APPENDIX C

MANUSCRIPT

EXPLORING G-PROTEIN COUPLED RECEPTOR (GPCR) AS THERAPEUTIC POTENTIAL OF LONG COVID

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ABSTRACT: Post-acute sequelae of SARS-CoV-2 infection, commonly known as long COVID, have been reported to affect patients well after the acute infection with long-lasting symptoms in up to 30% of those who initially presented with a mild-to-moderate form, significantly impacting their quality of life. The symptoms involved a wide range of problems related to physical and mental health and posed great challenges for healthcare systems, requiring an interdisciplinary approach to management. This study aims to explore the possibility of G-protein-coupled receptor-targeting (GPCR) therapeutics for long COVID using advanced bioinformatics tools to identify the association of these receptors with different disease processes and symptom management. Multiple bioinformatics tools and resources were utilized throughout this study such as genetic association, functional and sequence similarities, and molecular interaction analysis. This study identified and documented 21 genes associated with GPCRs for further exploration as potential targets. Notably, GPCR genes such as HRAS, KRAS, GNAQ, and GNA11 were determined to have replicability across this study; therefore, indicating a potential therapeutic target for the pathophysiological mechanisms of long COVID. The FDA-approved drugs targeting these genes include Binimetinib, Cabozantinib, Selumetinib, Panitumumab, Cetuximab, Adagrasib, Tipifarnib, and Sotorasib. Additionally, Naltrexone and Tipifarnib have emerged as potential new treatment options for Long COVID. Further research is required to translate the findings into the development of therapeutics.

Keywords: Long COVID, GPCR, Symptoms and Therapeutic drug

INTRODUCTION

The COVID-19 pandemic has uncovered numerous immediate and long-term health challenges, most commonly being Long COVID, or post-acute sequelae of SARS-CoV-2 infection (PASC) (Sisó-Almirall et al., 2021). This condition, defined by the long-term persistence of symptoms lasting over 12 weeks post-recovery and not explained by an alternative diagnosis, has been officially recognized by the World Health Organization (Gültekin & Özçelik, 2022). Long COVID is elucidated by symptoms persisting or arising for a time period of at least 2 months following the onset of the viral infection and not otherwise explained. Long COVID has been described with a wide variety of symptoms, from severe fatigue and breathing difficulties to important neurological problems, reducing the quality of life, and overloading the burden on persons and healthcare systems (S. T. Liu et al., 2023) (Kamamuta et al., 2022). The proportion of individuals developing Long COVID after mild-to-moderate COVID-19 infection is approximately between 10% and 30%, with the majority experiencing prolonged health complications and suffering from high resource use in healthcare (O' Mahony et al., 2022).

Amid these challenges, the largest gene family encoded in the genome, G-protein-coupled receptors (GPCRs) offer new hope in finding working drugs. GPCRs transduce extracellular signals to mediate cellular actions and are important mediators of a host of physiological processes: immune responses, neurotransmission, and cellular metabolism. The human genome encodes over 800 GPCRs, mediating nearly every form of human physiology, including diseases (Shen et al., 2023). Over one-third of currently FDA-approved drugs target such receptors, and this is not unfounded, given the tremendous potential in therapeutics (D. Yang et al., 2021).

Given the complex symptomatology of Long COVID, cutting across multiple organ systems and biological functions, GPCRs are uniquely placed for exploitation toward therapeutic benefit. It is in this regard that the possibility of repurposing existing drugs targeting GPCRs finds special mention, as it offers a means of rapid translation from bench to bedside using known pharmacodynamics and pharmacokinetics toward rapid availability to the Long COVID patient (X. Li et al., 2021) (Rodrigues et al., 2022). It also gives a pragmatic approach to the management of these complex and diverse presentations in Long COVID, saving time and investment in bringing new therapies to the market. On the other hand, focusing on GPCRs allows the creation of a multitarget strategy to decrease such general effects of Long COVID that would result

in relief and improvement in the quality of life of millions of sufferers. This becomes imperative since the newly found interdisciplinary collaborative innovations need to tackle these widespread and long-lasting impacts of the COVID-19 pandemic.

METHODOLOGY

This study on genetic association and potential therapeutic targets of GPCR in Long COVID used a range of bioinformatics tools and databases, such as BioRxiv, Guide to Pharmacology, ToppGene Suite, EMBOSS Needle, PA-Pred2, UCSF Chimera, and PyRx. It thereby permitted the retrieval of comprehensive data, right from functional and sequence analysis to molecular interactions simulated to identify key genes and pathways for the GPCR and Long COVID. The overall approach is summarized to be streamlined in a flowchart, as depicted in Figure 3.1.

Data Retrieval

The gene association data to Long COVID were taken from the very comprehensive study of Das and Kumar (2023), which derived the relationship among 331 genes with 255 symptoms of Long COVID from a systematic review and meta-analysis of a large compilation of published literature and reviews of PubMed, LitCovid, Embase, and other related databases. The study meticulously cataloged the associations of each gene with specific Long COVID symptoms from peer-reviewed journals, thus providing comprehensive genetic information about the disease Long COVID (Das & Kumar, 2023). Simultaneously, a vast data set from the GtoPdb database of 3,142 G-protein-coupled receptors with curated pharmacological, chemical, and genomic data on human drug targets was sourced to investigate the presence of any role of GPCRs in Long COVID. Preliminary in silico analysis reported the role of GPCRs in Long COVID. The dataset was cleaned of duplications and classification discrepancies to cut down to 2,949 GPCR sequences. Data collection and curation followed appropriate hygiene practices and provided the basis for in-depth analysis of the genetic and receptor-based mechanisms of Long COVID.

Functional Similarity Association

The ToppGene Suite was used to functionally link Long COVID genes to G-protein-coupled receptors. A tool that helps to perform gene list enrichment analysis and rank candidate genes using functional annotation and network data jointly. Two

datasets were prepared: a training set of all the Long COVID-associated genes and a test set of GPCR genes identified by their HGNC symbols. The training set was meant for the development of a gene function, interaction, and pathway detailed profile that was linked to Long COVID and used as a basis for analysis. For the test set, we aimed to find overlaps and functional links to genes associated with Long COVID. ToppGene analysis was run with default parameters for homogeneity and led to common pathways, followed by download in Excel format for readability of pathways and how GPCR modulation might influence Long COVID.

Sequence Similarity

Long COVID-related genes and G-protein Coupled Receptors (GPCRs) were retrieved in FASTA format using the National Center for Biotechnology Information (NCBI). Using the EMBOSS Needle tool, the percentage similarity between the query sequence of target Long COVID-related genes and G-protein Coupled Receptors (GPCRs) was ascertained. This tool does pairwise alignment of two nucleotide or protein sequences. It returns a detailed report on the quality of the alignment produced for two sequences, and it can explain the conservation and similarity percentage of the sequences. The Long COVID-related genes and the genes coding for GPCRs were aligned with human genes to assess the degree of conservation of the said genes in humans. All those GPCR genes that showed a minimum of 60% similarity in sequence with the Long COVID-related genes were shortlisted for further study. This percentage of similarity was considered because it would be relevant biologically, and one could predict the exact function and association of the gene in the backdrop of Long COVID.

Protein-Protein Affinity Prediction

The pairs of Long-COVID genes and GPCRs with a similarity percentage larger than 60% are further selected for binding free energy analysis between these genes and GPCR sequences using the PA-Pred2 tool for this protein-protein affinity (PPA-Pred) prediction. This tool is particularly useful as it approximates the strength of the interaction between a pair of proteins and tries to identify those that would produce the least binding free energy. Thus, the top 3 protein pairs for each pathway with the lowest binding free energy were chosen for the next stage of analysis. This is critical because lower energy values typically signify more stable and biologically significant interactions, which are essential for understanding the functional dynamics of protein complexes. PA-Pred2, which applies sophisticated algorithms to derive fast and accurate predictions for the affinity between protein-proteins, aided in isolating those

major interactions that are to be participating in the molecular mechanisms for long COVID more specifically through pathways of GPCR sequences.

Network Analysis

The relations between long COVID genes and GPCRs were queried with the help of Cytoscape, a bioinformatics software for the visualization of complex networks and their integration with any sort of attribute data. More specifically, the analysis was focused on the main interactions within the network, with the use of the Cytoscape plugin CytoHubba, ranking the top 10 nodes based on the Maximal Clique Centrality (MCC). MCC was used in such a way that it effectively pointed out the most influential nodes in the network, most of which are important to the biological processes under this study. CytoHubba furthers this analysis by providing a set of powerful network analysis tools that help to decipher the most significant nodes (genes and proteins) that may act as potential drug targets or may be pivotal in the pathology of the disease. This will allow the detailed examination of the interactome of the long COVID genes and GPCRs within the context of broader biological networks, potentially identifying key components of the disease mechanism and providing insights into new therapeutic avenues.

Molecular Docking

Molecular docking was performed to find the best molecular orientation and form stable complexes. One of the most important features of drug design is the intermolecular interaction between drugs (ligands) and their targets (receptors) to form stable complexes. The 3D structures of Long COVID-related genes and GPCR were downloaded from PubChem in PDB format since all docking simulations required an exact. The necessary modification with UCSF Chimera in receptor preparation was used for the refinement of structures. All extra material and nonstandard residues were deleted at first. Docking simulation was carried out in PyRx software by integrating AutoDock Vina, which was used for high-throughput virtual screenings. Two sets of dockings were then performed: first, in which PPA Pred selected the top three genes from each pathway, and second, in which Cytoscape selected the top ten genes along with the CytoHubba plugin. All receptor and ligand structures were converted to PDBQT format to be docked. A fully automated docking of ligand molecules within the PyRx was carried out to find the best orientation and position of the ligands to reduce the binding energy. It was also used to sort results according to the lowest binding energy and analysis in UCSF Chimera. This step was vital to visualize the most stable

receptor-ligand complexes and rationalize physical interactions and binding conformations to provide an insight into the potential efficacy of these complexes as therapeutic agents against Long COVID.

Post-Docking Analysis

Further, after the molecular docking of the genes, the genes targeted by the GPCR genes are obtained given the ligand interaction. Then, the top docked genes are listed in an integrative table, and the corresponding symptoms, relevant HPO IDs, and repurposed FDA-approved drugs to mitigate symptoms were obtained from Das & Kumar (2023) to identify further possible treatment from this study. For this purpose, the virus-host interactions with SARS-CoV-2 have been classified and tabulated using the Enrichr tool with the adjusted p-value stating the significance of the interaction. All these will be very handy to conceptualise Long COVID pathogenesis and to pick up the possible therapeutic targets.

RESULTS

Notably, a study by Das and Kumar (2023) recently found a complex linkage among 331 long COVID genes and 255 associated symptoms and conditions, such as those related to autoimmunity and cardiovascular diseases. The importance of these gene-symptom mapping in understanding these genetic influences on the risk and severity of long COVID had been previously raised. Among the others, 2949 GPCR genes sourced from the GtoPDB database have been of considerable help in revealing the genetic and pharmacological treatment mechanisms of Long COVID and giving insights into symptoms and possible therapeutic targets.

The analysis of functional enrichment for long COVID with the GPCR genes found a statistically significant relationship for both groups, focusing on signal transduction over four major pathways (Table 1.1). The ToppGene Suite analysis underlines the highly significant functional enrichment of "WP G Protein Signaling Pathways" and "Reactome G Protein-Mediated Events," which suggests the close relationship of long COVID genes with GPCR signaling components. The genes HRAS, GNA11, GNAQ, KRAS, and PRKACA were over-represented in long COVID relative to the GPCRs in a context of possible overlapping GPCR-mediated signaling by symptom severity and persistence (Table 1.2).

Further scrutiny of 275 initially mapped GPCR genes with 30 long COVID-associated genes across four pathways showed a refined focus on 111 GPCR genes of higher biological significance after applying a 60% sequence similarity threshold (Table 1.3). This selective approach revealed major reductions in gene counts, pointing to specific pathways where only a few genes had potential disease mechanisms. The high sequence conservation among critical genes such as the RAS and GNA families and the protein kinase family indicated functional interactions crucial in long COVID (Table 1.4).

Moreover, Cytoscape software, MCC module, and CytoHubba plug-in further assisted in identifying the statistically significant genes, GNA11 and GNAQ, respectively, in which both played pivotal roles in the GPCR signalling pathway associated with the pathobiology of long COVID (Figure 4.1 and Figure 4.2). Studies on molecular docking in terms of strength and stability concerning protein-ligand complexes were validated by these observations, thus emphasizing high binding affinities and potential targets in the case of Long COVID in therapeutic management. Furthermore, HRAS and KRAS were also identified within the WP G protein signalling pathway, attributing them to have their roles in terms of cell growth and cell survival, while PRKAR1A and PRKAR1B, the protein kinase A regulatory subunits, were identified to have wide effects on numerous pathways that would modulate cellular functions affecting the long COVID pathology (Table 1.5) (Figure 4.3 and Figure 4.4).

As the final step of this study, the post-docking analysis further illustrates the central roles that GPCR genes and their ligands play in the pathogenesis leading to Long COVID. Some of the remarkable characteristics of these genes pertain to how they might be helping the SARS-CoV-2 virus by boosting its replication or dampening the host's immune responses (Table 1.10). It further associated the genes with clinical symptoms and discussed possible therapeutics, further pointing toward continued research and clinical trials to be able to treat the long-term effects of Long COVID. Thorough research has not only pinpointed critical gene interactions but also suggested repurposing FDA-approved drugs associated with certain genes, including those of the HRAS, KRAS, GNAQ, and GNA11 variety, with symptoms such as edema, pain, and venous thrombosis and suggested treatment with Binimetinib, Cabozantinib, Selumetinib, Panitumumab, Cetuximab, Adagrasib, Tipifarnib, and Sotorasib. (Tables 1.6 – Table 1.9)

DISCUSSION

Initially, the results indicated 21 significant genes connected with the G protein-coupled receptors, indicative of their potential therapeutic targets for Long Covid. The said genes include members of the RAS family (HRAS, KRAS), the G protein family (GNAQ, GNAZ, GNA11, GNA13, GNA14, GNA15, GNAO1, GNAT3), a series of PRK—protein kinases (PRKCA, PRKCB, PRKCG, PRKACA, PRKACG, PRKACB, PRKAR1A, PRKAR1B) and calcium/calmodulin-dependent protein kinases (CAMK2A, CAMK2B, CAMKK1).

These gene groups are highly involved in general cellular and physiological processes. The Ras family of small GTPases involves molecular switches controlling cell growth, differentiation, and survival activity (Killoran & Smith, 2019). The G proteins bridge signal transduction from receptors to a large variety of effectors to influence processes ranging from sensory perception to hormonal responses (Hepler et al., 1993). Protein kinases are enzymes that modify proteins by phosphorylation, thus keying on and off important cellular functions, such as gene expression, secretion, growth, and apoptosis (Taylor et al., 2021). Lastly, CAMKs play a role in regulating several steps within the cell cycle, memory forming, and muscle contraction—processes very important to the functioning of living organisms—in addition to the many other functions they perform. These gene families dispose of the highly evolved cellular signalling networks of utmost importance in healthy and disease conditions, thus representing highly valuable therapeutic targets (Yasuda et al., 2022).

However, only 12 among these genes have been identified as pertinent to Long COVID symptoms since they participate in the receptor G protein-coupled cascade. These include HRAS, KRAS, GNAQ, GNA11, GNAO1, PRKCB, PRKCG, PRKACA, PRKACG, PRKAR1A, CAMK2A, and CAMK2B. As a whole, these genes account for the varied and long-lasting COVID symptoms in several systems. For example, an HRAS mutation can lead to anaphylactic shock and alopecia, while KRAS disruption leads to chronic pain and the presence of autoimmune problems. GNAQ and GNA11, on the other hand, lead to ocular pain and other vascular issues while GNAO1 can lead to neurotransmission problems, hence neuropsychiatric complications. On the other hand, PRKCB and PRKCG play important roles in inflammation and synaptic signalling, which can account for pleural thickening and neurologic symptoms, respectively. On the other hand, PRKACA and PRKACG also play an important role in the process of cellular signalling and control of reproductive functions, while PRKAR1A is implicated in metabolic and cognitive dysfunctions, and CAMK2A and CAMK2B are

involved in neuropsychiatric and gastroesophageal disorders. It is through these interrelations that the potential to target these genes for Long COVID interventions is realized.

Consequently, the symptoms of Long COVID persist, because of immune dysregulation, in which multiple proteins and tissue regions are targeted by autoantibodies, leading to gross inflammation of multiple tissues and, ultimately, organ failure. With multisystem activity, these antibodies can cause anaphylactic shock or gut-related symptoms by targeting G-protein-coupled receptors, neuronal proteins such as CAMK2A and CAMK2B, which ultimately leads to neuropsychiatric disorders, or hormonal regulators such as PRKACA and PRKAR1A and disrupt endocrine function and cause metabolic dysregulation. Such symptom persistence and severity are features of chronic fatigue syndrome and rheumatoid arthritis, amongst autoimmune illnesses, that clarify the imperative of precise therapy targeting immune system modulation to enhance outcomes in the spectrum of long COVID and other post viral conditions.

Furthermore, these identified GPCRs genes are highly involved in immune modulation, inflammation, and respiratory functions, which are very key in the pathology and recovery of Long COVID. To give examples, some of these identified genes are associated with the RAS/MAPK signaling pathways, such as HRAS and KRAS, central in immune cell proliferation and survival against viral infections, including the causative agent of COVID-19. Meanwhile, all three members of the Protein Kinase C family belong to the immune and inflammatory response mediators: PRKCA, PRKCB, and PRKCG. Members of the GNAQ family—GNA11 and GNA13—modify the movement of immune cells and the production of cytokines. GPCR pathways also regulate some very important functions in the respiratory system, including the level of airway muscle contraction and mucosal secretion. These are central to the management of Long COVID with respiratory symptoms. CAMK2A and CAMK2B regulate calcium signaling in inflammation processes, which is central in the characteristic chronic inflammation of Long COVID. Although these identified functions regarding these genes are important for the development of targeted treatments to enhance the management of Long COVID by improving symptoms such as cognitive impairments and fatigue, some gaps need to be shown on the specific impacts of some genes.

Based on this, the Drug repurposing for the Long COVID symptoms is targeting certain genes with the FDA-approved drugs for the management of the symptoms

(Figure 5.1). Precisely, the medications Verteporfin and Selumetinib are found to target the gene GNAQ, influencing symptoms of ocular and cellular proliferation, whereas Binimetinib, Cabozantinib, and Selumetinib target GNA11 for dermatologic and ocular issues. For the gene KRAS, there is Panitumumab, Cetuximab, Adagrasib, and Sotorasib. All these medications are effective for autoimmunity and cellular growth. Another set of rich promise came through the targets Tipifarnib for immune modulation and tissue repair, whereas G-protein coupled receptor signaling is important for immune and inflammatory responses (Odeniyide et al., 2022). Moreover, the anti-inflammatory and immune-regulatory effects of Naltrexone, which was originally used to treat addiction, are currently a new therapeutic avenue in easing symptoms associated with long COVID by modifying opioid receptors and important signaling pathways during the therapy (Choubey et al., 2022). It must be noted that not all genes have drugs of high value/interest because of the specificity of the gene action concerning disease mechanisms. High clinical testing is needed the guarantee safety and efficacy since, as discussed, Long COVID is a complex problem, and personalized medicine approaches are needed.

An observation that should be made from these results is noting that identification of the genes such as HRAS, KRAS, GNAQ, and GNA11 has been consistent across the studies, testifying to the credibility of methodologies and suggesting a central role in the underlying pathology and therapy of Long COVID. These four genes also represent the only genes compared among the rest which are linked to FDA-approved drugs, underlining the enormous potential these genes have in clinical applications.

CONCLUSION

This identified 21 genes associated with GPCRs, which could be the potential targets for treating Long COVID. The results have been validated with the robust methodology of molecular docking which resulted in high binding affinities but only 12 genes were found to be associated with several Long COVID symptoms. Major genes such as HRAS, KRAS, GNAQ, and GNA11 have been obtained throughout different research phases, which gives more credit to the findings and their possible importance in the pathology and treatment of Long COVID. Of those, several FDA-approved drugs are well documented, such as Binimetinib, Cabozantinib, Selumetinib, Panitumumab, Cetuximab, Adagrasib, Tipifarnib, and Sotorasib. Among these, Selumetinib is shown to target both GNAQ and GNA11, while Sotorasib targets both KRAS and HRAS.

Moreover, drugs like Naltrexone and Tipifarnib are advised for GPCR targeting on account of their immune-regulatory and anti-inflammatory characteristics, ability to aid tissue repair as well as modulation of essential signaling pathways. It is, therefore, recommended that detailed functional studies of these genes, including gene silencing and overexpression, and longitudinal studies be carried out to monitor changes throughout the disease. It also recommends the expedited process of drug discovery through high-throughput screening and AI modelling while paralleling the repurposing of FDA-approved drugs. For this, global research collaboration, data-sharing, and standardization of study protocols are required to make it efficient and the result reproducible. Targeted funding help in navigating regulatory pathways and fostering interdisciplinary collaboration will be necessary for fully understanding Long COVID and the development of appropriate treatments. Collaboration with patient advocacy groups is advised to align research and therapies with patient needs.

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FIGURES

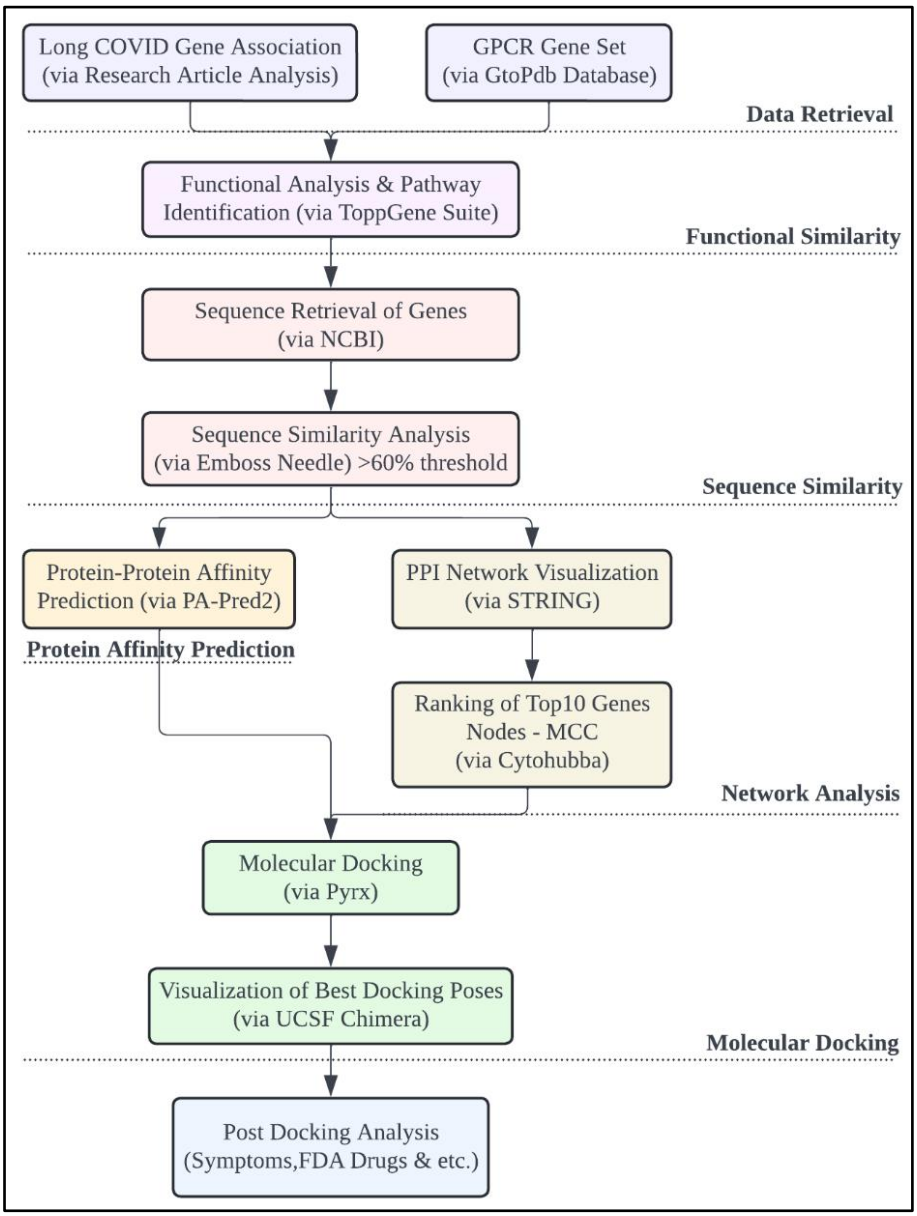

Figure 3.1: The overall workflow of this study.

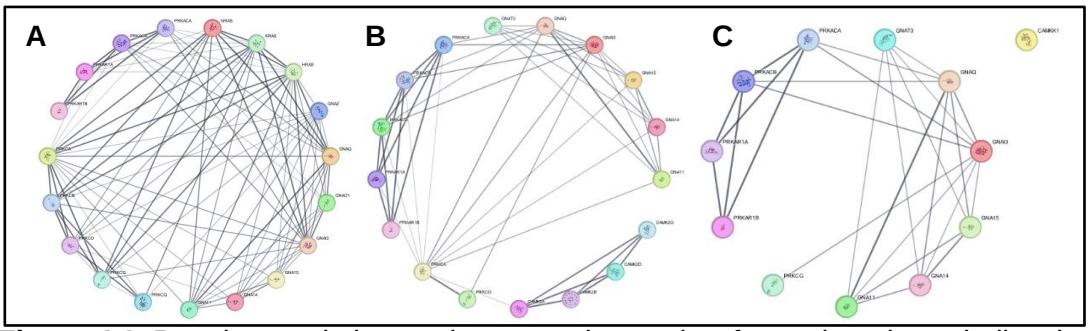

Figure 4.1: Protein-protein interaction network overview for each pathway built using STRING in Cytoscape. **(A)** MM15882 & M39426 **(B)** M26911 **(C)** MM14496.

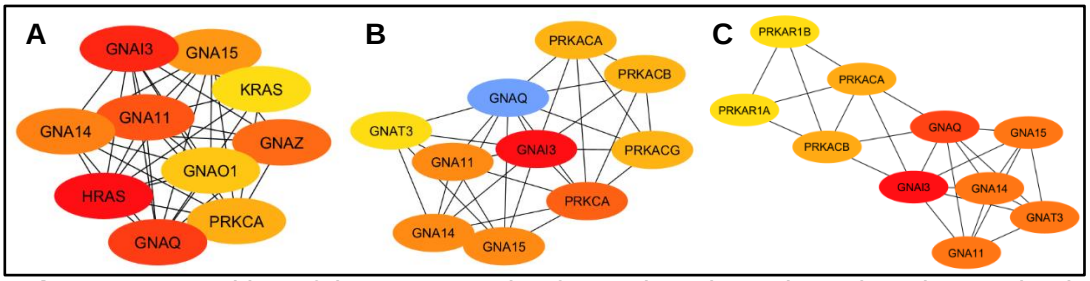

Figure 4.2: Ranking of the top 10 nodes for each pathway based on the Maximal Clique Centrality (MCC) with Cytohubba plugin. **(A)** MM15882 & M39426 **(B)** M26911 **(C)** MM14496.

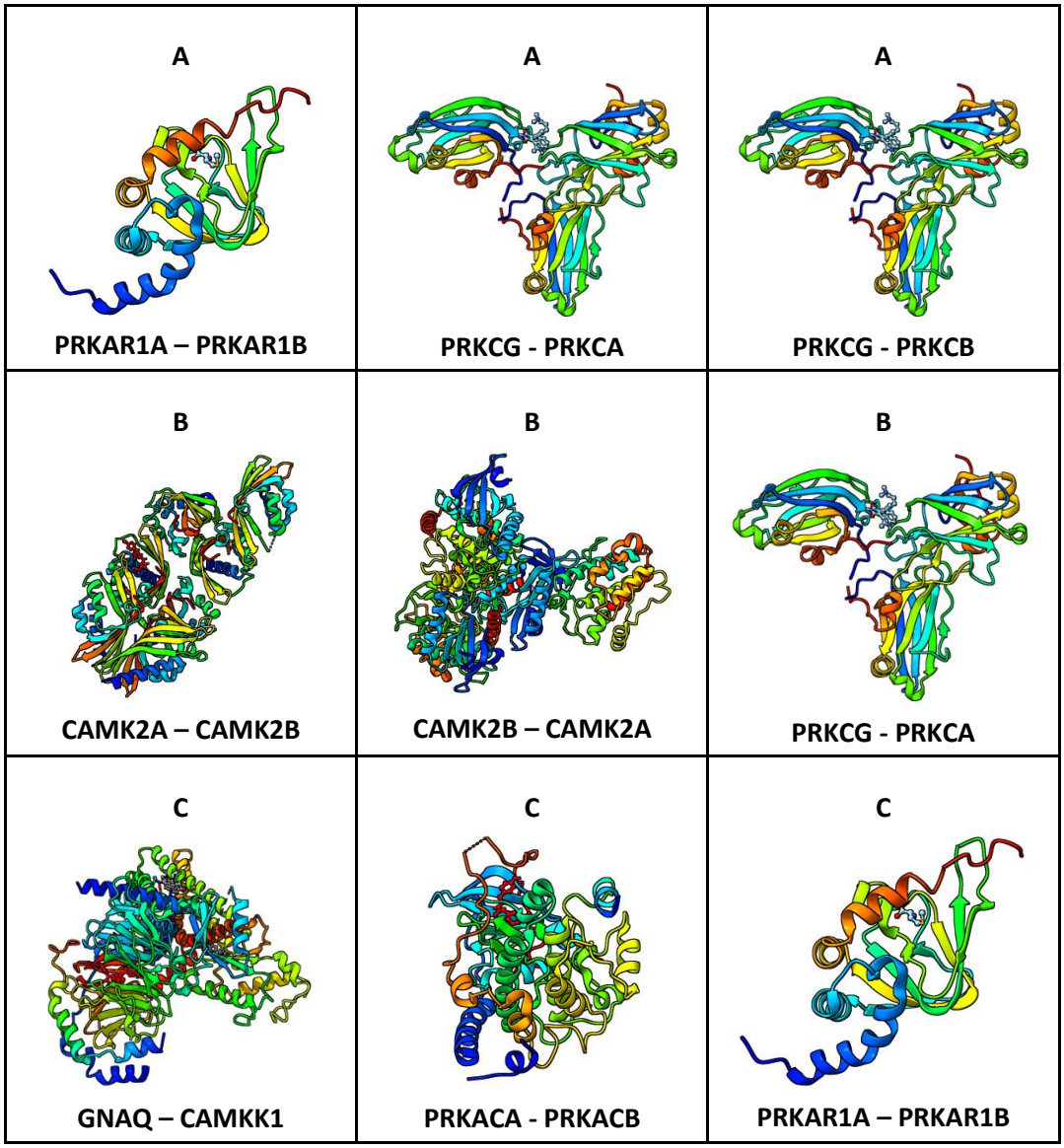

Figure 4.3: Best pose of the Top 3 PPA-Pred docked genes for each pathway. **(A)** MM15882 & M39426 **(B)** M26911 **(C)** MM14496

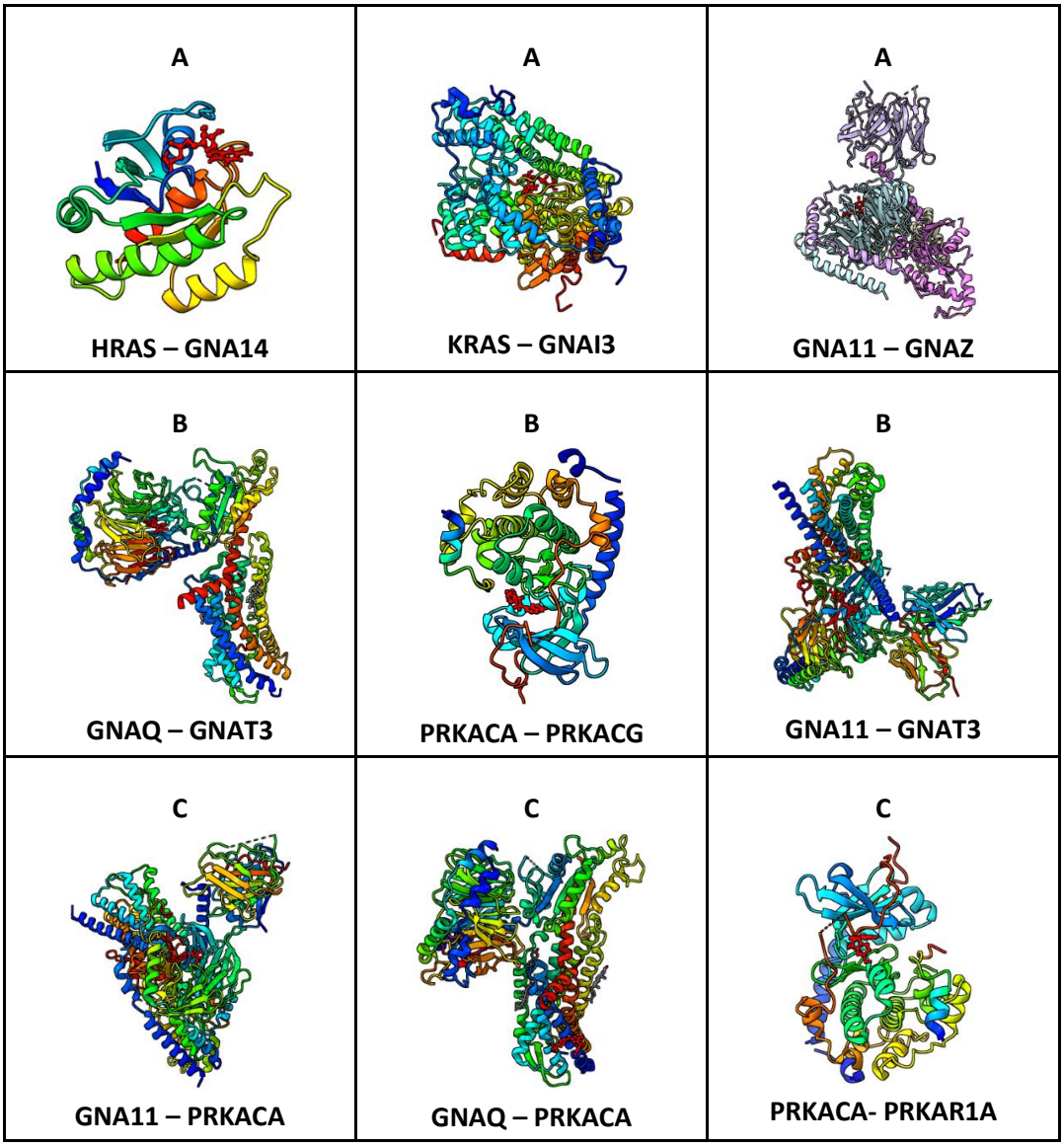

Figure 4.4: Best pose of the Top 3 Cytoscape docked genes for each pathway. **(A)** MM15882 & M39426 **(B)** M26911 **(C)** MM14496

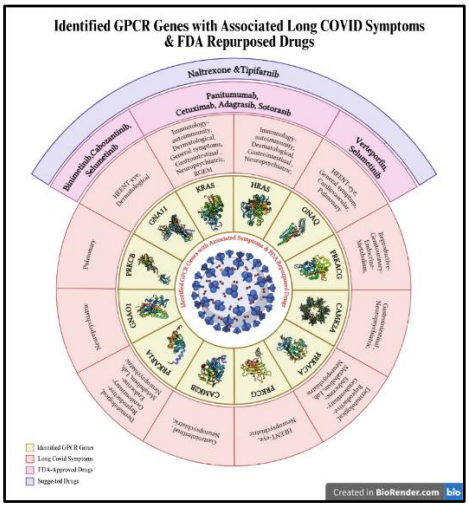

Figure 5.1: Identified GPCR genes with associated Long Covid Symptoms & FDA Repurposed Drugs, created with BioRender.com.

TABLES

Table 1.1: Summary of Long COVID Genes and Associated GPCR Genes Across Four Identified GPCR Pathways in ToppGene

Pathway ID	Name	Gene from Input (Long COVID)	Gene from Annotations (GPCR)
MM15882	Wp G Protein Signaling Pathways	9	90
M26911	Reactome G Protein Mediated Events	7	54
M39426	Wp G Protein Signaling Pathways	9	91
MM14496	Reactome G Protein Mediated Events	5	40
	Total	30	275

Table 1.2: Long COVID Genes and Associated GPCR Genes Across Four Identified GPCR Pathways

Pathway ID	Gene from Input (long covid)		Gene from Annontations (GPCR)	
	Gene Id	Symbol	Gene ID	Symbol
MM15882	3265	HRAS	9472	AKAP6
	2767	GNA11	387	RHOA
	2776	GNAQ	23683	PRKD3
	3845	KRAS	3845	KRAS
	4893	NRAS	5136	PDE1A
	5566	PRKACA	196883	ADCY4
	5573	PRKAR1A	5137	PDE1C
	5582	PRKCG	6548	SLC9A1
	5588	PRKCQ	8852	AKAP4
			5141	PDE4A
			5142	PDE4B
			5143	PDE4C

9495	AKAP5
5144	PDE4D
5530	PPP3CA
94235	GNG8
4893	NRAS
5533	PPP3CC
10142	AKAP9
5150	PDE7A
10270	AKAP8
9630	GNA14
5151	PDE8A
801	CALM1
5153	PDE1B
55970	GNG12
805	CALM2
8622	PDE8B
10672	GNA13
3760	KCNJ3
9138	ARHGEF1
51764	GNG13
10681	GNB5
5566	PRKACA
5567	PRKACB
3265	HRAS
5573	PRKAR1A
10566	AKAP3
5575	PRKAR1B

5576	PRKAR2A
5577	PRKAR2B
5578	PRKCA
5579	PRKCB
5580	PRKCD
5581	PRKCE
5582	PRKCG
11214	AKAP13
2767	GNA11
11215	AKAP11
5583	PRKCH
5584	PRKCI
2768	GNA12
11216	AKAP10
2769	GNA15
2770	GNAI1
2771	GNAI2
5331	PLCB3
5587	PRKD1
5588	PRKCQ
2773	GNAI3
5590	PRKCZ
2774	GNAL
2775	GNAO1
2776	GNAQ
2778	GNAS
2781	GNAZ

			6237	RRAS
			2782	GNB1
			2784	GNB3
			2785	GNG3
			2786	GNG4
			2787	GNG5
			2788	GNG7
			8165	AKAP1
			2790	GNG10
			2791	GNG11
			2792	GNGT1
			2793	GNGT2
			107	ADCY1
			27115	PDE7B
			108	ADCY2
			109	ADCY3
			111	ADCY5
			112	ADCY6
			113	ADCY7
			114	ADCY8
			115	ADCY9
			9590	AKAP12
			9465	AKAP7
			3708	ITPR1
M26911	2767	GNA11	5136	PDE1A
	2776	GNAQ	196883	ADCY4
	815	CAMK2A	10768	AHCYL1

816	CAMK2B	5137	PDE1C
5566	PRKACA	10645	CAMKK2
5573	PRKAR1A	156	GRK2
5582	PRKCG	9630	GNA14
		84254	CAMKK1
		5153	PDE1B
		801	CALM1
		814	CAMK4
		815	CAMK2A
		816	CAMK2B
		817	CAMK2D
		818	CAMK2G
		5566	PRKACA
		5567	PRKACB
		5568	PRKACG
		23236	PLCB1
		5573	PRKAR1A
		5575	PRKAR1B
		346562	GNAT3
		5576	PRKAR2A
		5577	PRKAR2B
		5321	PLA2G4A
		5578	PRKCA
		5580	PRKCD
		5582	PRKCG
		2767	GNA11
		26960	NBEA

			2769	GNA15
			2770	GNAI1
			5330	PLCB2
			2771	GNAI2
			5331	PLCB3
			5332	PLCB4
			2773	GNAI3
			2774	GNAL
			2776	GNAQ
			5594	MAPK1
			1385	CREB1
			107	ADCY1
			108	ADCY2
			109	ADCY3
			5613	PRKX
			111	ADCY5
			112	ADCY6
			113	ADCY7
			114	ADCY8
			115	ADCY9
			3708	ITPR1
			3709	ITPR2
			3710	ITPR3
			3838	KPNA2
M39426	3265	HRAS	9472	AKAP6
	2767	GNA11	387	RHOA
	2776	GNAQ	23683	PRKD3

3845	KRAS	3845	KRAS
4893	NRAS	5136	PDE1A
5566	PRKACA	196883	ADCY4
5573	PRKAR1A	5137	PDE1C
5582	PRKCG	6548	SLC9A1
5588	PRKCQ	8852	AKAP4
		5141	PDE4A
		5142	PDE4B
		5143	PDE4C
		9495	AKAP5
		5144	PDE4D
		5530	PPP3CA
		94235	GNG8
		4893	NRAS
		5533	PPP3CC
		10142	AKAP9
		5150	PDE7A
		10270	AKAP8
		9630	GNA14
		5151	PDE8A
		801	CALM1
		5153	PDE1B
		55970	GNG12
		805	CALM2
		8622	PDE8B
		10672	GNA13
		3760	KCNJ3

9138	ARHGEF1
51764	GNG13
10681	GNB5
5566	PRKACA
5567	PRKACB
3265	HRAS
5573	PRKAR1A
10566	AKAP3
5575	PRKAR1B
5576	PRKAR2A
5577	PRKAR2B
5578	PRKCA
5579	PRKCB
5580	PRKCD
5581	PRKCE
5582	PRKCG
11214	AKAP13
2767	GNA11
11215	AKAP11
5583	PRKCH
5584	PRKCI
2768	GNA12
11216	AKAP10
2769	GNA15
2770	GNAI1
2771	GNAI2
5331	PLCB3

5587	PRKD1
5588	PRKCQ
2773	GNAI3
5590	PRKCZ
2774	GNAL
2775	GNAO1
2776	GNAQ
2778	GNAS
2781	GNAZ
6237	RRAS
2782	GNB1
2784	GNB3
2785	GNG3
2786	GNG4
2787	GNG5
2788	GNG7
8165	AKAP1
2790	GNG10
2791	GNG11
2792	GNGT1
2793	GNGT2
107	ADCY1
27115	PDE7B
108	ADCY2
109	ADCY3
111	ADCY5
112	ADCY6

			113	ADCY7
			114	ADCY8
			115	ADCY9
			9590	AKAP12
			9465	AKAP7
			3708	ITPR1
			2783	GNB2
MM14496	2767	GNA11	23236	PLCB1
	2776	GNAQ	5573	PRKAR1A
	5566	PRKACA	346562	GNAT3
	5573	PRKAR1A	5575	PRKAR1B
	5582	PRKCG	5321	PLA2G4A
			5577	PRKAR2B
			5580	PRKCD
			5582	PRKCG
			2767	GNA11
			5136	PDE1A
			196883	ADCY4
			2769	GNA15
			5137	PDE1C
			5330	PLCB2
			2770	GNAI1
			2771	GNAI2
			5331	PLCB3
			5332	PLCB4
			2773	GNAI3
			10645	CAMKK2

2774	GNAL
2776	GNAQ
5594	MAPK1
156	GRK2
9630	GNA14
84254	CAMKK1
801	CALM1
5153	PDE1B
805	CALM2
808	CALM3
107	ADCY1
108	ADCY2
109	ADCY3
111	ADCY5
112	ADCY6
113	ADCY7
114	ADCY8
115	ADCY9
5566	PRKACA
5567	PRKACB

Table 1.3: Summary of GPCR and Long COVID Genes after sequence similarity analyses

Pathway ID	Name	Gene from Input (Long COVID)	Gene from Annotations (GPCR)
MM15882	Wp G Protein Signaling Pathways	9	33
M26911	Reactome G Protein Mediated Events	7	27
M39426	Wp G Protein Signaling Pathways	9	33
MM14496	Reactome G Protein Mediated Events	5	18
	Total	30	111

Table 1.4: Results of Sequence Similarity Analysis (>60% Threshold) and Protein-Protein Affinity Prediction (3 Lowest Binding Affinities per Gene)

	ToppGene Pathway				Sequence Similarity	Protein-protein Affinity	
Pathway ID	Gene from Input (long covid)		Gene from Annontations (GPCR)		Similarity Percentage	Binding Free Energy (kcal/mol)	Dissociation Value (M)
	Gene Id	Symbol	Gene ID	Symbol			
MM15882	3265	HRAS	3845	KRAS	94.2	-8.55	5.33e-07
			4893	NRAS	92.6	-8.37	7.26E-07
			3265	HRAS	100.0	-8.17	1.02E-06
	2767	GNA11	9630	GNA14	90.9	-6.51	1.67E-05
			2767	GNA11	100.0	-6.39	2.05E-05
			2769	GNA15	71.9	-7.25	4.81E-06
			2773	GNAI3	64.5	-7.46	3.40E-06
			2775	GNAO1	64.8	-7.84	1.78E-06
			2776	GNAQ	96.1	-6.38	2.11E-05

			2781	GNAZ	61.3	-7.75	2.06E-06
	2776	GNAQ	9630	GNA14	90.0	-6.49	1.73E-05
			2767	GNA11	96.1	-6.38	2.11E-05
			2769	GNA15	70.9	-7.21	5.12E-06
			2773	GNAI3	64.0	-7.44	3.51E-06
			2775	GNAO1	64.0	-7.82	1.84E-06
			2776	GNAQ	100.0	-6.36	2.18E-05
			2781	GNAZ	60.3	-7.74	2.12E-06
	3845	KRAS	3845	KRAS	100.0	-7.75	2.07e-06
			4893	NRAS	94.2	-7.22	5.05E-06
			3265	HRAS	94.2	-8.55	5.33E-07
	4893	NRAS	3845	KRAS	94.2	-7.57	2.82E-06
			4893	NRAS	100.0	-7.37	3.96E-06
			3265	HRAS	92.6	-8.37	7.26E-07
	5566	PRKACA	5566	PRKACA	100.0	-10.75	1.32E-08
			5567	PRKACB	77.5	-9.89	5.61E-08
			2773	GNAI3	64.0	-9.24	1.67E-07
	5573	PRKAR1A	5573	PRKAR1A	100.0	-10.61	1.64E-08
			5575	PRKAR1B	90.6	-10.23	3.14E-08
	5582	PRKCG	5578	PRKCA	80.0	-15.87	2.31e-12
			5579	PRKCB	78.0	-16.48	8.21E-13
			5582	PRKCG	100.0	-20.77	5.84E-16
	5588	PRKCQ	5580	PRKCD	68.5	-7.3	4.45E-06
			5588	PRKCQ	100.0	-4.54	4.68E-04
M26911	2767	GNA11	9630	GNA14	90.9	-6.51	1.67E-05

		346562	GNAT3	63.3	-8.08	1.19E-06
		2767	GNA11	100.0	-6.39	2.05E-05
		2769	GNA15	71.9	-7.25	4.81E-06
		2773	GNAI3	64.5	-7.46	4.00E-07
		2776	GNAQ	96.1	-6.38	2.11E-05
2776	GNAQ	9630	GNA14	90.9	-6.49	1.73E-05
		346562	GNAT3	63.3	-8.06	1.23E-06
		2767	GNA11	100.0	-6.38	2.11E-05
		2769	GNA15	71.9	-7.21	5.12E-06
		2773	GNAI3	64.5	-7.44	3.51E-06
		2776	GNAQ	96.1	-6.36	2.18E-05
815	CAMK2A	815	CAMK2A	100.0	-10.21	3.27E-08
		816	CAMK2B	67.9	-12.35	8.72E-10
		817	CAMK2D	88.3	-10.06	4.19E-08
		818	CAMK2G	82.7	-10.9	1.02E-08
816	CAMK2B	815	CAMK2A	67.9	-15.45	4.68e-12
		816	CAMK2B	100.0	-12.35	8.72E-10
		817	CAMK2D	88.3	-11.49	3.77E-09
		818	CAMK2G	82.7	-11.45	3.99E-09
5566	PRKACA	5566	PRKACA	100.0	-10.75	1.32E-08
		5567	PRKACB	77.5	-9.89	5.61e-08

			5568	PRKACG	74.5	-9.98	4.79E-08
	5573	PRKAR1A	5573	PRKAR1A	100.0	-10.52	1.93E-08
			5575	PRKAR1B	90.6	-9.99	4.68E-08
	5582	PRKCG	5578	PRKCA	80.0	-15.87	2.31e-12
			5582	PRKCG	100.0	-20.77	5.84E-16
M39426	3265	HRAS	3845	KRAS	94.2	-8.55	5.33e-07
			4893	NRAS	92.6	-8.37	7.26E-07
			3265	HRAS	100.0	-8.17	1.02E-06
	2767	GNA11	9630	GNA14	90.9	-6.51	1.67E-05
			2767	GNA11	100.0	-6.39	2.05E-05
			2769	GNA15	71.9	-7.25	4.81E-06
			2773	GNAI3	64.5	-7.46	3.40E-06
			2775	GNAO1	64.8	-7.84	1.78E-06
			2776	GNAQ	96.1	-6.38	2.11E-05
			2781	GNAZ	61.3	-7.75	2.06E-06
	2776	GNAQ	9630	GNA14	90.0	-6.49	1.73E-05
			2767	GNA11	96.1	-6.38	2.11E-05
			2769	GNA15	70.9	-7.21	5.12E-06
			2773	GNAI3	64.0	-7.44	3.51E-06
			2775	GNAO1	64.0	-7.82	1.84E-06
			2776	GNAQ	100.0	-6.36	2.18E-05
			2781	GNAZ	60.3	-7.74	2.12E-06
	3845	KRAS	3845	KRAS	100.0	-7.75	2.07e-06
			4893	NRAS	94.2	-7.22	5.05E-06
			3265	HRAS	94.2	-8.55	5.33E-07

	4893	NRAS	3845	KRAS	94.2	-7.57	2.82E-06
			4893	NRAS	100.0	-7.37	3.96E-06
			3265	HRAS	92.6	-8.37	7.26E-07
	5566	PRKACA	5566	PRKACA	100.0	-10.75	1.32E-08
			5567	PRKACB	77.5	-9.89	5.61E-08
			2773	GNAI3	64.0	-9.24	1.67E-07
	5573	PRKAR1A	5573	PRKAR1A	100.0	-10.61	1.64E-08
			5575	PRKAR1B	90.6	-10.23	3.14E-08
	5582	PRKCG	5578	PRKCA	80.0	-15.87	2.31e-12
			5579	PRKCB	78.0	-16.48	8.21E-13
			5582	PRKCG	100.0	-20.77	5.84E-16
	5588	PRKCQ	5580	PRKCD	68.5	-7.3	4.45E-06
			5588	PRKCQ	100.0	-4.54	4.68E-04
MM14496	2767	GNA11	346562	GNAT3	63.3	-8.08	1.19E-06
			2767	GNA11	100.0	-6.39	2.05E-05
			2769	GNA15	71.9	-7.26	4.81E-06
			2773	GNAI3	64.5	-7.46	3.40E-06
			2776	GNAQ	96.1	-6.38	2.11E-05
			9630	GNA14	90.9	-6.51	1.67E-05
	2776	GNAQ	346562	GNAT3	63.3	-8.08	1.23E-06
			2767	GNA11	96.1	-6.38	2.11E-05
			2769	GNA15	70.9	-7.21	5.12E-06
			2773	GNAI3	64.0	-7.44	3.51E-06
			2776	GNAQ	100.0	-6.36	2.18E-05

		9630	GNA14	90.0	-6.49	1.73E-05
		84254	CAMKK1	91.7	-8.15	1.06E-06
5566	PRKACA	5566	PRKACA	100.0	-10.75	1.32E-08
		5567	PRKACB	77.5	-10.13	3.74E-08
5573	PRKAR1A	5573	PRKAR1A	100.0	-10.61	1.64E-08
		5575	PRKAR1B	90.6	-10.23	3.14E-08
5582	PRKCG	5582	PRKCG	100.0	-20.77	5.84e-16

Table 1.5: Top 10 GPCR genes associated with Long COVID genes from each pathway identified using network analysis in Cytoscape and CytoHubba plugin

Pathway ID	**Receptor (Long COVID)**		**Ligand (GPCR)**	
	Gene Id	**Symbol**	**Gene ID**	**Symbol**
MM15882 & M39426	3265	HRAS	3265	HRAS
	2767	GNA11	3845	KRAS
	2776	GNAQ	9630	GNA14
	3845	KRAS	2767	GNA11
			2769	GNA15
			2773	GNAI3
			2775	GNAO1
			2776	GNAQ
			2781	GNAZ
			5578	PRKCA
M26911	2767	GNA11	9630	GNA14
	2776	GNAQ	346562	GNAT3
	5566	PRKACA	2767	GNA11

			2769	GNA15
			2773	GNAI3
			2776	GNAQ
			5566	PRKACA
			5567	PRKACB
			5568	PRKACG
MM14496	2767	GNA11	346562	GNAT3
	2776	GNAQ	2767	GNA11
	5566	PRKACA	2769	GNA15
	5573	PRKAR1A	2773	GNAI3
			2776	GNAQ
			9630	GNA14
			5566	PRKACA
			5567	PRKACB
			5573	PRKAR1A
			5575	PRKAR1B

Table 1.6.1: Molecular docking results of top 3 protein-protein affinity prediction for pathway ID MM15882 (Wp G Protein Signaling Pathways)

Gene from Input (Long COVID)		**Gene from Annotation (GPCR)**		**Similarity Percentage**	**Binding Free Energy (kcal/mol)**	**Dissociation Value (M)**	**Binding Affinity (kcal/mol)**	**RMSD Lower Bound**	**RMSD Higher Bound**
Gene Id	**Symbol**	**Gene ID**	**Symbol**						
5573	PRKAR1A	5575	PRKAR1B	90.6	-10.23	3.14E-08	-4.8	0.0	0.0
5582	PRKCG	5578	PRKCA	80.0	-15.87	2.31e-12	-4.8	0.0	0.0
		5579	PRKCB	78.0	-16.48	8.21E-13	-7.0	0.0	0.0

Table 1.6.2: Molecular docking results of top 3 protein-protein affinity prediction for pathway ID M26911 (Reactome G Protein Mediated Events)

Gene from Input (Long COVID)		Gene frm Annotation (GPCR)		Similarity Percentage	Binding Free Energy (kcal/mol)	Dissociation Value (M)	Binding Affinity (kcal/mol)	RMSD Lower Bound	RMSD Higher Bound
Gene Id	Symbol	Gene ID	Symbol						
815	CAMK2A	816	CAMK2B	67.9	-12.35	8.72E-10	-7.3	0.0	0.0
816	CAMK2B	815	CAMK2A	67.9	-15.45	4.68e-12	-4.2	0.0	0.0
5582	PRKCG	5578	PRKCA	80	-15.87	2.31e-12	-4.7	0.0	0.0

Table 1.6.3: Molecular docking results of top 3 protein-protein affinity prediction for pathway ID M39426 (Wp G Protein Signaling Pathways)

Gene from Input (Long COVID)		Gene from Annotation (GPCR)		Similarity Percentage	Binding Free Energy (kcal/mol)	Dissociation Value (M)	Binding Affinity (kcal/mol)	RMSD Lower Bound	RMSD Higher Bound
Gene Id	Symbol	Gene ID	Symbol						
5573	PRKAR1A	5575	PRKAR1B	90.6	-10.23	3.14E-08	-4.8	0.0	0.0
5582	PRKCG	5578	PRKCA	80.0	-15.87	2.31e-12	-4.8	0.0	0.0
		5579	PRKCB	78.0	-16.48	8.21E-13	-7.0	0.0	0.0

Table 1.6.4: Molecular docking results of top 3 protein-protein affinity prediction for pathway ID MM14496 (Reactome G Protein Mediated Events)

Gene from Input (Long COVID)		Gene from Annotation (GPCR)		Similarity Percentage	Binding Free Energy (kcal/mol)	Dissociation Value (M)	Binding Affinity (kcal/mol)	RMSD Lower Bound	RMSD Higher Bound
Gene Id	Symbol	Gene ID	Symbol						
2776	GNAQ	84254	CAMKK1	91.7	-8.15	1.06E-06	-9.6	0.0	0.0

5566	PRKACA	5567	PRKACB	77.5	-10.13	3.74E-08	-8.7	0.0	0.0
5573	PRKAR1A	5575	PRKAR1B	90.6	-10.23	3.14E-08	-4.8	0.0	0.0

Table 1.7.1: Molecular docking results of top 10 Cytoscape for pathway ID MM15882 (Wp Protein Signaling Pathways)

Receptor (Long COVID)		**Ligand (GPCR)**		**Binding Affinity (kcal/mol)**	**RMSD Lower Bound**	**RMSD Upper Bound**
Gene Id	**Symbol**	**Gene ID**	**Symbol**			
3265	HRAS	3265	HRAS	-10.6	0.0	0.0
		3845	KRAS	-8.0	0.0	0.0
		9630	GNA14	-10.3	0.0	0.0
		2767	GNA11	-5.4	0.0	0.0
		2769	GNA15	-4.1	0.0	0.0
		2773	GNAI3	-10.2	0.0	0.0
		2775	GNAO1	-6.7	0.0	0.0
		2776	GNAQ	-8.5	0.0	0.0
		2781	GNAZ	-8.9	0.0	0.0
		5578	PRKCA	-4.8	0.0	0.0
2767	GNA11	3265	HRAS	-8.4	0.0	0.0
		3845	KRAS	-6.3	0.0	0.0
		9630	GNA14	-7.4	0.0	0.0
		2767	GNA11	-5.0	0.0	0.0
		2769	GNA15	-3.7	0.0	0.0
		2773	GNAI3	-9.3	0.0	0.0
		2775	GNAO1	-6.8	0.0	0.0
		2776	GNAQ	-8.1	0.0	0.0
		2781	GNAZ	-8.6	0.0	0.0

		5578	PRKCA	-4.6	0.0	0.0
2776	GNAQ	3265	HRAS	-9.0	0.0	0.0
		3845	KRAS	-5.7	0.0	0.0
		9630	GNA14	-9.1	0.0	0.0
		2767	GNA11	-4.7	0.0	0.0
		2769	GNA15	-3.8	0.0	0.0
		2773	GNAI3	-8.7	0.0	0.0
		2775	GNAO1	-6.4	0.0	0.0
		2776	GNAQ	-9.0	0.0	0.0
		2781	GNAZ	-8.4	0.0	0.0
		5578	PRKCA	-4.3	0.0	0.0
3845	KRAS	3265	HRAS	-8.7	0.0	0.0
		3845	KRAS	-6.5	0.0	0.0
		9630	GNA14	-10.2	0.0	0.0
		2767	GNA11	-5.6	0.0	0.0
		2769	GNA15	-4.1	0.0	0.0
		2773	GNAI3	-9.8	0.0	0.0
		2775	GNAO1	-6.8	0.0	0.0
		2776	GNAQ	-8.3	0.0	0.0
		2781	GNAZ	-7.4	0.0	0.0
		5578	PRKCA	-4.9	0.0	0.0

Table 1.7.2: Molecular docking results of top 10 Cytoscape for pathway ID M26911 (Reactome G Protein Mediated Events)

Receptor (Long COVID)		**Ligand (GPCR)**		**Binding Affinity (kcal/mol)**	**RMSD Lower Bound**	**RMSD Upper Bound**
Gene Id	**Symbol**	**Gene ID**	**Symbol**			
2767	GNA11	9630	GNA14	-9.2	0.0	0.0

		346562	GNAT3	-9.0	0.0	0.0
		2767	GNA11	-4.9	0.0	0.0
		2769	GNA15	-3.6	0.0	0.0
		2773	GNAI3	-8.4	0.0	0.0
		2776	GNAQ	-8.3	0.0	0.0
		5566	PRKACA	-8.4	0.0	0.0
		5567	PRKACB	-7.9	0.0	0.0
		5578	PRKCA	-4.2	0.0	0.0
		5568	PRKACG	-8.8	0.0	0.0
2776	GNAQ	9630	GNA14	-8.9	0.0	0.0
		346562	GNAT3	-9.2	0.0	0.0
		2767	GNA11	-4.8	0.0	0.0
		2769	GNA15	-4.2	0.0	0.0
		2773	GNAI3	-8.9	0.0	0.0
		2776	GNAQ	-8.7	0.0	0.0
		5566	PRKACA	-9.0	0.0	0.0
		5567	PRKACB	-6.5	0.0	0.0
		5578	PRKCA	-4.3	0.0	0.0
		5568	PRKACG	-8.6	0.0	0.0
5566	PRKACA	9630	GNA14	-9.0	0.0	0.0
		346562	GNAT3	-9.0	0.0	0.0
		2767	GNA11	-5.1	0.0	0.0
		2769	GNA15	-3.9	0.0	0.0
		2773	GNAI3	-8.5	0.0	0.0
		2776	GNAQ	-9.3	0.0	0.0
		5566	PRKACA	-8.9	0.0	0.0
		5567	PRKACB	-8.7	0.0	0.0

		5578	PRKCA	-4.4	0.0	0.0
		5568	PRKACG	-9.6	0.0	0.0

Table 1.7.3: Molecular docking results of top 10 Cytoscape for pathway ID M39426 (Reactome G Protein Mediated Events)

Receptor (Long COVID)		**Ligand (GPCR)**		**Binding Affinity (kcal/mol)**	**RMSD Lower Bound**	**RMSD Upper Bound**
Gene Id	**Symbol**	**Gene ID**	**Symbol**			
3265	HRAS	3265	HRAS	-10.6	0.0	0.0
		3845	KRAS	-8.0	0.0	0.0
		9630	GNA14	-10.3	0.0	0.0
		2767	GNA11	-5.4	0.0	0.0
		2769	GNA15	-4.1	0.0	0.0
		2773	GNAI3	-10.2	0.0	0.0
		2775	GNAO1	-6.7	0.0	0.0
		2776	GNAQ	-8.5	0.0	0.0
		2781	GNAZ	-8.9	0.0	0.0
		5578	PRKCA	-4.8	0.0	0.0
2767	GNA11	3265	HRAS	-8.4	0.0	0.0
		3845	KRAS	-6.3	0.0	0.0
		9630	GNA14	-7.4	0.0	0.0
		2767	GNA11	-5.0	0.0	0.0
		2769	GNA15	-3.7	0.0	0.0
		2773	GNAI3	-9.3	0.0	0.0
		2775	GNAO1	-6.8	0.0	0.0
		2776	GNAQ	-8.1	0.0	0.0
		2781	GNAZ	-8.6	0.0	0.0
		5578	PRKCA	-4.6	0.0	0.0

2776	GNAQ	3265	HRAS	-9.0	0.0	0.0
		3845	KRAS	-5.7	0.0	0.0
		9630	GNA14	-9.1	0.0	0.0
		2767	GNA11	-4.7	0.0	0.0
		2769	GNA15	-3.8	0.0	0.0
		2773	GNAI3	-8.7	0.0	0.0
		2775	GNAO1	-6.4	0.0	0.0
		2776	GNAQ	-9.0	0.0	0.0
		2781	GNAZ	-8.4	0.0	0.0
		5578	PRKCA	-4.3	0.0	0.0
3845	KRAS	3265	HRAS	-8.7	0.0	0.0
		3845	KRAS	-6.5	0.0	0.0
		9630	GNA14	-10.2	0.0	0.0
		2767	GNA11	-5.6	0.0	0.0
		2769	GNA15	-4.1	0.0	0.0
		2773	GNAI3	-9.8	0.0	0.0
		2775	GNAO1	-6.8	0.0	0.0
		2776	GNAQ	-8.3	0.0	0.0
		2781	GNAZ	-7.4	0.0	0.0
		5578	PRKCA	-4.9	0.0	0.0

Table 1.7.4: Molecular docking results of top 10 Cytoscape for pathway ID MM14496 (Wp Protein Signaling Pathways).

Receptor (Long COVID)		**Ligand (GPCR)**		**Binding Affinity (kcal/mol)**	**RMSD Lower Bound**	**RMSD Upper Bound**
Gene Id	**Symbol**	**Gene ID**	**Symbol**			
2767	GNA11	346562	GNAT3	-9.5	0.0	0.0
		2767	GNA11	-4.8	0.0	0.0

		2769	GNA15	-3.7	0.0	0.0
		2773	GNAI3	-9.2	0.0	0.0
		2776	GNAQ	-8.0	0.0	0.0
		9630	GNA14	-9.7	0.0	0.0
		5566	PRKACA	-9.4	0.0	0.0
		5567	PRKACB	-8.1	0.0	0.0
		5573	PRKAR1A	-7.9	0.0	0.0
		5575	PRKAR1B	-4.8	0.0	0.0
2776	GNAQ	346562	GNAT3	-9.1	0.0	0.0
		2767	GNA11	-4.8	0.0	0.0
		2769	GNA15	-3.8	0.0	0.0
		2773	GNAI3	-8.7	0.0	0.0
		2776	GNAQ	-8.0	0.0	0.0
		9630	GNA14	-9.3	0.0	0.0
		5566	PRKACA	-9.1	0.0	0.0
		5567	PRKACB	-6.8	0.0	0.0
		5573	PRKAR1A	-7.5	0.0	0.0
		5575	PRKAR1B	-4.2	0.0	0.0
5566	PRKACA	346562	GNAT3	-8.7	0.0	0.0
		2767	GNA11	-4.7	0.0	0.0
		2769	GNA15	-4.4	0.0	0.0
		2773	GNAI3	-8.4	0.0	0.0
		2776	GNAQ	-9.5	0.0	0.0
		9630	GNA14	-8.8	0.0	0.0
		5566	PRKACA	-7.7	0.0	0.0
		5567	PRKACB	-8.7	0.0	0.0
		5573	PRKAR1A	-8.8	0.0	0.0

		5575	PRKAR1B	-4.1	0.0	0.0
5573	PRKAR1A	346562	GNAT3	-6.3	0.0	0.0
		2767	GNA11	-6.0	0.0	0.0
		2769	GNA15	-4.2	0.0	0.0
		2773	GNAI3	-6.7	0.0	0.0
		2776	GNAQ	-5.8	0.0	0.0
		9630	GNA14	-8.5	0.0	0.0
		5566	PRKACA	-6.7	0.0	0.0
		5567	PRKACB	-9.0	0.0	0.0
		5573	PRKAR1A	-6.8	0.0	0.0
		5575	PRKAR1B	-3.9	0.0	0.0

Table 1.8: 21 identified genes from molecular docking with associated symptoms and repurposed FDA drugs

Identified Genes	**Symptoms**	**HPO ID**	**Symptoms Category**	**FDA-Approved Repurposed Drugs**
HRAS	Anaphylactic shock	HP:0100845	Immunology-autoimmunity	/
	Anti-thyroid peroxidase antibody positivity	HP:0025379	Immunology-autoimmunity	
	Alopecia	HP:0001596	Dermatological	
	Fragile nails	HP:0001808	Dermatological	
	Gastroesophageal reflux	HP:0002020	Gastrointestinal	
	Sleep apnea	HP:0010535	Neuropsychiatric	
GNAQ	Ocular pain	HP:0200026	HEENT-Eye	Verteporfin, Selumetinib
	Pain	HP:0012531	General symptom	

	Venous thrombosis	HP:0004936	Cardiovascular	
	Pulmonary embolism	HP:0002204	Pulmonary	
GNA11	Alopecia	HP:0001596	Dermatological	Binimetinib, Cabozantinib, Selumetinib
	Ocular pain	HP:0200026	HEENT-Eye	
GNAO1	Hyperkinetic movements	HP:0002487	Neuropsychiatric	/
KRAS	Anti-thyroid peroxidase antibody positivity	HP:0025379	Immunology-autoimmunity	Panitumumab, Cetuximab, Adagrasib, Sotorasib
	Alopecia	HP:0001596	Dermatological	
	Edema	HP:0000969	Reproductive-Genitourinary-Endocrine-Metabolism	
	Back pain	HP:0003418	General symptom	
	Pain	HP:0012531	General symptom	
	Fatigue	HP:0012378	General symptom	
	Abdominal pain	HP:0002027	Gastrointestinal	
	Anorexia	HP:0002039	Gastrointestinal	
PRKCB	Pleural thickening	HP:0031944	Pulmonary-imaging	/
PRKCG	Gaze-evoked nystagmus	HP:0000640	HEENT-Eye	/
	Memory impairment	HP:0002354	Neuropsychiatric	
	Dysmetria	HP:0001310	Neuropsychiatric	
PRKACA	Alopecia	HP:0001596	Dermatological	/

	Irregular menstruation	HP:0000858	Reproductive-Genitourinary-Endocrine-Metabolism	
	Mania	HP:0100754	Neuropsychiatric	
	Memory impairment	HP:0002354	Neuropsychiatric	
PRKACG	Menorrhagia	HP:0000132	Reproductive-Genitourinary-Endocrine-Metabolism	/
PRKAR1A	Alopecia	HP:0001596	Dermatological	/
	Irregular menstruation	HP:0000858	Reproductive-Genitourinary-Endocrine-Metabolism	
	Elevated circulating thyroid-stimulating hormone concentration	HP:0002925	Lab	
	Hypofibrinogenemia	HP:0011900	Lab	
	Mania	HP:0100754	Neuropsychiatric	
	Memory impairment	HP:0002354	Neuropsychiatric	
CAMK2A	Gastroesophageal reflux	HP:0002020	Gastrointestinal	/
	Abnormal emotion/affect behavior	HP:0100851	Neuropsychiatric	
	Sleep disturbance	HP:0002360	Neuropsychiatric	
	Dystonia	HP:0001332	Neuropsychiatric	
	Gait disturbance	HP:0001288	Neuropsychiatric	

CAMK2B	Gastroesophageal reflux	HP:0002020	Gastrointestinal	/
	Sleep disturbance	HP:0002360	Neuropsychiatric	
GNAQ, GNA13, GNA14, GNA15, GNAT3, PRKCA, PRKACB, PRKAR1, CAMKK1	/	/	/	/

Table 1.9: Common genes identified in more than one Long COVID symptoms

Identified Genes	Occurrences	Symptoms Category
HRAS	6	Immunology-autoimmunity, Dermatological, Gastrointestinal, Neuropsychiatric
GNAQ	4	HEENT-eye, General symptom, Cardiovascular, Pulmonary
GNA11	2	Dermatological, HEENT-eye
GNAO1	1	Neuropsychiatric
KRAS	7	Immunology-autoimmunity, Dermatological, General symptoms, Gastrointestinal , Neuropsychiatric, Reproductive-Genitourinary-Endocrine-Metabolism
PRKCB	1	Pulmonary
PRKCG	3	HEENT-eye, Neuropsychiatric
PRKACA	4	Dermatological, Reproductive-Genitourinary-Endocrine-Metabolism, Neuropsychiatric
PRKACG	1	Reproductive-Genitourinary-Endocrine-Metabolism
PRKAR1A	6	Dermatological, Reproductive-Genitourinary-Endocrine-Metabolism, Lab, Neuropsychiatric

CAMK2A	5	Gastrointestinal, Neuropsychiatric
CAMK2B	2	Gastrointestinal, Neuropsychiatric

Table 1.10: Virus-host interaction table identified through Enrichr tool through COVID-19 related gene sets analysis

SARS-CoV-2 Gene	Human Genes	Adjusted P-Value
SARS coronavirus protein E (gene: E)	HRAS	0.3499
	GNAO1	0.4334
	GNAZ	0.4017
SARS coronavirus P2 envelope protein	HRAS	0.3499
	GNAO1	0.4334
	GNAZ	0.4017
SARS coronavirus Tor2 small envelope E protein	HRAS	0.3499
	GNAO1	0.4334
	GNAZ	0.4017
SARS coronavirus formerly known as growth-factor-like protein (gene: orf1ab)	HRAS	0.3499
	KRAS	0.1345
SARS coronavirus nsp7-pp1a/pp1ab (gene: orf1ab)	HRAS	0.3499
	KRAS	0.1345
	PRKCA	0.3479
SARS coronavirus hypothetical protein sars7a	HRAS	0.3499
	KRAS	0.2006
SARS coronavirus P2 hypothetical protein sars7a	HRAS	0.3499
	KRAS	0.2006
SARS coronavirus Tor2 Orf8	HRAS	0.3499
	KRAS	0.2006
SARS coronavirus nsp3-pp1a/pp1ab (gene: orf1ab)	HRAS	0.5045
	KRAS	0.157

	GNAQ	0.5409
	PRKCA	0.3286
	PRKCG	0.3064
	PRKACB	0.4816
SARS coronavirus excised_polyprotein 1..4369 (gene: orf1ab)	HRAS	0.6646
	KRAS	0.09531
	GNAQ	0.4168
	PRKCA	0.04058
	PRKCG	0.6548
	PRKACB	0.4816
SARS coronavirus P2 full_polyprotein 1..4382	HRAS	0.6713
	KRAS	0.09531
	GNAQ	0.4181
	PRKCA	0.04146
	PRKCG	0.6616
	PRKACB	0.4816
SARS coronavirus nsp13-pp1ab (ZD, NTPase/HEL; RNA (gene: orf1ab)	KRAS	0.089
SARS coronavirus RNA-dependent RNA polymerase (gene: orf1ab)	KRAS	0.1109
SARS coronavirus nsp4-pp1a/pp1ab (gene: orf1ab)	KRAS	0.1316
SARS coronavirus hypothetical protein sars9b	KRAS	0.1345
SARS coronavirus Tor2 Orf13	KRAS	0.1345
SARS coronavirus 3C-like proteinase (gene: orf1ab)	KRAS	0.1406
SARS coronavirus leader protein (gene: orf1ab)	KRAS	0.1428

SARS coronavirus nucleocapsid protein (gene: N)	KRAS	0.1714
SARS coronavirus P2 nucleocapsid protein	KRAS	0.1714
SARS coronavirus Tor2 nucleocapsid protein	KRAS	0.1714
SARS coronavirus nsp8-pp1a/pp1ab (gene: orf1ab)	KRAS	0.2234
	PRKCA	0.3479
	PRKAR1A	0.2813
SARS coronavirus P2 spike glycoprotein precursor	KRAS	0.3121
	GNAQ	0.4168
	GNAO1	0.4334
	PRKCB	0.5624
	PRKAR1A	0.3559
SARS coronavirus E2 glycoprotein precursor (gene: S)	KRAS	0.3152
	GNAQ	0.4168
	GNAO1	0.4334
	PRKCB	0.5624
	PRKAR1A	0.3575
SARS coronavirus Tor2 spike glycoprotein	KRAS	0.3152
	GNAQ	0.4168
	GNAO1	0.4334
	PRKCB	0.5624
	PRKAR1A	0.3575
SARS coronavirus excised_polyprotein 1..4369 (gene: orf1ab)	GNAQ	0.4168
	GNAO1	0.4334
	GNAZ	0.4017
	PRKCB	0.5624

	PRKCG	0.6548
	PRKACB	0.4816
SARS coronavirus P2 full_polyprotein 1..4382	GNAQ	0.4181
	GNAO1	0.4334
	GNAZ	0.4017
	PRKCB	0.5624
	PRKCG	0.6616
	PRKACB	0.4816
SARS coronavirus Tor2 replicase 1AB	GNAQ	0.5281
	GNAZ	0.4017
	GNA11	0.2203
	GNA13	0.4737
	PRKCA	0.3286
	PRKAR1A	0.4509
SARS coronavirus P2 full_polyprotein 1..7073	GNAQ	0.53
	GNAZ	0.4017
	GNA11	0.2203
	PRKCA	0.3286
	PRKAR1A	0.4529