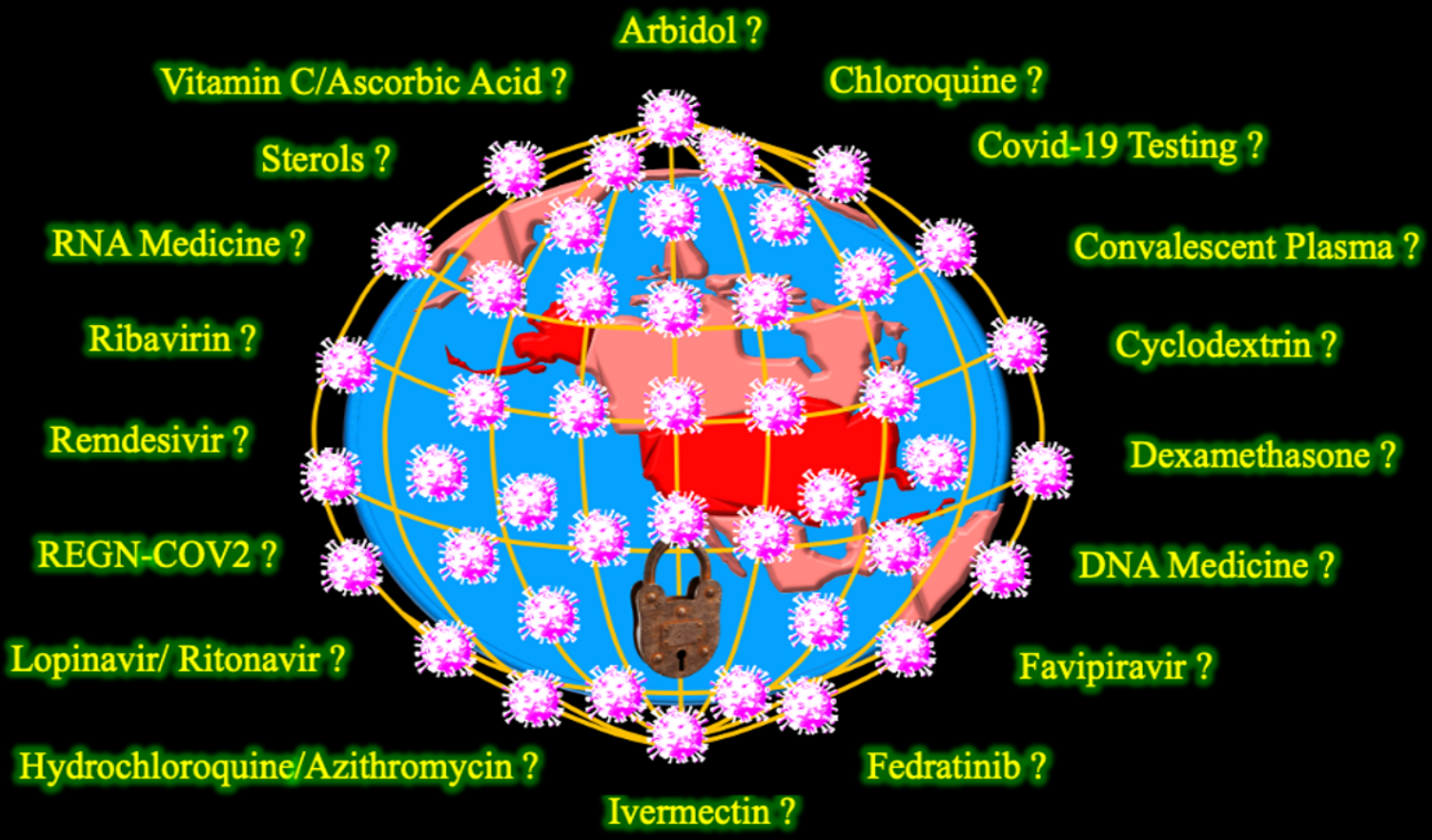

Fear of coronavirus locks down the world and resets activities on the earth

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Review Article

Neuro-COVID-19: a meta-analysis of COVID-19-induced neuroinflammation and its implications

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As the COVID-19 pandemic continues to impact the world, it has become clear that the disease caused by SARS-CoV-2 is not only a respiratory illness. In addition to respiratory symptoms and multiorgan failure, neurological symptoms have also been observed in clinics. Moreover, neurological manifestations, in the form of cognitive deficits, have persisted even in the recovered patients. We therefore hypothesize that COVID-19 causes neuroinflammation, which could in turn contribute to the formation of the neurological symptoms. In the present meta-analysis study, we identified and analyzed the molecules affected by COVID-19 using the bioinformatics tool QIAGEN Ingenuity Pathway Analysis (IPA) to demonstrate possible pathogenic mechanism underlying COVID-19-induced neuroinflammation. Using the Core Analysis and My Pathway tools of IPA, our data demonstrated a strong association between the molecules affected by COVID-19 and the canonical pathway of Neuroinflammation Signaling Pathway, as well as three neurological diseases associated with inflammatory response (Inflammation of central nervous system, Encephalitis and Experimental autoimmune encephalomyelitis). Lipopolysaccharide (LPS) and Interferon gamma (IFNG) were identified to be the top two upstream regulators that may account for the change of the molecules affected by COVID-19. Regulatory networking of LPS and IFGN counteracted with each other. In line with the fact that SARS-CoV-2 binds and downregulates angiotensin-converting enzyme 2 membrane receptor (mACE2), our data also demonstrated that downregulation of mACE2 activated the Neuroinflammation Signaling Pathway, through activation of Nuclear factor kappa B (NFκB)-induced elevation of cytokines and inhibition of IFNγ-induced antiviral response via direct interaction and indirect effects. Together, our meta-analysis has suggested that COVID-19 causes neuroinflammation leading to neurological pathogenesis, via modulating the major inflammatory pathways like NF-κB, IFNγ, Jak-STAT that is observed in the disease.

Introduction

Coronavirus Disease 2019 (COVID-19) pandemic is a severe global health crisis caused by the severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2). As a respiratory illness, COVID-19 exhibits symptoms similar to influenza, including fever, cough, fatigue, sore throat, muscle pain, and headache.[1] Cases of this disease range from mild to severe. Older patients with pre-existing conditions are especially at risk of developing the severe form of the disease. Pre-existing conditions such as hypertension, diabetes mellitus, and any respiratory illness are some of the underlying conditions that have been linked with a poorer prognosis.[1] In severe COVID-19 cases, SARS-CoV-2 infection triggers intensive immune responses with high levels of inflammatory cytokines in blood plasma that is highlighted as "cytokine storm".[2,3] As shown in Fig. 1, via the Spike (S) protein, the SARS-CoV-2 binds to the transmembrane receptor angiotensin-converting enzyme 2 (mACE2), with its co-receptor, the transmembrane serine protease2 (TMPRSS2), to initiate fusion of the viral and plasma membrane into the host cells.[4-7] As any cell surface receptor, mACE2 mediates endocytosis of its receptor-ligand complex. By the receptor-mediated endocytosis, entry of SARS-CoV-2 into the host cell takes place with internalization of mACE2. Inside cytoplasm, virion releases its genomic RNA (gRNA). Host replication and transcription complexes are hijacked to synthesize SARS-CoV-2 RNA and proteins. Viral proteins and RNA are assembled, and new SARS-CoV-2 are released. Internalization of mACE2 leads to downregulation of mACE2 and possible decrease of soluble ACE2 (sACE2) that are known to be released from mACE2.[8,9] With COVID-19, binding of SARS-CoV-2 to its cell surface receptor mACE2 could lead to downregulation of mACE2. Downregulation of mACE2 has been reported to contribute to the severe acute lung injury in SARS.[8,9]

One of the clinical characteristics of the lethal cases of COVID-19 is multi-organ failure. Damage to several organs has been observed in severe cases of the disease, especially those with co-morbidities. Systemic inflammation is believed to be a major factor contributing to this symptom. Inflammatory cellular infiltration, for instance, has been commonly observed in multiple organs of COVID-19 patients, including lung, heart, liver, and kidney, clinical characteristics that suggest that SARS-CoV-2 not only induces direct organ damage, but also aggravates the condition through pro-inflammatory action.[10] Similarly, in a retrospective study analyzing clinical data from 113 deceased patients who died of COVID-19, sepsis was found as a symptom in all patients, who also exhibited elevation of IL 2 receptor, IL6, IL8, IL10, and TNFα in circulation showing that systemic inflammation was a leading factor in the onset of sepsis

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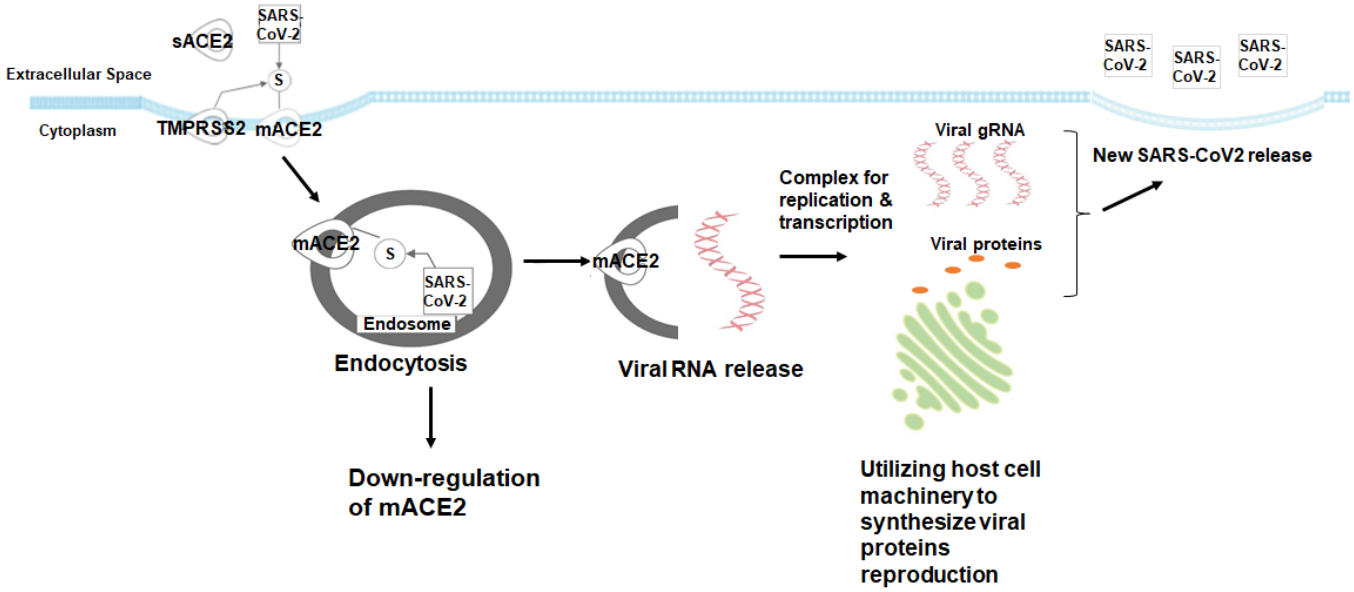

Fig. 1. SARS-CoV2 entry into host cell. Via the viral Spike (S) protein, SARS-CoV-2 binds to the transmembrane receptor angiotensin-converting enzyme 2 (mACE2), with its co-receptor, the transmembrane serine protease2 (TMPRSS2), to initiate fusion of the viral and plasma membrane into the host cells. By the receptor-mediated endocytosis, entry of SARS-CoV-2 into the host cell takes place with internalization of mACE2. Inside cytoplasm, SARS-CoV-2 releases its genomic RNA (gRNA). Host replication and transcription complexes are hijacked to synthesize viral RNA and proteins. Viral proteins and RNA are assembled, and new SARS-CoV-2 are released. Internalization of mACE2 leads to downregulation of mACE2 and possible decrease of soluble ACE2 (sACE2) that is released from mACE2. Binding of SARS-CoV-2 to its cell surface receptor mACE2 could lead to downregulation of mACE2.

of these patients.[11] Recently, ample studies have demonstrated systemic inflammation could lead to neuroinflammation.[12-14]

Patients afflicted by this disease have also manifested neurological symptoms in addition to the various other symptoms described above.[15,16] These neurological manifestations range from fairly specific symptoms, such as loss of sense of smell or taste, myopathy, and stroke, to more nonspecific symptoms, such as headache, depressed level of consciousness, dizziness, seizure,[17,18] along with neurodegeneration, neuroinflammation, and demyelination signs.[19] It has been also reported that some recovered and discharged patients have cognitive deficits, such as dysexecutive syndrome, signs of inattention, disorientation, and poorly organized movements and response.[20,21] The systemic inflammation symptom, called "cytokine storm", observed in COVID-19 has been demonstrated to induce neuroinflammation in mice models.[14] Moreover, downregulation of mACE2 mediated by the binding of the SARS-CoV-2 S protein to the receptor has been linked to the appearance of the "cytokine storm".[22,23] With these premises, we have hypothesized that COVID-19 could cause neuroinflammation leading to neurological manifestations that may be caused by direct infection of SARS-CoV-2 in the CNS or induced by systematic inflammation. In this study, we conducted a network meta-analysis using QIAGEN Ingenuity Pathway Analysis (IPA) and QIAGEN Knowledge Base (QKB) data resource to examine how COVID-19 binding to mACE2 causes neuroinflammation and explore how downregulation of mACE2 enhances neuroinflammation.

IPA is a bioinformatics software that uses computational algorithms to analyze the functional connectivity of genes through QKB, which is a comprehensive repository for manually curated information identified from over seven million individually modeled relationships among diseases, drugs, genes, proteins, and metabolites, as well as published results from omics experiments. Molecules affected by COVID-19 were obtained from QKB. Network meta-analysis, referred to as "Core Analysis" in this study, using IPA was then performed to investigate the inflammatory processes associated with COVID-19. IPA analysis using the My Pathway tool was also performed to determine the effects of downregulation of mACE2 on the neuroinflammation pathway. Our Core Analysis demonstrated a strong association between the molecules affected by COVID-19 and the canonical pathway of "Neuroinflammation Signaling Pathway," as well as biological functions and diseases associated with inflammatory response. Similarly, downregulation of mACE2 resulted in the activation of the "Neuroinflammation Signaling Pathway" and other functions associated with CNS damage.

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Materials & Methods

Ingenuity Pathway Analysis (IPA) Software

The Ingenuity Pathway Analysis (IPA) Analysis Match CL license was purchased from QIAGEN (QIAGEN, Germantown, MD;QIAGEN Inc., https://www.QIAGENbioinformatics.com/products).

IPA is a bioinformatics tool based on computational algorithms that analyzes the functional connectivity of molecules from information in the comprehensive, manually curated QKB which is a horizontally and vertically structured repository database composed of curated information created from over seven million individually modeled relationships between diseases, drugs, biological entities (e.g., genes, proteins, and metabolites) and processes, including the type of process (e.g., expression, molecular cleavage, phosphorylation, etc.) and the published results of omics experiments (e.g., increased or decreased expression). The information is manually curated and extracted primarily from scientific literature including journal articles, publicly available molecular content databases, and textbooks over the past two decades. The information used in this study were retrieved from QKB from December 14, 2020 to January 12, 2021. As shown in Fig. 2, the IPA tools were used to identify and analyze the molecules affected by COVID-19 with respect to the known signaling pathways and biological functions and diseases.

Identification of Molecules Impacted by COVID-19 from QKB

IPA's "Pathway" feature is to identify and organize molecule and biological relationship information from published research data from *in vitro* and *in vivo* research of human, rat, and mice subjects from a variety of biological contexts stored in the QKB. Using IPA's "Build"-"Grow" tool in a Pathway window, 535

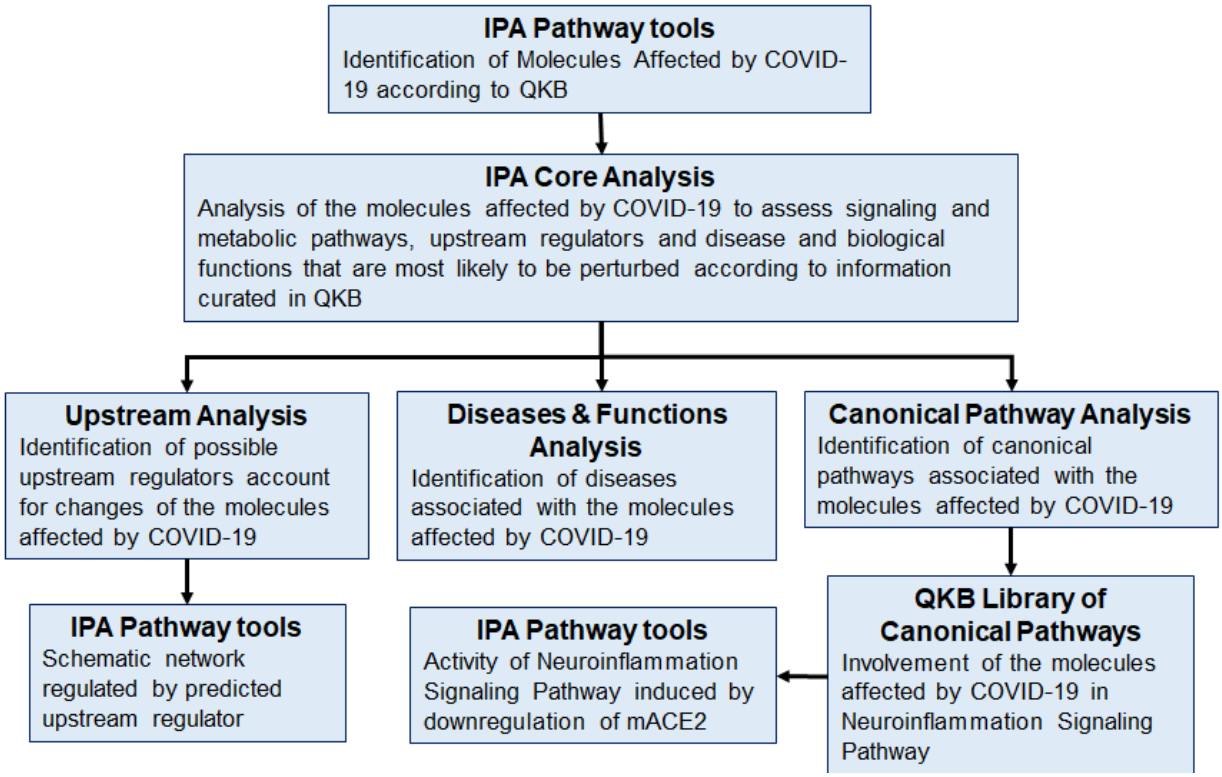

Fig. 2. The flow of information from bioinformatics, IPA tools used to analyze the molecules affected by COVID-19. Using the IPA Pathway tool, molecules affected by COVID-19 were identified. IPA Core Analysis was then performed on the dataset of molecules affected by COVID-19. The Core Analysis included Upstream Analysis, Diseases & Functions Analysis and Canonical Pathway Analysis. Upstream Analysis identified possible upstream regulators account for changes of the molecules affected by COVID-19, and IPA's Pathway tools were further used to show effects of the predicted regulator on the molecules being affected in its regulatory network. Diseases & Functions Analysis was used to identify diseases associated with the molecules affected by COVID-19. Canonical Pathway Analysis identified canonical pathways associated with the molecules affected by COVID-19. Among the top 10 pathways, Neuroinflammation Signaling Pathway was the only pathway relevant to neurological manifestations, and thus Neuroinflammation Signaling Pathway map was obtained from QKB library of canonical pathways and highlight the molecules affected by COVID-19 in Neuroinflammation Signaling Pathway and. IPA Pathway tools were used to generate connectivity map of mACE2 and Neuroinflammation Signaling Pathway to illustrate the relationships, and to demonstrate activity of Neuroinflammation Signaling Pathway induced by downregulation of mACE2.

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molecules affected by COVID-19 were obtained from QKB. Using Trim tool, drugs and toxicants were removed from the above list. As shown in Fig. 3, there were 375 molecules including genes, proteins, and complexes that exist in organisms in nature were further analyzed in the present study.

IPA Core Analysis

The 375 molecules affected by COVID-19 were uploaded into IPA for "Core Analysis". The analysis included Upstream Analysis, Diseases & Functions Analysis and Canonical Pathway Analysis (Fig. 2). Upstream Analysis was used to identify possible upstream regulators that may account for observed changes of queried molecules by overlaying onto global molecular network developed from Expression, Transcription, and Protein-DNA Bindings relationships contained in the QKB. The significance of the upstream regulators was measured using an overlap p value calculated by the right-tailed Fisher's Exact Test using a suite of algorithms reported by Krämer et al.[24] Diseases & Functions Analysis was used to identify the biological functions and/or diseases that are significantly correlated with the 375 molecules affected by COVID-19. As done in the Upstream Analysis, a right-tailed Fisher's Exact Test was used to calculate a p-value determining the probability that each disease annotation assigned to the 375 molecules affected by COVID-19 was due to chance alone. Based on the annotations, each disease category was then identified to be linked with the 375 molecules based on the biological relationships curated in the QKB. Canonical Pathway Analysis was used to identify the pathways from the QKB library of 712 canonical pathways that were most significant to the data set of molecules affected by COVID-19. The significance of the association between the data set of 375 molecules and the canonical pathway was measured using a p-value calculated by right-tailed Fisher's Exact Test. The p-value determined the probability that the association between the 375 molecules in the dataset and the canonical pathway is explained by chance alone.

Highlighting Molecules Affected by COVID-19 among the Molecular Components of Neuroinflammation Signaling Pathway

As noted above, IPA Core Analysis of the 375 molecules affected by COVID-19 was conducted to reveal ranking of the Neuroinflammation Signaling Pathway among the the Neuroinflammation Signaling Pathway including its p value, ratio of the number of the molecules affected by COVID-19 versus all molecules within the top 10

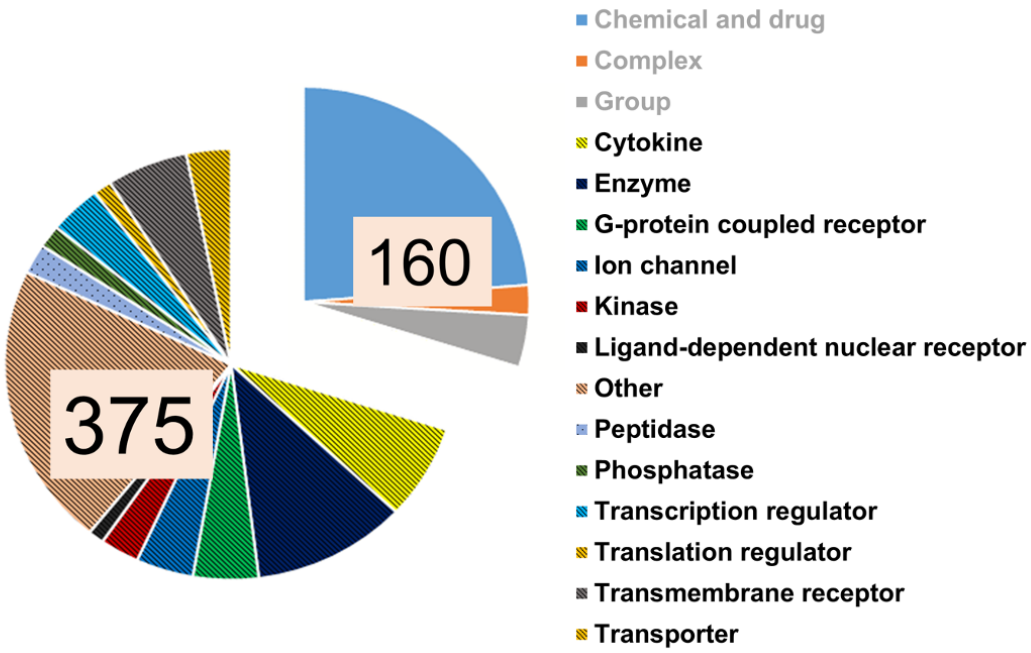

Fig. 3. The molecules affected by COVID-19. Using IPA, there were 535 molecules identified to be affected by COVID-19, according to QIAGEN Ingenuity Knowledge Base. Among the 535 molecules, there were 160 chemicals and drugs (24%), 12 complexes (2%), 21 groups (4%), 38 cytokines (7%), 59 enzymes (11%), 25 G-protein coupled receptors (5%), 22 ion channels (4%), 15 kinases (3%), 6 ligand-dependent nuclear receptors (1%), 12 peptidases (2%), 9 phosphatases (2%), 20 transcription regulators (4%), 7 translation regulators (1%), 31 transmembrane receptors (6%), 17 transporters (3%) and 114 others (21%). Among the 535 molecules, 375 molecules in the groups of cytokines, enzymes, G-protein coupled receptors, ion channels, kinases, ligand-dependent nuclear receptors, peptidases, phosphatases, transcription regulators, translation regulators, transmembrane receptors, transporters and other were used further for IPA Core Analysis.

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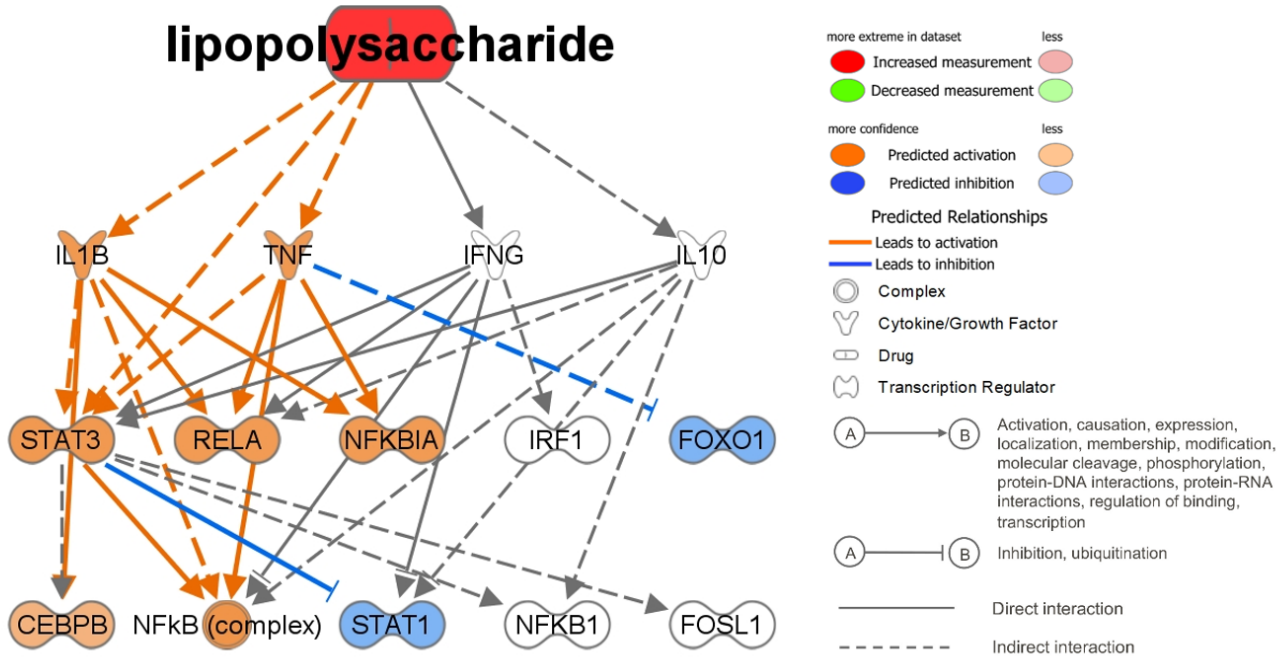

Figure 4. Schematic network regulated by lipopolysaccharide, the top predicted upstream regulator of the molecules affected by COVID-19. Upstream Analysis of the 375 molecules affected by COVID-19 revealed lipopolysaccharide (LPS) as the top predicted regulator with lowest p value of 7.75E-86 [-log(p-value) of 85.11]. Schematic network induced by activation of LPS was presented: LPS was predicted to elevate the expression of IL1B, TNF, which in turns increased STAT3, RELA Proto-Oncogene NF-KB Subunit (RELA), NFKB Inhibitor alpha (NFKBIA) while inhibited forkhead box O1 (FOXO1); STAT3 increase further led to activation of CCAAT Enhancer Binding Protein Beta (CEBPB) and nuclear factor kappa (NFκB) and inhibition of STAT1, while affected Nuclear Factor Kappa B Subunit 1 (NFKB1) and FOS Like 1 AP-1 Transcription Factor Subunit (FOSL1). LPS also affected IFNG and IL10, which regulated STAT3, RELA, NFκB, STAT1, interferon regulatory factor (IRF)1 and NFKB1. LPS does not affect expression of IFNG, the second top predicted upstream regulator of the molecules affected by COVID-19.

canonical pathways. In addition, the parameters of Neuroinflammation Signaling Pathway as well as characters of these molecules were obtained to define possible involvement Neuroinflammation Signaling Pathway in COVID-19 pathogenesis.

Membrane ACE2 modulation of Neuroinflammation Signaling Pathway

Neuroinflammation Signal Pathway network was taken from the IPA library of canonical pathways. By using IPA's Connect tool, membrane ACE2 (mACE2) was connected to the components of the Neuroinflammation Signaling Pathway according to the relationships curated in QKB. IPA's Overlay-Molecular Activity Predictor (MAP) was used to highlight the effects of downregulation of mACE2 on Neuroinflammation Signaling Pathway. This simulates that SARS-CoV-2 binding to mACE2 would have decreased expression or activity of ACE2.[25-27] The molecules associated with mACE2 among components of the Neuroinflammation Signaling Pathway were then highlighted using the Trim tool and the molecules not associated with mACE2 were removed by using "Not in overlaid dataset" option was selected under the Node Fill overlay feature under the Trim tool. The intensity of the node color was taken to reflect degree of up-(red/orange) or down-(green/blue) regulation of Neuroinflammation Signaling Pathway included by mACE2 downregulation. Nodes were displayed using various shapes that represent the functional class of each gene product.

Statistical Analysis

All statistical analyses were part of the IPA analyses using various tools in our study, according to the built-in algorithm suites as described.

Results

The Molecules Affected by COVID-19

A total of 535 molecules were found to be affected by COVID-19 by searching Qiagen Knowledge Base (QKB). As shown in Fig. 3, there were 127 chemicals and drugs (24%), 12 complexes (2%), 21 groups (4%), 38 cytokines (7%), 59 enzymes (11%), 25 G-protein coupled receptors (5%), 22 ion channels (4%), 15 kinases (3%), 6 ligand-dependent nuclear receptors (1%), 12 peptidases (2%), 9 phosphatases (2%), 20 transcription regulators (4%), 7 translation regulators (1%), 31 transmembrane receptors (6%), 17 transporters (3%) and 114 others

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(21%). Among the 535 molecules, the 160 molecules of chemical and drug, complex and group categories, as shown in the small segment, were trimmed off. The big segment showed the 375 molecules in the groups of cytokines, enzymes, G-protein coupled receptors, ion channels, kinases, ligand-dependent nuclear receptors, peptidases, phosphatases, transcription regulators, translation regulators, transmembrane receptors, transporters and other. Only the 375 molecules were used further for IPA Core Analysis.

elevate the expression of IL1B, TNF, which in turns increased STAT3, RELA Proto-Oncogene NF-KB Subunit (RELA), NFKB Inhibitor alpha (NFKBIA) while inhibited forkhead box O1 (FOXO1); STAT3 increase further led to activation of CCAAT Enhancer Binding Protein Beta (CEBPB) and nuclear factor kappa (NFκB) and inhibition of STAT1, while affected Nuclear Factor Kappa B Subunit 1 (NFKB1) and FOS Like 1 AP-1 Transcription Factor Subunit (FOSL1). LPS also affected IFNG and IL10, which regulated STAT3, RELA, NFκB, STAT1, interferon regulatory factor (IRF)1 and NFKB1,

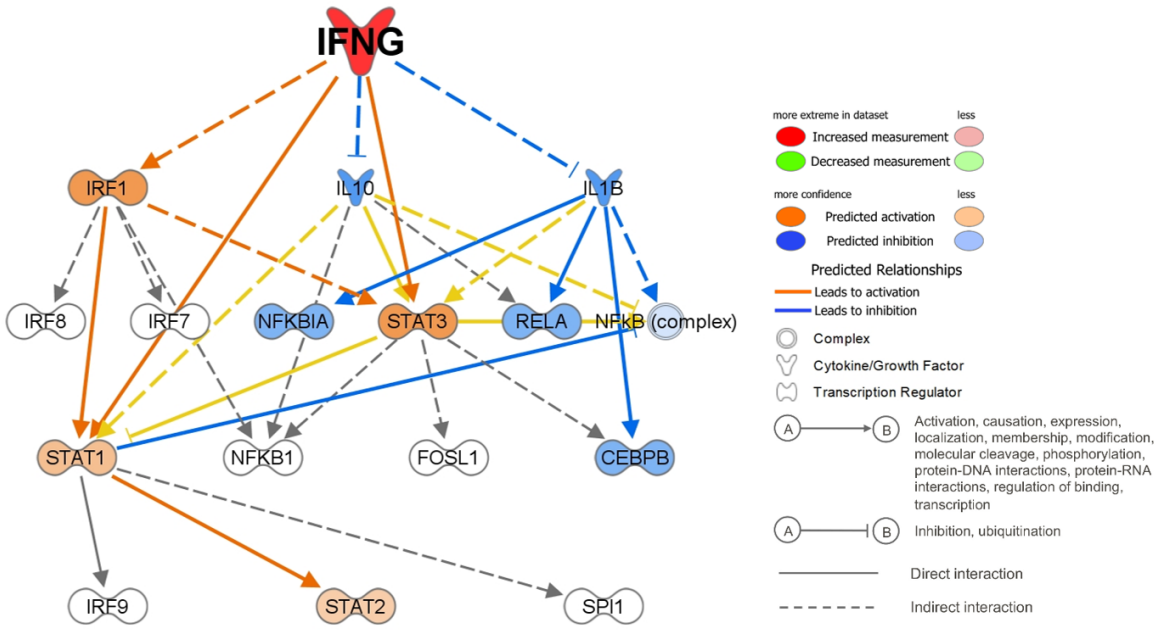

Fig. 5. Schematic network regulated by IFNG, the second top predicted upstream regulator of the molecules affected by COVID-19. Upstream Analysis of the 375 molecules affected by COVID-19 revealed IFNG as No 2 top predicted upstream regulator, with a p-value of 2.94E-73[-log(p-value) of 72.53]. Schematic network regulated by IFNG was presented: activation of IFNG was predicted to increase IRF1 and decreased IL10 and IL1B, which in turns affected IRF8, IRF7, inhibited NFKBIA, RELA, CEBPB and NFκB while activated STAT1 and STAT3. Activation of STAT1 and STAT3 further regulated IRF9, STAT2, Spi-1 proto-oncogene (SPI1), NFKB1, FOSL1 and CEBPB.

Analysis of the Molecules Affected by COVID-19

The molecules (375 as noted in Fig. 3) were uploaded into IPA, and Core Analysis was performed on these molecules. Upstream Analysis was performed on the 375 molecules affected by COVID-19, the top ten molecules predicted as upstream regulators were lipopolysaccharide (LPS), Interferon gamma (IFNG), dexamethasone, Interferon alpha, signal transducer and activator of transcription (STAT) 1, resiquimod, Interleukin (IL)1B, Tumor necrosis factor (TNF), poly rI:rC-RNA and STAT3. Their p values were ranged from 7.75E-86 to 2.37E-47. LPS was the molecule with lowest p value (7.75E-86) and IFNG was the regulator with second lowest p value (2.94E-73).

Fig. 4 shows the schematic network regulated by LPS. As the master upstream regulator, LPS was predicted to however with no known activity direction. This was taken to suggest that LPS does not affect expression of IFNG. Fig. 5 shows the schematic network regulated by IFNG. As an upstream regulator, activation of IFNG was predicted to increase IRF1 and decreased IL10 and IL1B, which in turns affected IRF8, IRF7, inhibited NFKBIA, RELA, CEBPB and NFκB while activated STAT1 and STAT3. Activation of STAT1 and STAT3 further regulated IRF9, STAT2, Spi-1 proto-oncogene (SPI1), NFKB1, FOSL1 and CEBPB. Notably, the overall regulatory networking of IFNG (Fig. 4) and that of LPS (Fig. 3) appear to counteract with each other.

Diseases & Functions Analysis of the 375 molecules affected by COVID-19 revealed association of these molecules with most high-level disease categories (25 out of 29 in total) collected in QKB. (p-values of these 25

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categories ranged from 8.99E-116 to 1.09E-29). Neurological disease was listed as the 13th category. There were 32 disease annotations associated with the 375 molecules affected by COVID-19 being identified among all annotations of the high-level category "Neurological Diseases". The total number of the "Neurological Diseases" annotations is close to 9300. These 32 annotations had p-values ranged from 6.19E-74 to 5.91E-29. Among these 32 disease annotations with the category of Neurological disease, as listed on Table 1, three annotations were also affiliated with the disease category of Inflammatory Response. The first one was Inflammation of central nervous system with p-value of 4.21E-38. The second one was Encephalitis with p-value of 2.80E-35. The third one was with Experimental autoimmune encephalomyelitis with p-value of 5.91E-29.

Canonical Pathway Analysis identified 218 Canonical Pathways with p< 0.05 to be affected by the 375 molecules being affected by COVID-19. Fig. 6 shows the top ten pathways among these 218 pathways. The p values of these 10 pathways ranged from 1.44E-33 to 2.10E-21. The Neuroinflammation Signaling Pathway was found to be the fourth top pathway with p value 1.10E-25. These suggest close relationship between COVID-19 and Neuroinflammation Signaling Pathway, COVID-19 induced-neuroinflammation, and the neuronal manifestations of COVID-19.

Involvement of the Molecules Affected by COVID-19 in the Neuroinflammation Signaling Pathway

IPA Core Analysis of the molecules affected by COVID-19 revealed that Neuroinflammation Signaling Pathway was one of the top five pathways being associated with the 375 molecules affected by COVID-19. Neuroinflammation Signaling Pathway has a p-value of 1.10E-25 [-log(p-value)=25.00)]. As shown in Fig. 6, there were 41 molecules affected by COVID-19 being involved in Neuroinflammation Signaling Pathway, of which a total of 300 molecules resides in its molecular network. Fig. 7 also shows that the 41 molecules consisted of the two antiviral response cascades induced by COVID-19. The following molecules, IFNγ, JAK, STAT1, MHC1, AP1, COX2, TLR. IRF7, cytokines and chemokines (IL1β, IL4, IL6, IL10, IL18, TNFα, CCL2, CCL3, CCL5, CXCL8, CXCL10) along with their receptors (IL1R1, IL6R, CX3CR1), were involved interconnectedly within each of the two cascades or between the two cascades.

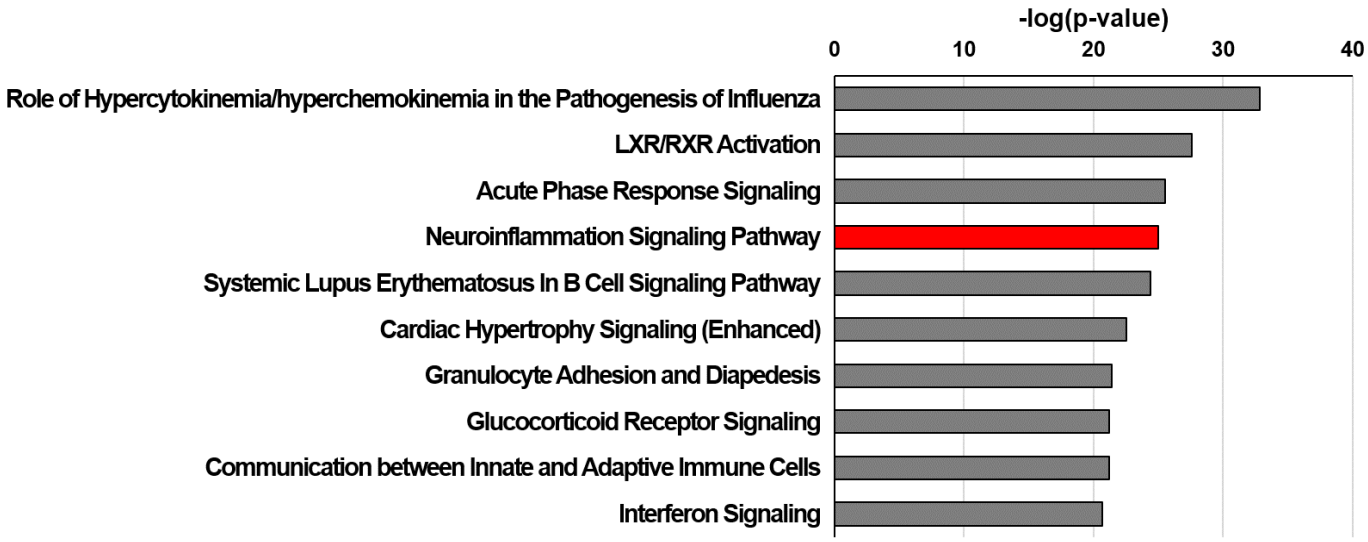

Fig. 6. IPA Canonical Pathway analysis of molecules affected by COVID-19. The 375 molecules affected by COVID-19 were uploaded into IPA for Canonical Pathway Analysis, to identify canonical pathways associated with the molecules affected by COVID-19. There were 218 Canonical Pathways with p< 0.05 identified by Canonical Pathway Analysis to be affected by COVID-19. Top ten pathways were presented, with p values in the range of 1.44E-33 to .2.10E-21 [-log(p-value) of 32.80 – 20.70].

Downregulation of mACE2 Activation of the Neuroinflammation Signaling Pathway

SARS-CoV-2 enters host cells via mACE2 as the receptor[4-7] and decreased mACE2 expression was reported in COVID-19 patients (Imai, Kuba et al. 2005, Chaudhry, Lavandero et al. 2020, Ni, Yang et al. 2020). Fig. 8 shows the effects of mACE2 downregulation on activity of Neuroinflammation Signaling Pathway.

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Downregulation of mACE2 activated Neuroinflammation Signaling Pathway mainly through activation of NFκB-induced elevation of cytokines and inhibition of IFNγ-induced antiviral response. Functions associated with central nervous system (CNS) damage were then induced. These functions have included BBB (blood-brain barrier) disruption, Neurons damage, Astrogliosis, ROS (reactive oxygen species) production, and Oxidative stress.

Involvement of the Molecules Associated with mACE2 in Activation of the Neuroinflammation Signaling Pathway To explore direct effects on Neuroinflammation Signaling Pathway by downregulation of mACE2, molecules not connected to mACE2 were removed from Neuroinflammation Signaling Pathway and effects of mACE2 was showed in Fig. 9. NFκB-induced elevation of cytokines and inhibition of IFNγ-induced antiviral response, with less components involved. This led to activation of Neuroinflammation Signaling Pathway, as well as BBB disruption, Neurons damage, Astrogliosis and Neurons apoptosis, however with less confidence based on the color of the Neuroinflammation Signaling Pathway node.

Discussion

COVID-19 is more than just a respiratory disease as initially described in late 2019. In severe cases, sepsis caused by systemic inflammation has led to multiple organ damage, including damage to the lungs, heart, liver, and kidneys. Neurological symptoms have also been observed in this disease.[15-16] However, only a certain percentage of patient groups have exhibited these neurological manifestations. For instance, in a study involving 214 COVID-19 patients from Wuhan, China, only 36% presented neurological manifestations.[28] However, it has been reported that certain percentages of recovered and discharged patients have cognitive deficits, such as dysexecutive syndrome, signs of inattention, disorientation, and poorly organized movements and response.[20,21] Clinical data have projected that neurological effects can continue in a patient who has recovered and been discharged, thus, understanding the pathophysiological mechanisms underlying the neurological effects is of pressing importance.

Enough COVID-19 publications have proposed different mechanisms underlying the pathophysiology of the neurological manifestations. The mechanisms fall into two categories: direct neuroinvasion of SARS-CoV-2 and COVID-19-induced "cytokine storm" leading to neurological damage. The literature has also proposed various mechanisms to explain neuroinvasion of SARS-CoV-2. SARS-CoV-2 could reach the CNS through neuron-to-neuron transport by infecting neurons through the olfactory route or the gut-brain axis.[29,30] The virus

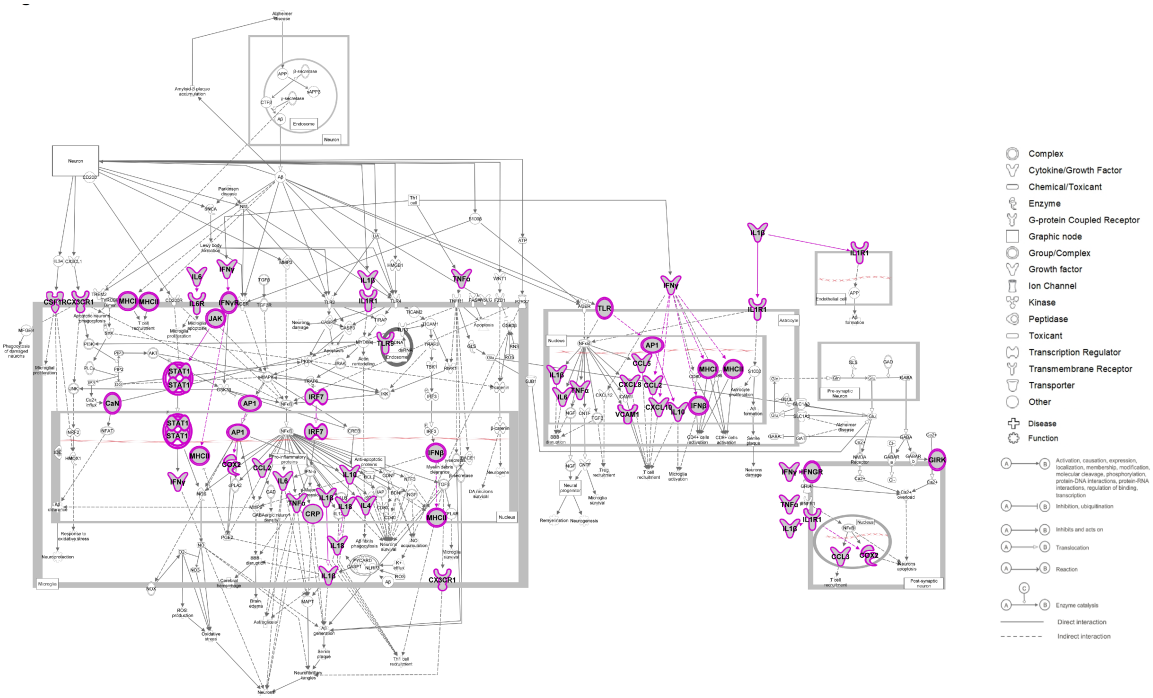

Fig. 7. Involvement of the molecules affected by COVID-19 in Neuroinflammation Signaling Pathway. There were 41 molecules affected by COVID-19 that were involved in Neuroinflammation Signaling Pathway that consists of a total of 300 molecules. The 41 molecules affected by COVID-19 were overlaid in Neuroinflammation Signaling Pathway from IPA library of canonical pathways. The overlaid molecules were highlighted in purple frame. Within Neuroinflammation Signaling Pathway network, COVID-19 affected two antiviral response cascades, highlighted by two red boxes, including IFNγ-JAK-STAT1 and MHC1, AP1-COX2, and TLR- IRF7), cytokines (IL1β, IL4, IL6, IL10, IL18, TNFα, CCL2) along with their receptors (IL1R1, IL6R, CX3CR1).

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could also reach the CNS through the hematogenous route in a "Trojan horse" mechanism, in which SARS-CoV-2 infected immune cells extravasate into the meninges and cerebrospinal fluid.[31,32] It has also been suggested that they may gain entry into the brain through the vasculature, the meninges, and the choroid plexus.[29] Finally, neurological damage could be caused by the "cytokine storm" induced after infection. In this mechanism, inflammation in periphery would disrupt the Blood-Brain Barrier (BBB) and induce neuroinflammation.[33]

The current study was undertaken to investigate the role of COVID-19 in inducing neuroinflammation. Systemic inflammation has already been identified as one highly critical symptom of COVID-19,[34] and it has already been demonstrated that systemic inflammation induces neuroinflammation.[12-14] These are the key premises leading to our central hypothesis that COVID-19 induces neuroinflammation possibly mediated via the systemic inflammation in the course of disease.

To substantiate our hypothesis, our Canonical Pathway Analysis revealed Neuroinflammation Signaling Pathway and Role of Hypercytokinemia in the Pathogenesis of Influenza to be among the top ten canonical pathways enriched by the 375 molecules affected by COVID-19 (Fig. 6). Neuroinflammation Signaling Pathway appeared as the fourth most significant pathway within the top ten canonical pathways, a fact that supports how COVID-19 may be indeed causing neuroinflammation and neuroinflammation is involved in the pathogenesis of the neurological manifestations. Neuroinflammation Signaling Pathway was also activated when downregulated mACE2, upon SARS-CoV-2 binding to its receptor on the host cells, was connected to the molecules associated with the pathway itself, which included various cytokines including IL-6, TNF-α, and IL-1β; all which were activated as well (Figs. 8 and 9). The activation of these cytokines suggests that systemic inflammation in COVID-19 should have been involved in COVID-19 induction of neuroinflammation. Indeed,

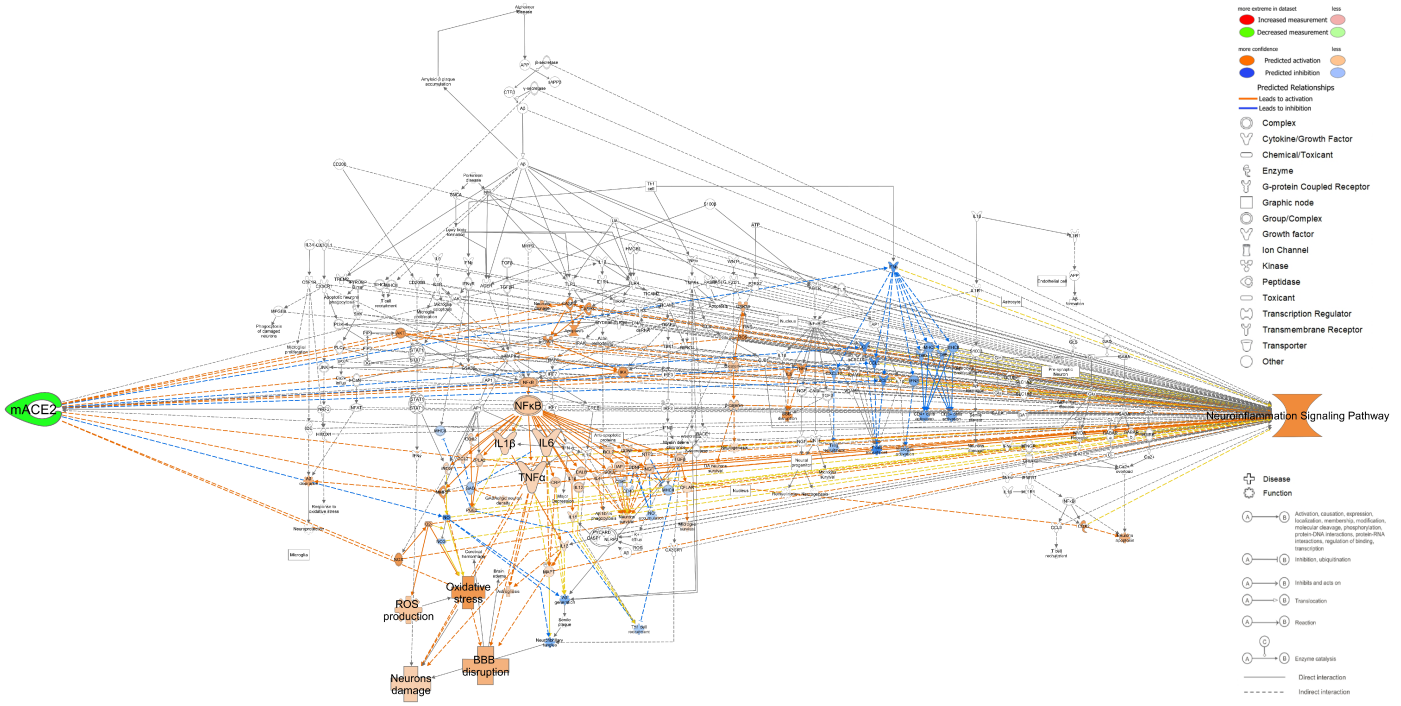

Fig. 8. Modulation of Neuroinflammation Signaling Pathway induced by downregulation of mACE2. Effects of mACE2 downregulation on Neuroinflammation Signaling Pathway were presented by connecting mACE2 to components of the Neuroinflammation Signaling Pathway according to the relationships curated in QKB and applying IPA's Overlay-Molecular Activity Predictor (MAP) to show effects of downregulation of mACE2 on Neuroinflammation Signaling Pathway based on the mapped relationships. A total of 20 molecules were affected by mACE2 among the 300 molecules associated with Neuroinflammation Signaling Pathway. Among these 20 molecules, 10 molecules were also affected by COVID-19. In Neuroinflammation Signaling Pathway, downregulation of mACE2 induced increase of caspases Caspase (CASP)8 and CASP3; increase of cytokines Interleukin (IL)6, TNFα; activation of kinases Protein kinase B (AKT) and NF-κB kinase (IKK); increase of Matrix metallopeptidase 9 (MMP9); increase of Reactive Oxygen Species (ROS);decrease of IFNγ and downstream chemokines C-C Motif Chemokine Ligand (CCL)2, CCL5, C-X-C motif chemokine ligand (CXCL) 8 and CXCL10. These modulations and resulting downstream effects led to activation of Neuroinflammation Signaling Pathway with functions associated with CNS damage were then inducted, which included BBB Disruption, Neurons Damage, Astrogliosis, ROS Production, and Oxidative Stress.

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researchers have proposed that when the SARS-CoV-2 S protein binds to and downregulates mACE2, the downregulation of mACE2 leads to overactivation of the Angiotensin (Ang) II/ Angiotensin II Receptor Type 1 (AT1R) axis, which in turn would lead to the "cytokine storm".[22,23] It thus follows that systemic inflammation induced by the "cytokine storm" could then generate neuroinflammation in COVID-19. Our results of Diseases & Functions Analysis revealed involvement of AT1R in the three inflammatory annotations of the neurological disease, Inflammation of central nervous system (CNS), encephalitis and experimental autoimmune encephalomyelitis (EAE). This information confirms that Angiotensin (Ang) II/AT1R axis may be involved in mACE2 modulation of neuroinflammation.

It should also be noted that downregulation of mACE2 connected to molecules associated with the "Neuroinflammation Signaling Pathway" not only activated the pathway itself and associated pro-inflammatory cytokines, it also activated functions associated with CNS damage, such as BBB disruption, Astrogliosis, and Neurons Damage (Figs. 8 and 9). These results thus further support the role neuroinflammation may be playing in generating the neurological symptoms observed in COVID-19. BBB disruption and astrogliosis, for instance, have been associated with spontaneous seizures in mice,[35] and seizure has been observed as one of the neurological symptoms of COVID-19.[17,18]

Our Canonical Pathway Analysis also revealed Role of Hypercytokinemia in the Pathogenesis of Influenza" to be top-most canonical pathway enriched by the 375 molecules affected by COVID-19 (Fig. 5). It is important to note that the association generated between COVID-19 and Role of Hypercytokinemia in the Pathogenesis of Influenza, which is the first pathway within the top ten, does not necessarily mean that COVID-19 would induce hyperctyokinemia in influenza, but rather suggests that the influenza cytokine storm pathway and COVID-19 share a common set of key mediating molecules and cells. This further supports the important role systemic inflammation has in COVID-19 pathogenesis, as the top-most enriched canonical pathway is one related to the "cytokine storm" that leads to systemic inflammation that ultimately elevate neuroinflammation.

Our Diseases & Functions Analysis of the 375 molecules affected by COVID-19 revealed association of these

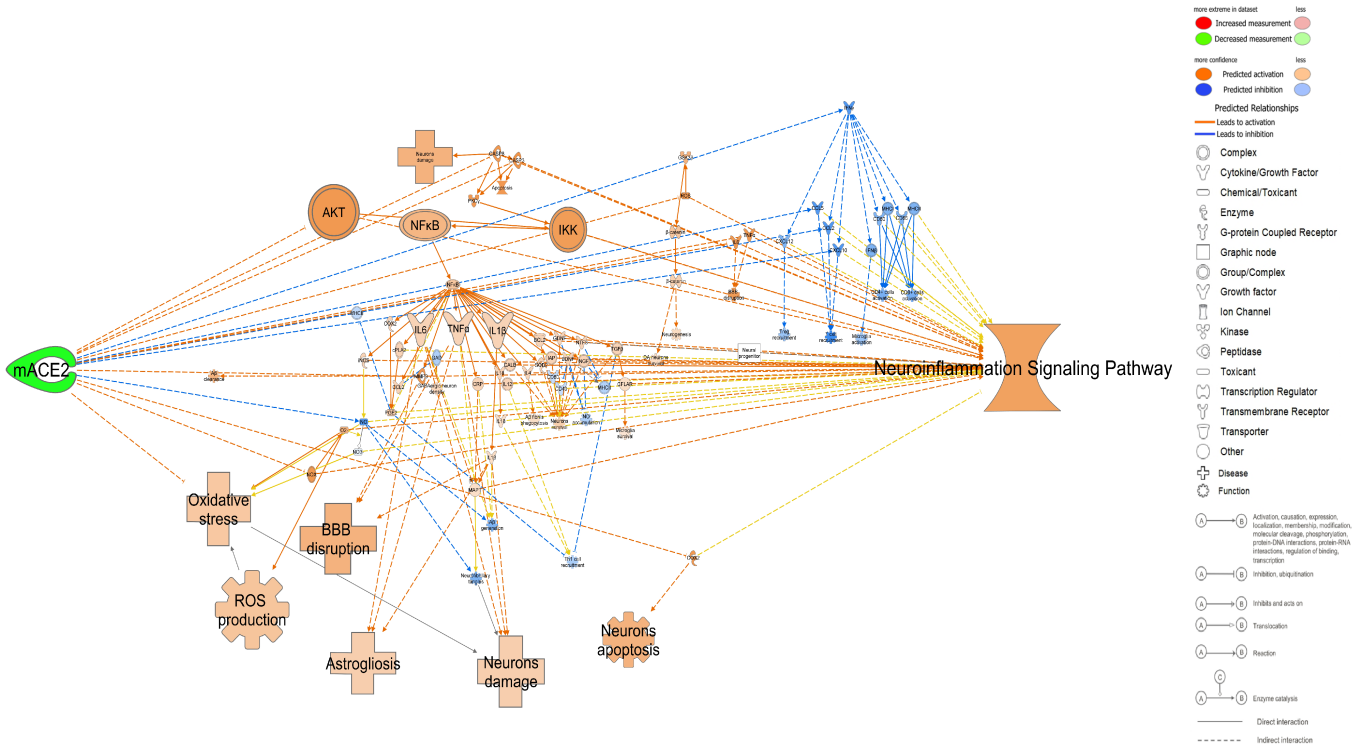

Fig. 9. Direct effects on Neuroinflammation Signaling Pathway induced by downregulation of mACE2. Effects of mACE2 downregulation on Neuroinflammation Signaling Pathway were presented by connecting mACE2 to components of Neuroinflammation Signaling Pathway according to the relationships curated in QKB and applying IPA's Overlay-Molecular Activity Predictor (MAP) to show the effects of downregulation of mACE2 on Neuroinflammation Signaling Pathway based on the mapped relationships. Direct effects of mACE2 downregulation were presented by removing the molecules not associated with mACE2 in Neuroinflammation Signaling Pathway.

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Table 1. Three disease annotations associated with Neurological Disease and Inflammatory Response

Annotation	Categories associated with annotation	p-value for annotation
Inflammation of central nervous system	**Inflammatory Response; Neurological Disease**	4.21E-38
Encephalitis	**Inflammatory Disease;** Inflammatory Response; **Neurological Disease;** Organismal Injury and Abnormalities	2.80E-35
Experimental autoimmune encephalomyelitis	Immunological Disease; Inflammatory Disease; **Inflammatory Response; Neurological Disease;** Organismal Injury and Abnormalities	5.91E-29

molecules with 25 disease categories curated in QKB, among which Neurological Disease was listed as the 13th category. There were 32 disease annotations significantly associated with the 375 molecules affected by COVID-19. And three among the 32 Neurological Disease annotations were also affiliated with the category of Inflammatory Response: 1) Inflammation of central nervous system (CNS) with a p-value of 4.21E-38; 2) Encephalitis with a p-value of 2.80E-35; and 3) Experimental autoimmune encephalomyelitis (EAE), an experimental model for multiple sclerosis (MS), with a p-value of 5.91E-29 (Table 1). Both encephalitis and MS are inflammatory diseases in the CNS.[36-38] Encephalitis is an acute inflammation of the brain most commonly caused by viral infection.[38] Notably, cases of COVID-19 related encephalitis were reported in fall of 2020.[39,40] MS is a chronic inflammatory disease caused by T-cell-mediated autoimmunity in the CNS.[36,37] The molecules affected by COVID-19 possibly leading to MS pathological features in the EAE has suggested that autoimmunity in CNS may be a risk factor by which COVID-19 would enhance onset and progression of MS.[41,42] The association of the 375 molecules affected by COVID-19 with inflammations in the CNS including encephalitis and MS further confirmed the involvement of neuroinflammation in neurological pathogenesis of COVID-19.

In addition to Diseases and Functions associated with inflammation, our Upstream Regulator Analysis also revealed the activation of LPS and IFN-γ as two upstream regulators of the molecules associated with COVID-19 (Figs. 3 and 4). Activation of LPS provides further evidence of systemic inflammation being involved in inducing neuroinflammation in COVID-19 because it suggests that LPS and COVID-19 share a common set of key mediating molecules and cells, and LPS has been demonstrated to induce systemic inflammation.[43] The upregulation of IFN-γ also confirms the presence of an anti-viral state being induced within the context of inflammation in COVID-19.

The results of the present meta-analysis suggest systemic inflammation is inducing neuroinflammation in COVID-19, which in turn may be contributing the manifestations of the neurological symptoms observed in the disease. However, it is possible that the pathophysiology of these symptoms is multifactorial, involving both direct viral invasion into the CNS and neuroinflammation caused by systemic inflammation. Further studies need to be performed to fully comprehend the pathophysiological mechanisms underlying these neurological manifestations. The fact that neurological symptoms continue after a person has recovered from the illness, in the form of cognitive deficits, make the need for further studies more pressing. A better understanding of the mechanisms underlying the neurological symptoms could lead to developing therapeutic strategy that could also take into the count the integrity of the COVID-19 patient's nervous system.

Limitations

The current meta-analysis has several limitations. First, the results of the study were produced through the analysis of molecules lists and associations retrieved from the published literature. New literature updates could alter some of the results presently analyzed. Secondly, data sets on differential molecule expression from COVID-19 patients who experienced neurological symptoms was not used in this study. Instead, the study relied on a list of molecules affected by COVID-19 collected from the QKB, whose literature sources were not analyzed to determine if the molecule associations were derived from studies that recorded whether the COVID-19 patients

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experienced neurological symptoms or not. Thirdly, our Core Analysis results, which did not rely on a list of quantitative data as previously explained, only yielded statistically significant associations based on the number of molecules associated with COVID-19 that overlapped with the molecules of canonical pathways. These associations did not project whether those pathways are activated or inhibited in COVID-19. Finally, My Pathway analyses performed on mACE2 and the Neuroinflammation Signaling Pathway relied on data that did not originate from the studies with COVID-19. The results of these analyses also did not provide quantitation of confidence level about the activation or inhibition of molecules and pathways observed. Only a color change was used to indicate activation (orange) or inhibition (blue). Although with these limitations with meta-analyses, the data presented in the current study still clearly can demonstrate the empirical evidence about the relationship of molecules and pathways being observed in COVID-19.

Conclusion

There is increasing acknowledgement that SARS-CoV-2 infection/COVID-19 can cause neurological pathologies. However, lack of study in mechanism how COVID-19 could impact on central nerve system (CNS). Our *in silico* study utilized a bioinformatics tool QIAGEN Ingenuity Pathway Analysis (IPA) to analyze the molecules affected by COVID-19 curated in QIAGEN Knowledge Base (QKB) based on published papers. The results of our meta-analysis demonstrate that COVID-19 causes neuroinflammation via modulating the major inflammatory pathways, which in turn may be contributing the manifestations of the neurological symptoms observed in the disease.

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Conflict of Interest

The authors declare that there is no conflict of interest.

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