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Immunoregulation of Ghrelin in neurocognitive sequelae associated with COVID-19: an *in silico* investigation

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ABSTRACT

Some patients suffering from the new Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2) develop an exaggerated inflammatory response triggered by a "cytokine storm" resulting in acute respiratory distress syndrome (ARDS) with the concomitant activation of non-specific inflammatory reactivity in the circulatory system and other organs, leading to multiorgan failure, leaky vasculature, coagulopathies and stroke. Impairment of brain functions may also occur as dysregulations in immune function resulting from neuroendocrine interactions. In this study, we explored, by bioinformatics approaches, the interaction between the multiple inflammatory agents involved in SARS-CoV-2 and Ghrelin (Ghre) together with its receptor GHSR-1A, which are described as anti-inflammatory mediators, in order to investigate what could trigger the hyper-inflammatory response in some SARS-CoV-2 patients. In our analysis, we found several interactions of Ghre and GHSR-1A with SARS-CoV-2 interacting human genes. We observed a correlation between Ghre, angiotensin-converting enzyme 2 ACE2, toll-like receptors 9 (TLR9), and Acidic chitinase (CHIA), whereas its receptor GHSR-1A interacts with chemokine receptor 3 (CXCR3), CCR3, CCR5, CCR7, coagulation factor II (thrombin) receptor-like 1 (F2RL1), vitamin D receptor (VDR), Nucleotide-binding oligomerization domain-containing protein 1 (NOD1) and DDP4 in receptor dipeptidyl peptidase-4. To our knowledge, our findings show, for the first time, that Ghre and GHSR-1A may exert an immunomodulatory function in the course of SARS-Cov-2 infection.

1. Introduction

The new Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2) is an unusual respiratory illness, named Coronavirus disease-2019 (COVID-19) (Yang et al., 2020). This disease is responsible for higher mortality than common influenza. In the past two years, the understanding of the action, pathogenicity and antigenic potential of SARS-CoV-2 has become the center of common interest. The tissues and organs involved are different, leading to a wide spectrum of clinical manifestations such as fever, cold, bronchiolitis, and pneumonia. The infection may cause headaches, hemoptysis, diarrhea, dyspnea, and lymphopenia. In the early stage of the infection, some patients show neurological symptoms, and ischemic stroke often occurs around 2 weeks after the onset of infection (Harapan and Yoo, 2021; Mussa

Abbreviations: ACE2, Angiotensin-converting enzyme 2; AMCase, CHIA - Active mammalian endochitinase; AMs, Alveolar macrophages; ANPEP, Alanyl Aminopeptidase, Membrane; ARDS, Acute respiratory distress syndrome; CCR, C-C Motif Chemokine Receptor; CHIA, Acidic chitinase; CNS, Central nervous system; COVID-19, Coronavirus disease-2019; CXCR3, Chemokine receptor 3; DDP4, Dipeptidyl peptidase-4; ENPEP, Glutamyl Aminopeptidase; F2RL1, Coagulation factor II (thrombin) receptor-like 1; Ghre, Ghrelin; GHSR-1A, GH secretagogue receptor-1a; GO, Gene Ontology; HMGB1, High Mobility Group Box 1; IFN-γ, Interferon gamma; PNS, Peripheral nervous system; IL, Interleukin; IRF2, Interferon Regulatory Factor 2; MAPK, Mitogen-activated protein kinase; MCP-1, Monocyte Chemoattractant Protein-1; NF–κB, Nnuclear factor–κB; NLR, Nucleotide-oligomer domain-like receptors; NLRP3, NLR Family Pyrin Domain Containing 3; NOD1, Nucleotide-binding oligomerization domain-containing protein 1; PAMPs, Pathogen-associated molecular patterns; PARs, Protease-activated receptors; PPI, Protein-protein interactions; PRR, Pattern Recognition Receptors; RDX, Radixin; SARS-CoV-2, Severe Acute Respiratory Syndrome Coronavirus 2; SERPING1, Serpin Family G Member 1; Th2, T-helper cell type 2; TLR, Toll-like receptor; TMPRSS2, Transmembrane serine protease 2; TNF–α, Tumor necrosis factor–α; VDR, Vitamin D receptor.

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et al., 2021). It has been reported that about the 34% of patients develop central and peripheral nervous system symptoms (CNS and PNS) reporting headache, dizziness, confusion, mild cognitive impairment, epilepsy, loss of smell, altered taste and loss of appetite, blurred vision, muscle pain, nerve pain, and ataxia. In the case of long COVID status, patients can develop neurocognitive disorders which include the "brain fog" and cognitive deficits which alterate the normal daily activities living. The brain fog state is a cognitive impairment condition which leads to confusion and memory problems including mental fatigue, intellectual confusion, low concentration and anxiety related to some brain areas such as cortex and hippocampus (Hugon et al., 2022; Asadi-Pooya et al., 2022).

The essential step to cause the SARS-CoV-2 infection and related pathogenesis is the binding between the COVID-19 virus and the host-cell receptors including angiotensin-converting enzyme 2 (ACE2) and in the dipeptidyl peptidase-4 (DPP4) receptor. Molecular study showed that also heparan sulfate is required as co-factor for SARS-CoV-2 entry. In particular, specific sulfonated heparin promotes the binding through a conformational change of ACE2 S-protein interacting with the receptor-binding domain (RBD) at the S1 subunit of the SARS-CoV-2 (Kalra and Kandimalla, 2021). The mucosa is rich in ACE2 receptors; therefore, the virus enters the host via eyes, nose and mouth (Hamming et al., 2004). Additionally, ACE2 is widely distributed in the epithelial cells of trachea, bronchi, alveoli, as well as alveolar monocytes and macrophages (Kuba et al., 2006) in tubular epithelial cells of kidneys and in endothelial cells of arteries and veins, cerebral neurons, mucosal cells of intestines, and in immune cells (Guo et al., 2008; Liu et al., 2011). In addition, transmembrane serine protease 2 (TMPRSS2) facilitates the entry of SARS-CoV-2 into host cells. by. It causes a proteolytic cleavage of the receptor-attached spike protein at Arg667 and Arg797. Other TMPRSS2 amino acid residues interacting with S-protein identified are: Ala262, Arg214, Arg246, Asp80, Asp215, Asp614, Gly261, His66, His245, Leu242, Lys187, Phe79, Trp64, Thr95, Val213 (Senapati et al., 2021). Due to the cytokine-mediated autoimmune storm, multi-organ failure may occur. Moreover, neuroinflammation caused by neurological dysfunction (Li et al., 2020) has been reported. The pathophysiology of acute respiratory distress syndrome (ARD) syndrome involves many of the pro-inflammatory pathways that are influenced by a small peptide known as Ghrelin (Ghre) (Narula and deBoisblanc, 2015).

Ghre is an acylated 28-amino acid peptide endogenous ligand for the GH secretagogue receptor-1a (GHSR-1A) (Kojima et al., 1999; Date et al., 2000). Both Ghre and GHSR-1A are widely distributed. Their expression has been detected in several tissues, such as brain, stomach, lung, intestine, kidney, testis, and ovary (Gnanapavan et al., 2002). Therefore, Ghre and GHSR-1A exert multiple biological activities including food-intake and metabolism stimulation, cardiovascular function, adipogenesis, synaptogenesis, learning and memory (Baatar et al., 2011). In addition, Ghre and GHSR-1A were also identified in lymphoid tissues and immune cells, such as B and T cells, monocytes and natural killer cells (Hattori et al., 2001). Consequently, acting as powerful anti-inflammatory mediators they could be regarded as promising therapeutic agents in the treatment of inflammatory diseases and injury. Ghre through its anti–inflammatory effects on the nuclear factor–κB (NF–κB) and the tumor necrosis factor–α (TNF–α) gene reduces the cortex inflammation in epileptic patients (Han et al., 2018).

The hypothalamic area is strongly related to the olfactory and hippocampal system also through the existence of the Ghre-direct-pathway (Russo et al., 2017; Russo et al., 2018; Beheshti and Dehestani, 2021).

Ghre regulates many functions of the neuro-immuno-endocrine axis and, as a result of the integration between Ghre, hormones and other cephalic cues, hippocampal neurons modify their synaptic plasticity and neurogenesis in order to promote spatial learning and memory (Carlini et al., 2010; Li et al., 2013).

Evidences suggest that SARS-CoV-2 infects also nerve cells underling a potential direct effect on CNS function (Yu et al., 2020). Therefore, in susceptible individuals, there are powerful and rapid COVID-19-CNS interactions mediated by the strong humoral immune responses elicited by the infection. Ghre is able to attenuate the initial inflammatory response induced by the virus (Russo et al., 2021). Pathogenic influences on brain functions may involve dysregulations of the immune system resulting from neuroendocrine interactions with several immune/inflammatory mediators. Hence, it is important to characterize and understand the neuroendocrine interactions with the immune and inflammatory molecules that trigger neuroinflammation. In this study, we explored, by bioinformatics approaches, the interaction between the multiple inflammatory mediators involved in COVID-19 and Ghre and GHSR-1A in order to investigate their contribution to the pathogenesis of SARS-CoV-2.

2. Materials and methods

2.1. Ethical compliance

This investigation did not contain any studies with human participants or animals performed by any of the authors, and therefore no ethical compliance is required.

2.2. Data selection

In order to get insights into the role of Ghre in SARS-Cov-2, we integrated the Ghre gene and GHSR-1A with the list of genes encoding SARS-CoV-2 interacting human proteins from the Human protein atlas (HPA, https://www.proteinatlas.org/humanproteome/sars-cov-2https://www.proteinatlas.org/humanproteome/sars-cov-2) following the same method of our recent paper (Franz et al., 20182018). The HPA is a large project that aims to develop a map of human proteins through several technologies such as imaging, proteomics and transcriptomics, providing freely available data. In particular, the SARS-CoV-2 HPA program includes transcriptomics and antibody-based proteomics data, offering information about the tissue- and cell-specific expression patterns of SARS-CoV-2 interacting human proteins. Moreover, genes encoding proteins involved in inflammatory processes, such as chemokines, cytokines, metalloproteinases, Serpin Family G Member 1 (SERPING1), Alanyl Aminopeptidase, Membrane (ANPEP), Glutamyl Aminopeptidase (ENPEP), DPP4, and Vitamin D receptor (VDR), were also considered. The gene list of SARS-CoV-2 HPA were integrated using the program filtered on the basis of their selective expression on cortex and hippocampus tissue (S1).

2.3. GeneMANIA/Cytoscape in silico gene-gene interaction

We performed an *in silico* network-based analysis to explore the Ghre and SARS-CoV-2 interacting human proteins network, as well as the possible related biological implications. The total list of selected genes was given to GeneMANIA/Cytoscape, which is a Gene Multiple Association Network Integration Algorithm. This software database consists of genomics and proteomics data from several sources, including gene and protein expression profiling studies and also primary and curated molecular interaction networks and pathways. GeneMANIA examinates the weights from data sources built on their predicted value in order to re-establish the query list. Analysing the gene lists and their functions it evaluates related genes which can be in the same pathway or co-expressed or have similar enzymatic function.

The protein-protein interactions (PPI) were studied through the Cytoscape database (version:3.8.2, http://www.cytoscape.org/) (Shannon et al., 2003). Through experimental and *in silico* interactions derived from a gene list, this application provides a combined gene-gene functional interaction network. Furthermore, it uses several association data, such as pathways, protein and genetic interactions, as well as co-expression, co-localization and protein domain similarity data. For clarity reasons, we decided to focus our analysis only on the direct interactions between Ghre and the other genes of our interest. Network

Analyzer in Cytoscape v 3.8.2. was used to analyze the network's topological parameters, with particular focus on the degree centrality value (Maere et al., 2005). The degree centrality of a node (gene) represents the number of edges (interactions) linked to a given node, and nodes having a high degree may represent the hub genes possessing important biological functions.

2.4. Gene Ontology enrichment analysis.

In order to investigate biological processes associated with the gene products under investigation and their interactions, we performed a Gene Ontology (GO) enrichment analysis, by using the Biological Networks Gene Ontology tool (BiNGO, version 3.0.3), another plugin in Cytoscape (Torsello et al., 2012).

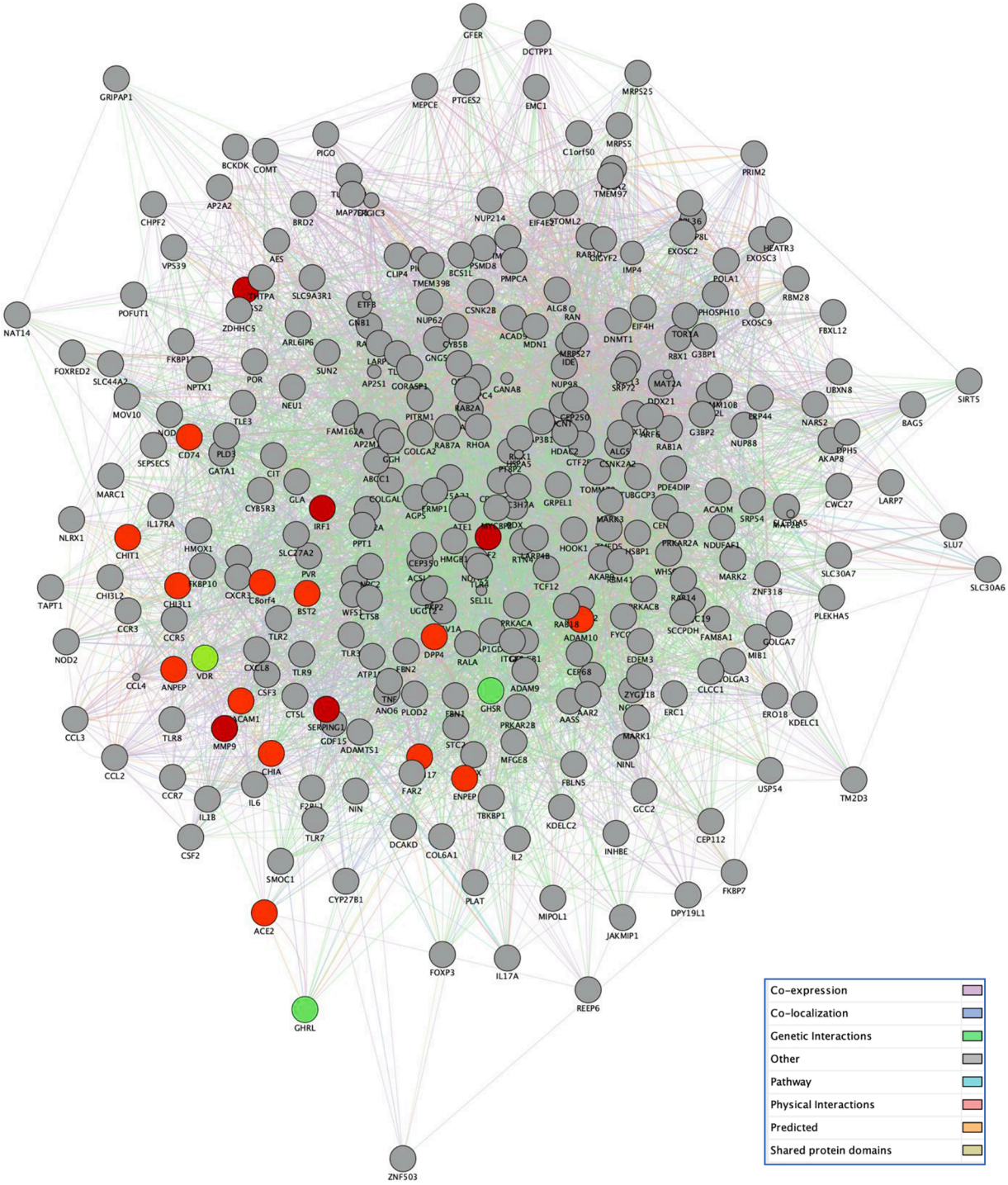

Figure 1. Interaction between our genes of interest and the gene networks of SARS-CoV-2 targeted human proteins. The PPI network made by GeneMANIA shows the interconnection for SARS-CoV-2 targeted human proteins and the genes of interest (nodes) connected (with edges) according to the functional association networks from the databases. The edges indicate the type of evidence supporting each interaction by using different colors: co-expression (light purple), physical interaction (pink), genetic interaction (green), shared protein domains (golden yellow), pathway (light blue), predicted (orange), and co-localization (blue).

The resulting p-values were calculated by the hypergeometric test and corrected by Benjamini and Hochberg False Discovery Rate (FDR) adjustment, with a significance level of $p \leq 0.05$ used as a threshold for statistical significance.

3. Results

Ghre and its receptor's involvement in SARS-CoV-2 was highlighted in our network analysis.

The PPI network obtained from GeneMANIA/Cytoscape was filtered firstly considering the direct connections between our genes of interest, which showed a total of 305 protein-encoding genes involved (Fig. 1).

In the next step, the PPI network was further filtered by focusing on the direct interactions between Ghre, its receptor and other genes in the network (Fig. 2).

The final network consisted of 81 nodes (proteins) and 570 edges (interactions). In particular, the majority of the connections were co-expression interactions (54%), followed by physical interactions (17%), 15% of predicted protein interactions, 8% with co-localization, 5% with genetic interactions and 1% pathway (Fig. 3).

4. Functional enrichment analysis and identification of key genes

GO enrichment analysis was performed using the BINGO plugin in Cytoscape in order to probe and understand the biological process of the entire PPI network. The over-represented GO terms (adjusted $p<0.05$) were mainly associated with the regulation of cell activation, leukocyte activation, peptide transport, and immune response (Table 1).

Among the nodes (genes) in the network, Radixin (RDX) showed the highest connectivity degree (n=101). Interestingly, among the top 10 gene nodes of the network, we also found some genes of interest, such as Interferon Regulatory Factor 2 (IRF2, node degree=93) and the High Mobility Group Box 1 (HMGB1, degree=92) (Table 2).

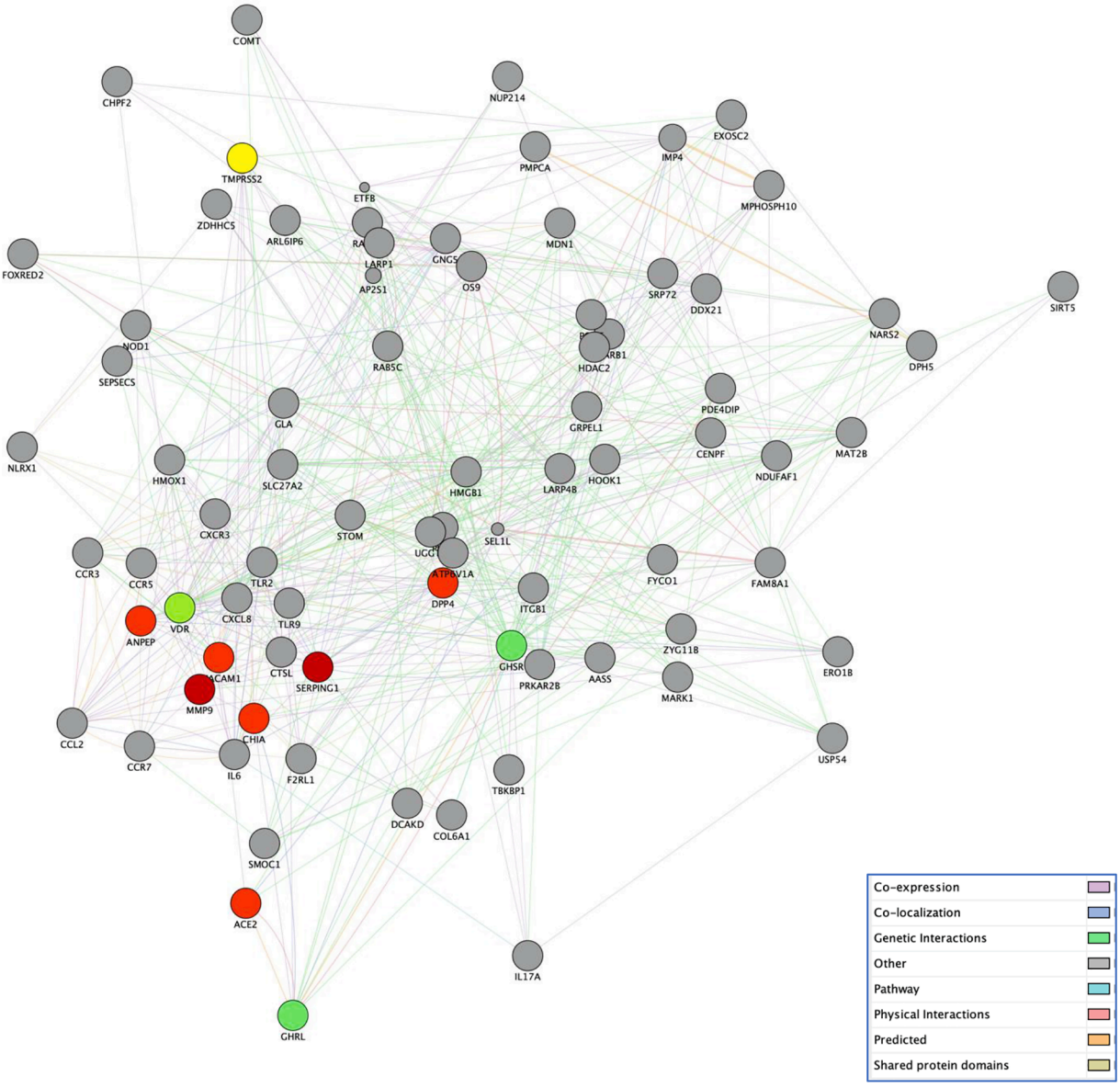

Figure 2. Gene networks of SARS-CoV-2 targeted human proteins interacting with genes encoding proteins involved in inflammatory processes. The PPI network constructed by GeneMANIA shows the relationships between SARS-CoV-2 targeted human proteins, the genes of interest and Ghrelin and GHSR-1A receptor. The edges between nodes (proteins) indicate interactions according to the GeneMANIA database information. The edges indicate the type of evidence supporting each interaction by using different colors: co-expression (light purple), physical interaction (pink), genetic interaction (green), shared protein domains (golden yellow), pathway (light blue), predicted (orange), and co-localization (blue).

Figure 3. Interaction-specific views of gene networks of SARS-CoV-2 targeted human proteins and their interactions with Ghrelin, and the list of genes of our interest. The figure shows different interaction-specific views of the PPI network constructed by GeneMANIA reporting the interconnection between SARS-CoV-2 targeted human proteins and their interactions with Ghrelin, and the list of genes encoding proteins involved in inflammatory processes. The edges between nodes (proteins) indicate interactions based on GeneMANIA database information. For each network, differently colored 'edges' indicate the type of evidence supporting each interaction: co-expression (light purple), physical interaction (pink), genetic interaction (green), shared protein domains (golden yellow), pathway (light blue), predicted (orange), and co-localization (blue).

5. Discussion

In this study, we examined the correlation between human proteins that are involved in the SARS-CoV-2 infection and an endogenous peptide, Ghre together with its receptor, in order to detect whether Ghre could be a potential candidate capable of blocking or ameliorating the pathological effects of the virus and symptom severity. Our network analysis highlighted a direct interaction between Ghre and ACE2, toll-like receptor (TLR) 9, Acidic chitinase (CHIA) and, of course, with its receptor GHSR-1A, which, in turn, links several SARS-CoV-2 interacting human genes: including the chemokine receptor 3 (CXCR3), CCR3, CCR5, CCR7, coagulation factor II (thrombin) receptor-like 1 (F2RL1), VDR, Nucleotide-binding oligomerization domain-containing protein 1 (NOD1), and DDP4. In detail, our analysis showed that Ghre and ACE2 are correlated through physical interactions and similar co-expression patterns. It was previously reported that Ghre and its synthetic analogues are effective ACE-inhibitors (Xia and Lazartigues, 2008). SARS-CoV-2 infects many cell types, including neurons, by interacting with ACE2 (Lambeir et al., 2003), suggesting a good number of possible pathological mechanisms by which CNS functions could be affected.

A genetic interaction link between Ghre signaling, through its receptor GHSR-1A, and DDP4 also emerged. The DPP4 enzyme is a type II transmembrane glycoprotein, which like ACE2 facilitates SARS CoV-2 viral entry. DDP4 is expressed in several tissues, including bone marrow, blood vessels, lungs, spleen, pancreas, kidney, intestines, and epithelial and immune cells (Iacobellis, 2020). DPP-4 inhibitor has anti-

Table 1
GO enrichment analysis Ghrelin Network. GO enrichment analysis of the biological process of the entire PPI networks.

GO I.D.	Background genes	Genes	Description	FDR value	P-value
GO.1902531	1764	47	Regulation of intracellular signal transduction	9.7e-4	7.53e-5
GO.0040012	881	32	Regulation of locomotion	9.76e-5	4.44e-6
GO.0051347	630	26	Positive regulation of transferase activity	9.61e-5	4.35e-6
GO.0006457	214	16	Protein folding	9.57e-6	2.96e-7
GO.0032880	901	40	Regulation of protein localization	9.51e-8	1.27e-9
GO.0065008	3559	96	Regulation of biological quality	9.49e-8	1.21e-9
GO.0051649	1616	57	Establishment of localization in cell	9.49e-8	1.22e-9
GO.0002237	317	23	Response to molecule of bacterial origin	9.49e-8	1.22e-9
GO.0002696	298	17	Positive regulation of leukocyte activation	9.43e-5	4.25e-6
GO.0051246	2668	79	Regulation of protein metabolic process	9.3e-8	1.17e-9
GO.0048523	4454	95	Negative regulation of cellular process	9.3e-4	7.17e-5
GO.2000377	169	11	Regulation of reactive oxygen species metabolic process	9.2e-4	7.1e-5
GO.0023057	1258	37	Negative regulation of signaling	9.2e-4	7.13e-5

Table 2
Top 10 gene nodes of the network. Connectivity degrees PPI network.

Gene name	Degree
RDX	**101**
IRF2	93
HMGB1	92
RAB5C	87
GTF2F2	82
RHOA	82
TOMM70	80
RAB2A	79
COLGALT1	79
TLR2	77

inflammatory, anti-fibrotic, and anti-adipogenic properties, which may be useful in delaying the hyper-inflammatory progression in SARS CoV-2 cases (Iwasaki and Medzhitov, 2004). It has been reported that the inhibition of DPP4 can antagonize hyper-inflammation, macrophage infiltration and polarization. Moreover, DPP4 modulates T-cell activity regulating the immune system, enhancing lymphocyte proliferation and promoting the function of chemokines, cytokines, and growth factors (Iwasaki and Medzhitov, 2004). Changes in DPP4 expression could contribute to cause the complications observed in the severe symptoms of SARS-CoV-2. Taken together, these findings support the possibility that Ghre and GHSR-1A could effectively block the virus-cell interaction and prevent multi-organ injury.

The network analysis also shows a co-expression relationship between Ghre and TLR9 gene. The TLRs are Pattern Recognition Receptors (PRR), and represent a family of transmembrane proteins responsible for adaptive immune responses (Takeda et al., 2003). These receptors trigger innate immune defense against pathogenic infections (Riley and Nelson, 2010). They play a crucial role in SARS-CoV-2 infection, probably because of their ability to recruit immune cells and secrete cytokines (Koga et al., 2014; Kawai and Akira, 2010). Among TLRs, TLR9 mRNA expression was detected on the membrane of endosomes in several tissues including the adrenal gland, brain, heart, liver, lung, small intestine, spinal cord, thymus and trachea (Acosta et al., 2020). The TLR9 signaling pathway can involve the activation of IRF in IRF-signaling endosomes (IRF-SE) or the activation of NF-κB in NF-κB-signaling endosomes (NF-κB-SE).

Specifically, the enrollment of TLR9 and its ligand to IRF-SE could play a critical role in regulating constitutive antiviral gene networks to confer resistance against viral infections by regulating the early expression of IFNs (Ziegler et al., 2021). Mysteriously, interferon activity is significantly reduced in COVID-19 patients (Li et al., 2021). Whereas, the TLR9 signaling pathway, involving NF-κB-SE, induces the expression of pro-inflammatory cytokines that are critical for the pathological conditions of SARS-CoV-2 (Wang et al., 2017).

Studies demonstrating the interaction between Ghre and TLR9 have not yet been carried out. Ghre treatment in human umbilical vein endothelial cells (HUVEC) reduced the secretion of IL-8 and MCP-1 and the activation of NFκB in response to TNF-α stimuli.

It has been reported that Ghre protects against ischemia/reperfusion injury by inhibiting oxidative stress and inflammation by the TLR4/NLRP3 signaling pathway (Deng et al., 2015; Peng et al., 2012). Moreover, Ghre also inhibits the translocation of LPS-induced NF-κB (p65) into the nucleus in murine macrophages, which explains the suppressive effects of ghrelin on pro-inflammatory cytokine production including IL-1 β, IL-6 and TNF- α (Dixit et al., 2004; Waseem et al., 2008; Birra et al., 2020). All these mediators and cell types contribute to the cytokine storm and thrombotic complications underlying the multi-organ pathological condition in patients with severe COVID-19 infections (Pashenkov et al., 2019). In some COVID-19 patients, the activation of TLR9 could be a silent but powerful force inducing hyper-inflammation and thrombotic complications seen in SARS-CoV-2. Considering that TLR9 and Ghre in our network were correlated through a similar co-expression pattern, they may be controlled by the same transcriptional regulation program, functionally related, or represent members of the same path or protein complex. The co-expression of Ghre and TLR9 also suggests that, in specific health conditions of virion endocytosis, Ghre could be an efficient TLR9 agonist, potentiating defense against SARS-CoV-2, as it may control TLR9 recognition to restrain immune cell responses. Severe COVID-19, ARDS and sepsis patients show the activation and increased expression of TLRs and NOD1 and their associated synergisms (Mahla et al., 2013).

Our analysis showed that Ghre genetically interacts also with a member of nucleotide-oligomer domain-like receptors (NLR), the nucleotide-binding oligomerization domain protein (NOD1), which is a ubiquitous intracellular sensor of pathogen-associated molecular patterns (PAMPs), mainly expressed on epithelial and immune cells, identifying bacterial molecules and stimulating an immune response (Moreira and Zamboni, 2012). NOD1 facilitates the assemblage of complexes that induce MyD88 activation via mitogen-activated protein kinase (MAPK) and the NF-κB signaling pathways, resulting in the release of type 1 interferon, as well as the process of cellular autophagy (Di Rosa et al., 2014). It could be supposed that Ghre acts as an antagonist acting upon NOD1 interaction, contravening its activation in the presence of viral infections, probably through TLRs, and in particular TLR9. Interestingly, Ghre is also an anti-inflammatory mediator and promotes the expression of anti-inflammatory cytokines such as IL-10 (Baatar et al., 2011).

Ghre is also co-localized and co-expressed with CHIA, also named

active mammalian endochitinase (AMCase). CHIA is one of the enzymes with true chitinase activity (Ohno et al., 2016). It is expressed by secretory cells, such as gastric chief cells, and preserves activity in the presence of stomach proteases (Zhu et al., 2004). CHIA is produced by secretory epithelial cells lining proximal and distal airways and type 2 alveolar cells and macrophages at sites of Th2 inflammation. CHIA is induced by STAT6-activating signals, such as IL-13 in the course of type 2 immune challenges or allergic lung disease (Madan et al., 2020). It modulates the immune response of T-helper cell type 2 (Th2) stimulating chemokine production by the pulmonary epithelial cells. Like Ghre, CHIA has also an anti-apoptotic effect (Malaguarnera, 2020). The presence of CHIA in the course of SARS-CoV-2 infection and its co-localization and co-expression with Ghre poses new questions and needs to be investigated to understand the relationship between these two proteins in particular on ARDS pathogenesis.

A genetic interaction between VDR and GHSR-1A was also found. It is now well-known that vitamin D modulates both innate and adaptive immune responses through VDR. This receptor is expressed in many cells of the immune system including T and B cells, monocytes, neutrophils, macrophages, and dendritic cells (Zeng et al., 2015). It has been demonstrated that GHSR-1A is expressed in alveolar macrophages (AMs) and that Ghre alleviates septic ARDS in rats (Valle et al., 2021) exerting an antiapoptotic effect on AMs. This effect is mediated by GHSR-1A. Like Ghrelin, vitamin D is able to decrease apoptosis (Malaguarnera, 2020). There is emerging evidence revealing the promising role of vitamin D in preventing the cytokine storm and, consequently, determining outcomes of SARS-CoV-2 (Yilmaz et al., 2015). Therefore, it is conceivable that the immune-modulating Ghre effects could be similar to the immunomodulatory functions exerted by Vitamin D (Fig. 4).

Indeed, both Ghre and VDR can modulate adaptive immunity by promoting Th2 cells-mediated production of cytokines and macrophage response, preventing the release of IL-1, IL-6, IL-8, IL-12 and TNFα by monocytes and increasing the expression of anti-inflammatory cytokines (Malaguarnera, 2020). Antiviral effects of vitamin D include direct interference with viral replication, acting as an immune-modulatory and anti-inflammatory agent. There are still few studies that show that Ghre can play an antiviral role (Ossovskaya and Bunnett, 2004). It would, therefore, be indispensable to investigate whether Ghre also fulfills this function.

GHSR-1A shared a protein domain with F2RL1, a transmembrane G-protein coupled receptor belonging to Protease-activated receptors (PARs), which can be activated by trypsin, and coagulation factors tryptase and matriptase (Mußbach et al., 2016). After its activation, it stimulates vascular smooth muscle relaxation, expands blood vessels, and increases the blood flow lowering blood pressure. F2RL1 gene expression has been associated with the activation and suppression of the inflammatory response and regulates innate and adaptive immunity. Pathophysiologic processes have been related to the impaired activation of F2RL1, such as inflammation, metabolic disorders, pain processing, cardiovascular diseases, neurological disorders, and cancers (Steinhoff et al., 2000). Overexpression of F2RL1 in allergic inflammation of the airway exacerbates eosinophil infiltration into the lumen and hyper-reactivity of the airway, while F2RL1 deletion reduces inflammatory cell infiltration and hyper-reactivity. Moreover, F2RL1 plays a protective role during influenza virus type A infection through IFN-gamma production, reducing excessive recruitment of inflammatory cells to lung alveoli. In both inflammation and neuro-immune communication possible roles for F2RL1 have been described (Romagnani et al., 2001). GHSR-1A may represent a potential regulator of F2RL1. Therefore, alterations in one of these receptors can lead to several disorders related to

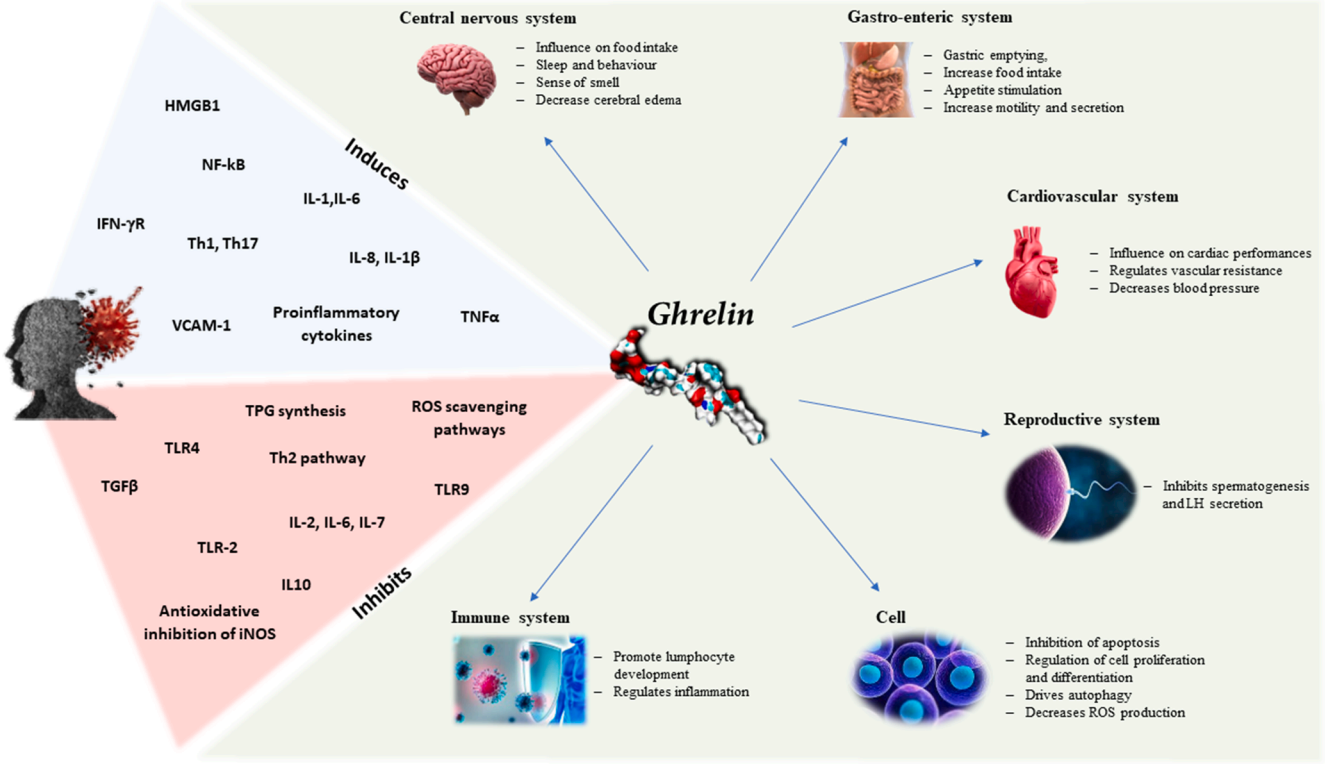

Figure 4. Immunomodulatory functions exerted by Ghrelin. The figure shows a schematic comparison of the immune-modulating effects exerted by Ghre. Abbreviations: NF-kB, nuclear factor kappa-light-chain-enhancer of activated B cells; IFN-γR, Interferon gamma; Th, T helper; HMGB1, High Mobility Group Box 1; VCAM-1, Vascular Cell Adhesion Molecule 1; IL, interleukin; TNFα, tumor necrosis factor-a.; TLR, toll-like receptor, PG, prostaglandins; TGFβ, Transforming Growth Factor-β; iNOS, Inducible nitric oxide synthase; ROS, Reactive oxygen species.

metabolism, blood vessels and neurological problems.

The network analysis revealed a link between GHSR-1A and some chemokines and cytokines. Specifically, we found a shared protein domain between GHSR-1A and CXCR3, CCR3, CCR5 and CCR7. CXCR3 is a chemokine receptor expressed by T-cells and takes part in several activities, such as migration, differentiation and development. CXCR3 is also able to recruit activated T-cells in the sites of the initial inflammatory damage. CXCR3 is activated by three interferon-inducible ligands: CXCL9, CXCL10 and CXCL11. CXCL10 is induced by several innate stimuli that induce IFN-α/β and the adaptive immune cell cytokine IFN-γ, although induction is restricted to IFN-γ (Yamaguchi et al., 2021). CCR3 and CCR5 are known as the receptors of the chemokine CCL5 (Cascieri and Springer, 2000). CCR3 is expressed by eosinophils Th2 cells, mast cells and basophils and it has been demonstrated that its downregulation alleviated nasal cavity symptoms (Malaguarnera, 2020). In particular, CCR5 promotes and regulates trafficking and effector functions of macrophages, T lymphocytes, and immature dendritic cells (Castagna et al., 2005). It also plays an important role in human immunodeficiency virus (HIV) infections, acting as a co-receptor for macrophage-tropic isolates of HIV (Salem et al., 2021). CCR7 regulates adaptive immune cell migration and is expressed by dendritic cells, B and T cells and acts by binding CCL21 and CCL19 (Yang et al., 2020; Phan-Lai et al., 2014; Kohout et al., 2004). Like other chemokine receptors, CCR7 activation induces the phosphorylation of PI3K-/PKB and the recruitment of β-arrestin, as well as the mobilization of intracellular calcium (Koo et al., 2001). Based on this observation, it would be useful to examine in more detail whether GHSR-1A influences the expression of CCR5 and other Th1 effector molecules, such as IFN-gamma and CXCR3, which are able to induce Th2 cytokines such as IL-4, IL-5, IL-13 and chemotactic receptors (CCR3 and CRTH2). Ghre is able to induce an increase in peripheral blood lymphocyte levels and thymic cellularity and differentiation (Hue et al., 2020). Therefore, Ghre, through GHSR-1A, may play a physiologic role in modulating COVID-19 susceptibility to lymphocytes that are all potential targets of viral infection and may decrease proinflammatory cytokine production, improving survival. Blocking CCR5 should promote the rapid reduction of IL-6 plasmatic levels, restore the CD4+/CD8+ ratio, and significantly decrease SARS-CoV-2 plasma viremia. A better understanding of the mechanism whereby GHSR-1A represses CCR5 expression may help to clarify the genetic regulation of CCR5 in COVID-19 target cells and may be useful to develop novel methods to antagonize CCR5 in patients with COVID-19.

6. Concluding remarks

Some patients suffering from severe COVID-19 sometimes develop brain function which may occur as dysregulations in immune functions resulting from neuroendocrine interactions.

It is not yet known what triggers a hyper-inflammatory response in a certain number of COVID-19 patients. Subgroups of patients experience neurocognitive manifestations related to the acute cytokine-mediated hyper-inflammatory process (Cheyuo et al., 2011). Ghre and GHSR-1A take a part in the SARS-Cov-2 network. Our observation that Ghre interacts with receptors involved in SARS-CoV-2 entry, such as ACE2 and DDP4, suggests that Ghre should have an antiviral activity. We found that Ghre is also co-expressed, co-localized or interacts with some PPRs (such as TLR9 and NOD1), with chemokine receptors that trigger or modulate inflammatory response (such as CXCR3, CCR3, CCR5 and CCR7), or with receptors that are able to control inflammation, such as VDR. We can hypothesize that during the SARS CoV-2 infection the activity of Ghre signaling could be reduced, contributing to functional impairment, possibly mediated by cytokines. These data suggest that, in healthy subjects, the good functioning of Ghre and GHSR-1A could block virus-cell interaction, preventing the cytokine storm, multi-organ injury and avoiding neurocognitive sequelae associated with COVID-19. Therefore, we can suppose that there are different possible mechanisms sustaining an exaggerated immune response and neurological manifestation. During COVID-19, in particular, three main features may be taken into consideration. The first possibility is that spike SARS-CoV-2, by binding to its specific receptors ACE2 and DPP4, may compete with Ghre and /or GHSR-1A and inhibits their action. The second scenario is that the agonist action of Ghre against some of the interacting factors examined is weakened, or completely damaged, due to polymorphisms in Ghre or in previously described interacting molecules impeding Ghre or GHS-1A to exert a protective function against SARS-CoV-2. The third hypothesis could be a lack of interaction between VDR and GHSR-1A caused by genetic alterations. However, it may also be possible that vitamin D deficiency or the absence of Ghre acylation, which is a process that confers to the molecule the ability to interact with its specific hypothalamic receptors, may damage their immunomodulatory ability to act in synergy. Interestingly, a hemorrhage-induced brain injury study highlights that Ghre administration alleviates oxidative brain damage, exerting neuroprotective effects through the suppression of the proinflammatory cytokine expression (Fisicaro et al., 2021).

Neurological alterations observed in patients with SARS-CoV-2, although mild, may also precede the onset of the most common symptoms of the disease and may sometimes prove fatal themselves. The symptomatology profile mainly concerns disorders such as dizziness, headache, dulling sensation, hypogeusia, ageusia, hyposmia, anosmia and myalgia. Some of these, such as anosmia and ageusia, are very frequent manifestations, especially in asymptomatic patients, and may also precede respiratory symptoms. The systemic unrestrained immune response, typical of the infection, can have delayed effects on neuronal cells, with manifestations both at the central and peripheral nervous system that, typically, they occur when the acute stage of infection is attenuated [75].

To date, few genetic studies have analyzed the relationship between circulating Ghre and functional SNPs but it could be possible that genetic alterations in the Ghre molecule could be responsible for neurological dysfunctions found in some COVID-19 patients. Since Ghre has multiple cellular and intracellular targets, additional studies are needed to clarify its role in the immune-response against SARS-CoV2 in order to achieve a significant insight into possible future prophylactic and therapeutic strategies for the prevention of this viral infection.

CRediT authorship contribution statement

Cristina Russo: Methodology, Conceptualization, Project administration. **Giovanna Morello:** Methodology, Validation. **Giuliana Mannino:** . **Antonella Russo:** Validation. **Lucia Malaguarnera:** Conceptualization, Project administration.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Competing Interests

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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