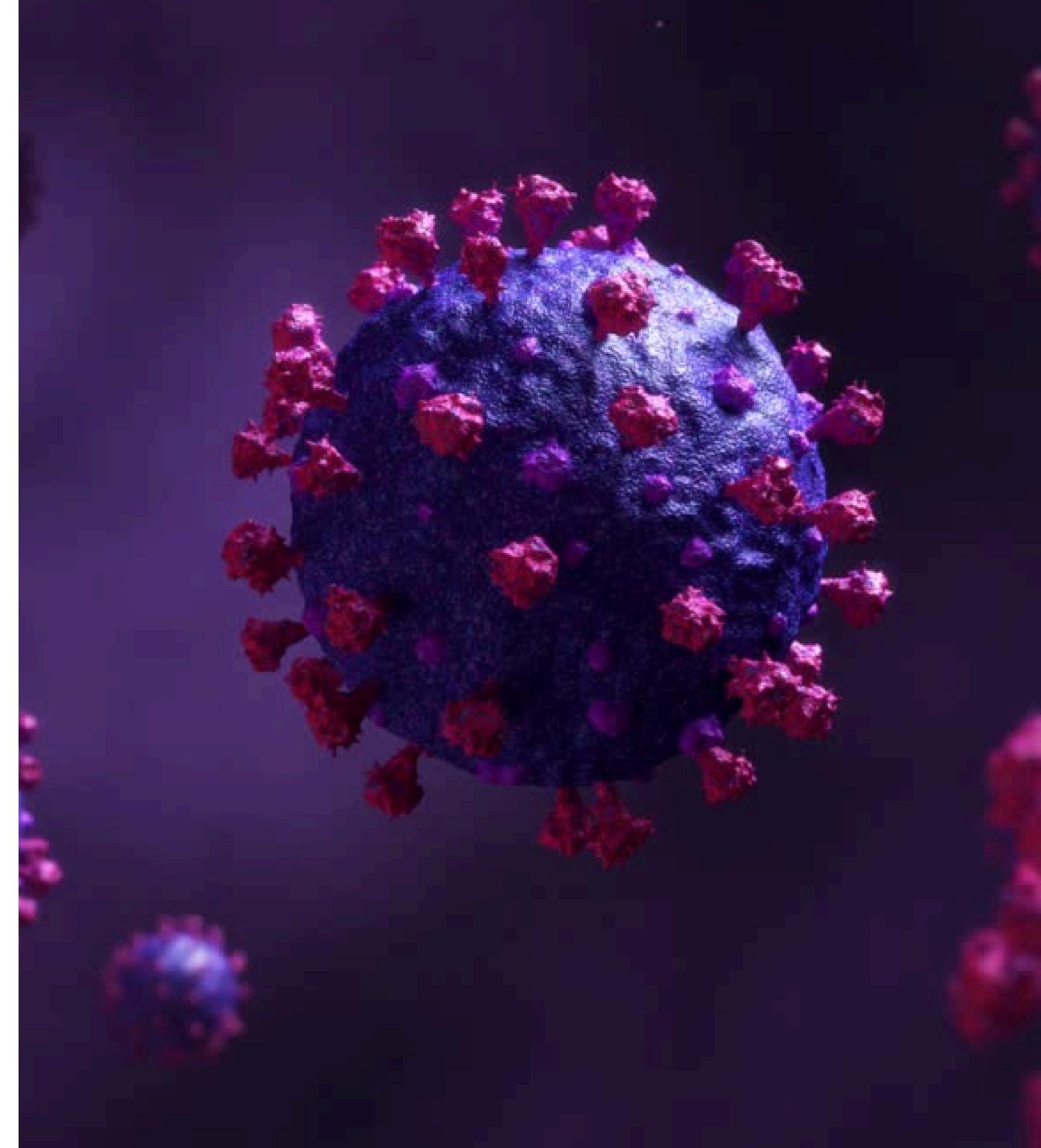


EXPLORING GPCR AS THERAPEUTIC POTENTIAL OF LONG COVID

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Overview

01
02
03
04
05
06

Introduction
Objectives
Hypothesis
Literature Review
Methodology
Results

07
08
09
10
11
12

Discussion
Limitation
Conclusion
Future Work
References
Thank You



1.0 Introduction

Definition: post-acute sequelae of SARS-CoV-2 infection (PASC), symptoms that persists usually more than 12 weeks in patients who have recovered from the acute phase of COVID-19 ((Sisó-Almirall et al., 2021) (Gültekin & Özçelik, 2022)

Symptoms: symptoms range from mild (fatigue, headache, pain), to severe brain fog, psychiatric symptoms (depression, anxiety, sleep disturbances, and cognitive impairments), affecting multiple vital organs c (S. T. Liu et al., 2023) (Kamamuta et al., 2022).

Statistics: 10%-30% of patients has mild-to-moderate post- acute clinical symptoms three months after SARS-CoV-2 infection & in the United Kingdom alone, 1.3 million people have been experiencing Long COVID, with over 500,000 displaying symptoms lasting for over a year (O' Mahony et al., 2022)

Challenges: Long Covid impacts various domains of quality of life, pointing out the mental element as particularly impacted (Fernandez et al., 2023) & burdens healthcare system.

Therapeutics: GPCRs are the largest family of cell surface receptors, essential in various physiological processes and the **WHY??** target of over a third of FDA-approved drugs. It plays roles in multiple physiological processes makes them promising targets for developing Long COVID therapies (Shen et al., 2023).

1.2 Introduction

Why is GPCR suitable as a therapeutic potential/drug target?

- **Central role in physiological function**

crucial in regulating sensory perceptions, moods, and defense reactions.

- **Diversity & Presence**

over 800 types, GPCRs form the largest family of membrane receptors in humans

- **Structural flexibility**

ability to bind various molecules from small molecules & peptides to large proteins

- **Allosteric Modulation**

can be modulated at multiple sites, not just the primary binding site, enhancing drug efficacy

- **Proven Clinical Success**

about 34% of FDA-approved medications target GPCRs, proven reliability in therapy.

- **Potential in Cancer Therapy**

involved in cancer-related processes such as tumor growth

1.3 Objectives

General Objective:

to explore the potentiality of G protein-coupled receptor (GPCR) as a therapeutic target for Long COVID infection using advanced bioinformatics tools to screen the role and the interaction of the target systematically.

Specific Objective 1:

To determine the presence of functional autoantibodies against GPCRs among persistent symptoms of Long COVID-19 and assess its correlation with disease severity.

Specific Objective 3:

To assess the diversity of GPCRs and their expression across different diseases to identify new therapeutic targets for Long COVID and related conditions

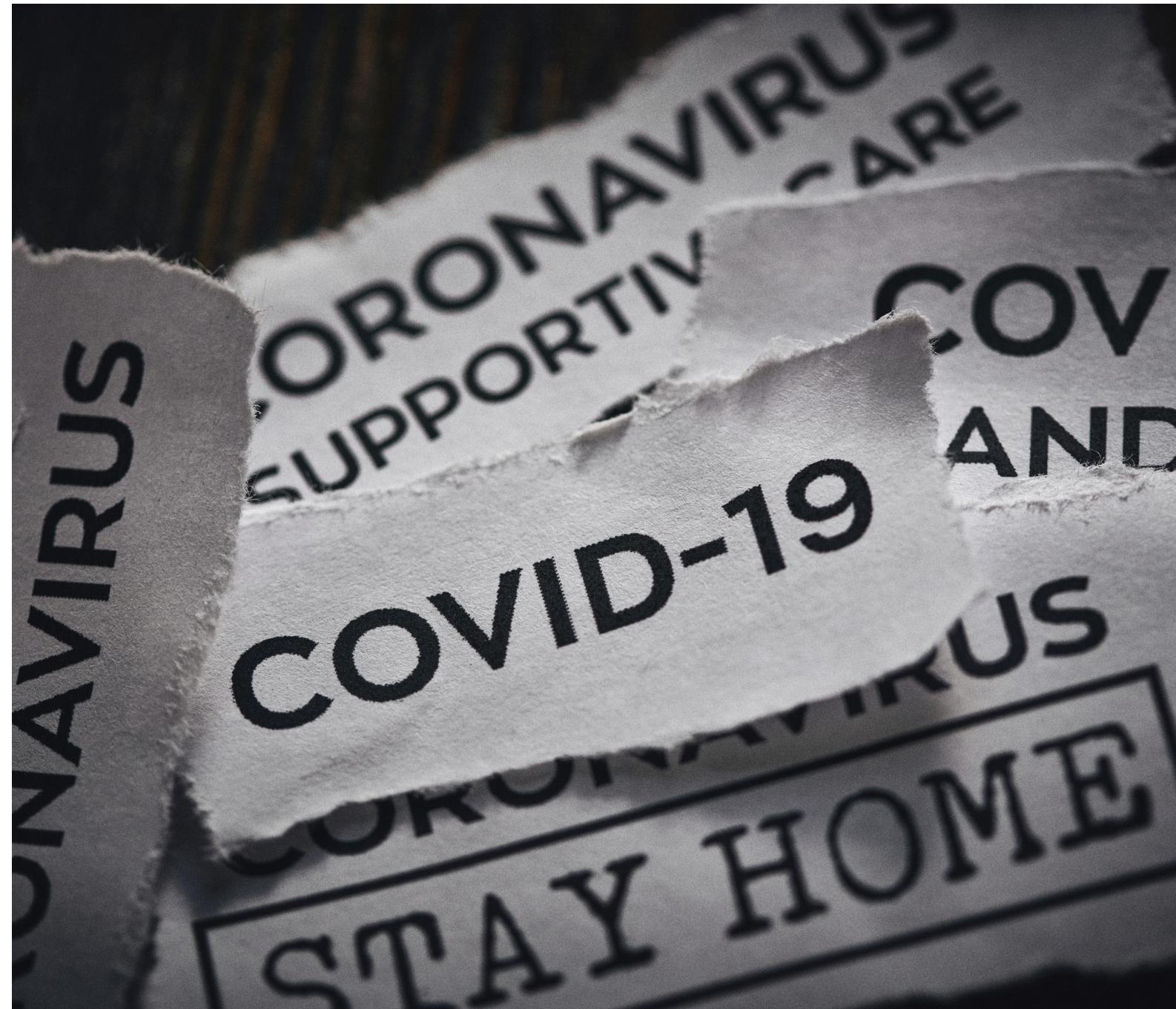
Specific Objective 2:

To analyse the role of GPCRs as key players in immune modulation, inflammation, and respiratory function, with an emphasis on involvement in the severity of COVID-19.

Specific Objective 4:

To evaluate the effects of GPCR on key physiological processes, (eg:immune response, inflammation, & respiratory function) in understanding the therapeutic potential Long COVID.

1.4 Hypothesis



The hypothesis of the project is to test out the potentiality of the G protein-coupled receptor (GPCR) for therapeutic purposes for Long COVID infection:

Null Hypothesis (H_0):

There are no prospective GPCR targets for Long COVID, and GPCRs lack clinical relevance in immune regulation, inflammation, or respiratory functions.

Alternative Hypotheses (H_1):

There are prospective GPCR targets for Long COVID, and GPCRs possess clinical relevance in immune regulation, inflammation, or respiratory functions.

2.0 Literature Review

1. Statistics on Long COVID case

global systematic analysis of the occurrence, severity, and recovery pattern among COVID in 2020 and 2021

Jah Wulf Hanson, PhD,¹ Cristiana Abbafati, PhD,² Prof. Joachim G Aerts, MD,³ Ziyad Al-Aly, MD,^{4,5}

An estimate of 144.7 million cases with Long symptoms from 2020 to 2021, which translates to 3.69% of the total global COVID-19 infections (Wulf Hanson et al., 2022).

2. Long COVID Symptoms

Long COVID: G Protein-Coupled Receptors (GPCRs) responsible for persistent post-COVID symptoms

Sanisha Das,  Suresh Kumar

255 Long COVID symptoms were associated with 331 Long Covid genes in various organ systems & their associated gene ontology and pathway insights (Das & Kumar, 2022).

3. Roles of GPCRs

Structure, function and drug discovery of GPCR signaling

Lin Cheng^{1,2†}, Fan Xia^{3†}, Zivan Li^{1†}, Chenglong Shen^{1†}, Zhiqian Yang^{1†}, Hanlin Hou^{1†}, Suvi

GPCR has been highly involved in regulating immune response, inflammation, and respiratory functions due to the recognition of different ligands that these proteins mediate (Cheng et al., 2023).

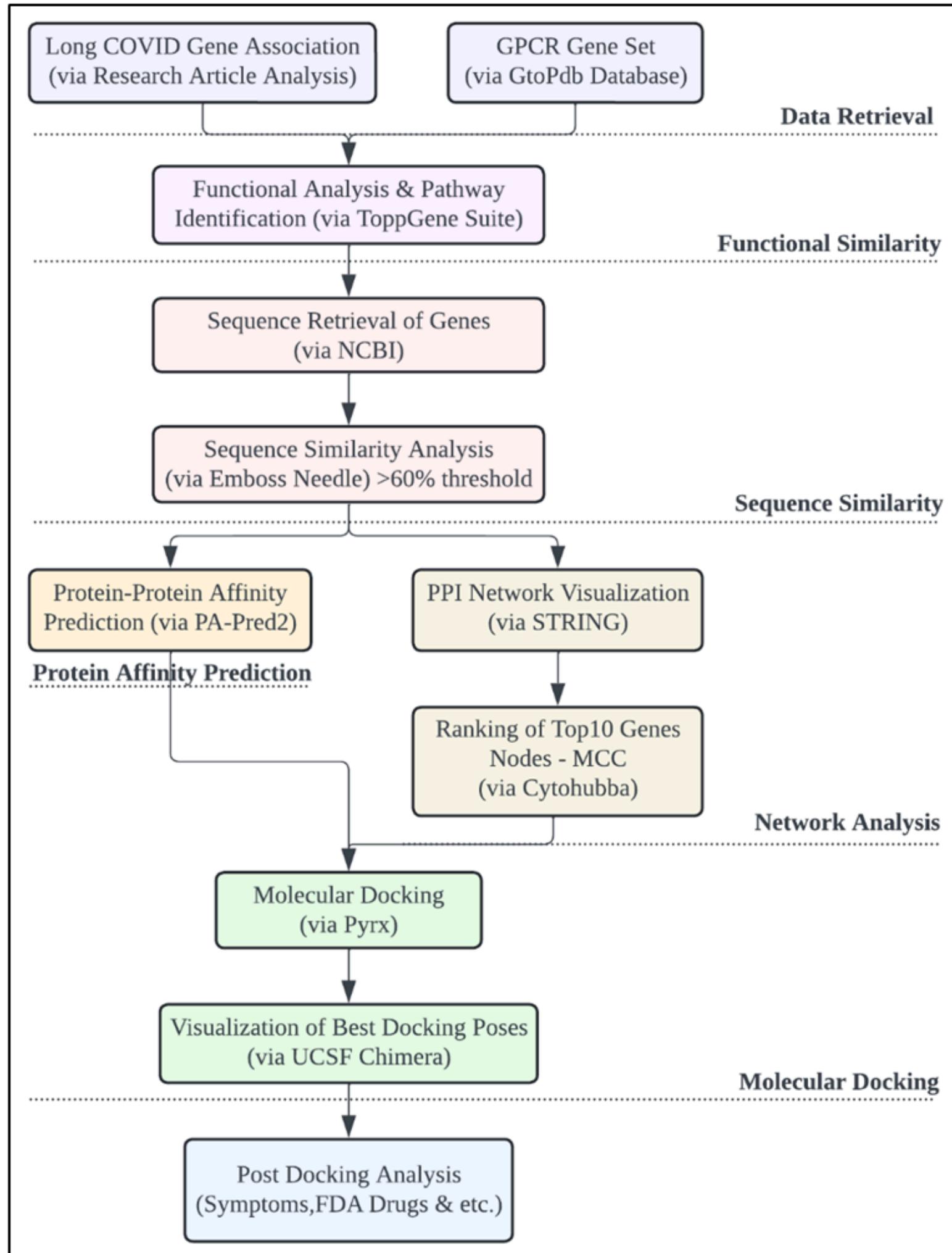
4. Suitability of GPCRs as Drug Targets

Trends in GPCR drug discovery: new agents, targets and indications

Alexander S. Hauser, Misty M. Attwood, Mathias Rask-Andersen, Helgi B. Schiöth & David E. Gloriam 

GPCR's involvement in critical pathways of physiology makes them an excellent target for the development of therapies that can be applied very widely for a whole range of different diseases (Hauser et al., 2017b).

Figure 3.1: Shows the overall workflow of the study.



3.0 Methodology

1. Research Article Analysis:

- 255 Long Covid genes and associated symptoms retrieved from a study (Das & Kumar , 2022)
- Other suggested repurposed drugs for Long Covid retrieved from several studies ((Lee, 2022; Odeniyide et al., 2022) & (Srivastava & Gold, 2018).

2. Bioinformatics Tools & Softwares:

- GtoPdb
- ToppGene Suite
- NCBI
- Emboss Needle
- PA-Pred2
- PubChem/Uniprot
- Cytoscape (STRING & Cytohubba)
- Pyrx (AutoDock Vina & OpenBabel)
- UCSF Chimera
- Enricher

4.0 Results

Functional Similarity:

Table 4.1: Summary of Long COVID Genes and Associated GPCR Genes Across 4 Identified Pathways in ToppGene

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

Sequence Similarity:

Table 4.2: Summary of GPCR and Long COVID Genes after sequence similarity analysis

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

Network Analysis:

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

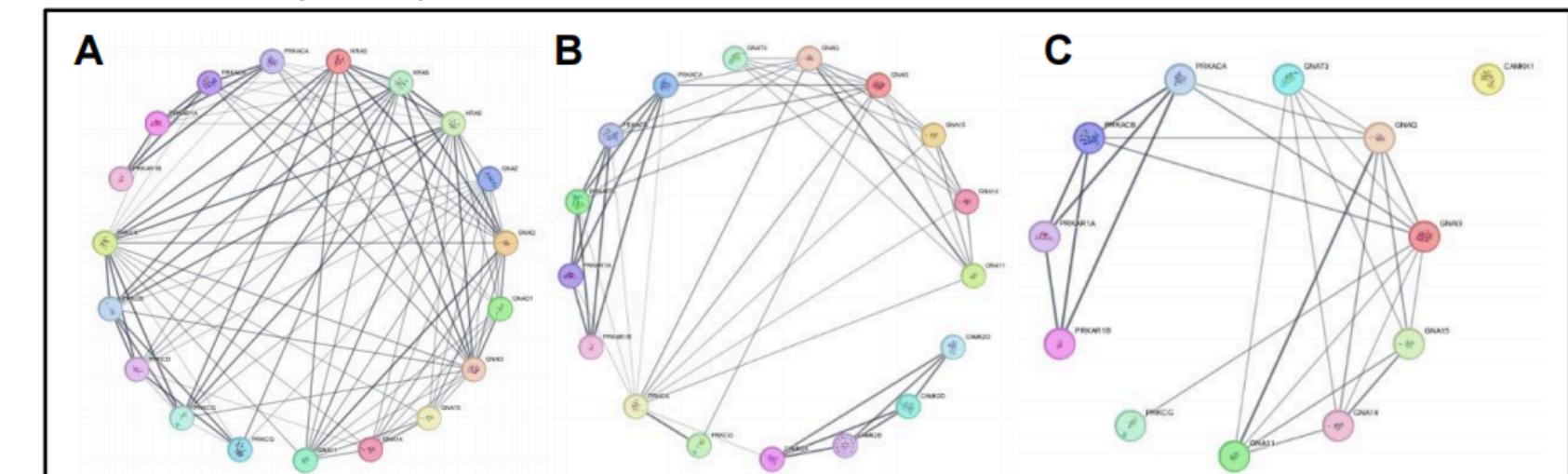
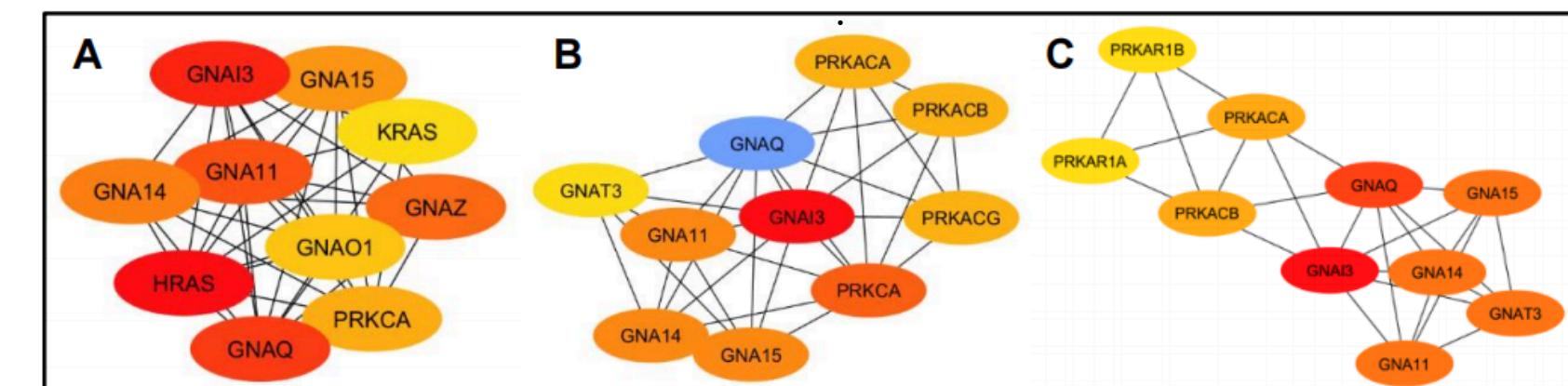


Figure 4.2: Ranking of the top 10 nodes for each pathway based on the Maximal Clique Centrality (MCC) with Cytohubba plugin. (A) MM15882 & M39426 (B) M26911 (C) MM14496.



4.0 Results

Molecular Docking

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

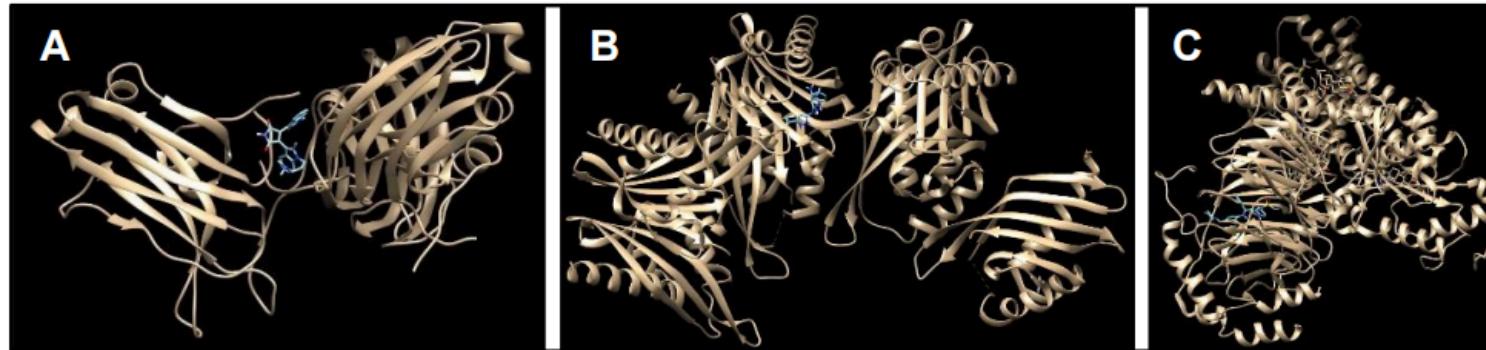


Figure 4.4: Best pose of the Top 3 Cytoscape docked genes for each pathway. (A) MM15882 & M39426 (B) M26911 (C) MM14496.

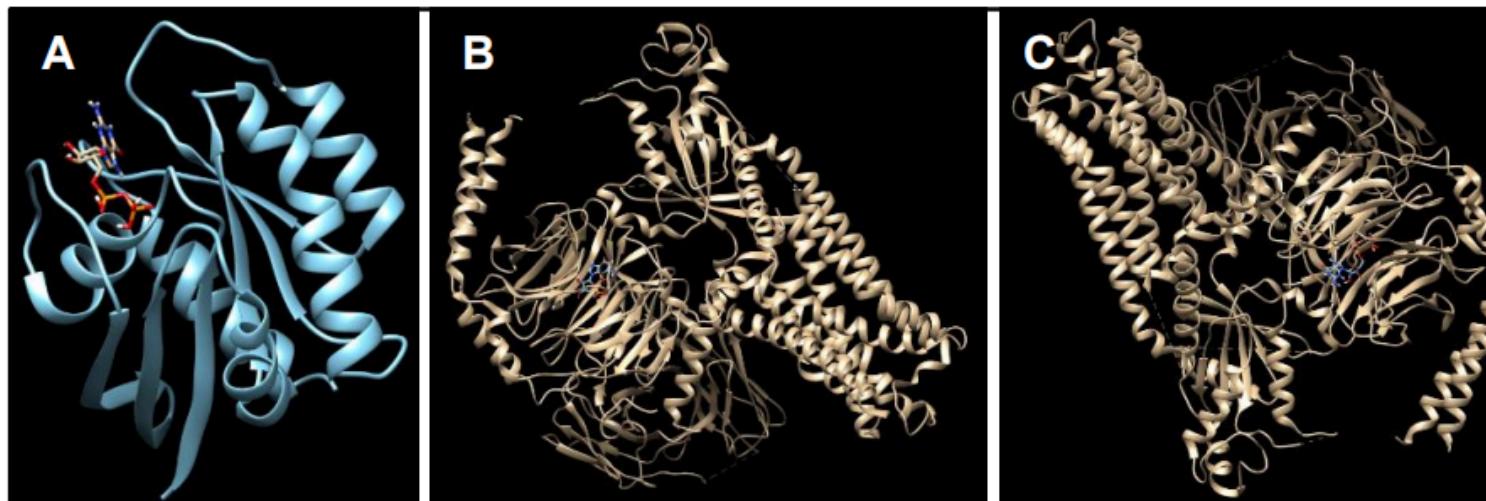
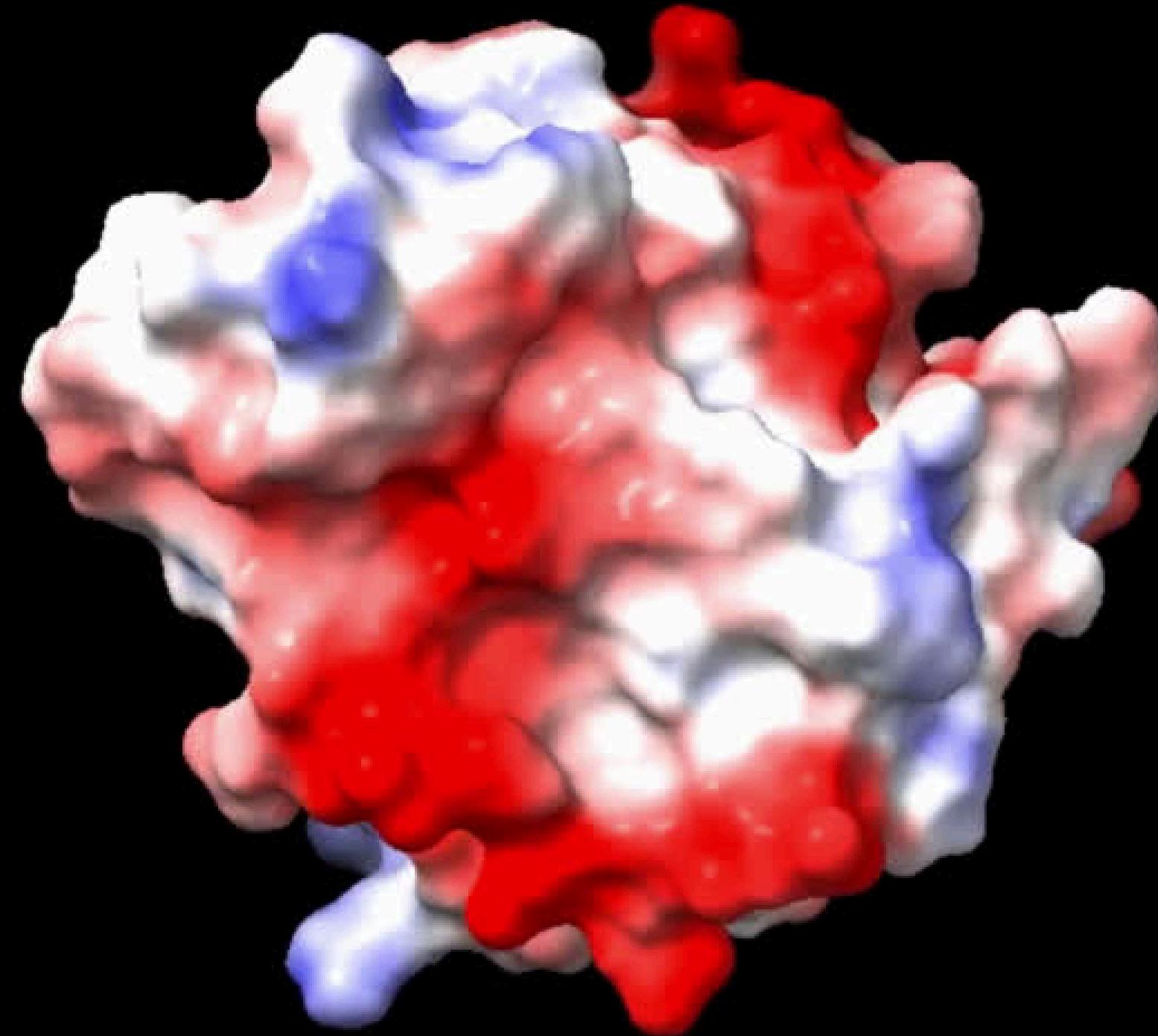


Table 4.3: Summary of the best docked genes for PPA-Pred & Cytoscape for each pathway.

Pathway ID	Receptor (Long COVID)			Ligand (GPCR)			Binding Affinity (kcal/mol)
	Gene Id	Symbol	Name	Gene ID	Symbol	Name	
MM15882 & M39426	3265	HRAS	HRas proto-oncogene, GTPase	2776	GNAQ	G protein subunit alpha q	-8.5
	2767	GNA11	G protein subunit alpha 11	3265	HRAS	HRas proto-oncogene, GTPase	-8.4
	3845	KRAS	KRas proto-oncogene, GTPase	3265	HRAS	HRas proto-oncogene, GTPase	-8.7
M26911	2767	GNA11	G protein subunit alpha 11	2776	GNAQ	G protein subunit alpha q	-9.3
MM14496	2767	GNA11	G protein subunit alpha 11	2776	GNAQ	G protein subunit alpha q	-8.0
	2776	GNAQ	G protein subunit alpha q	2776	GNAQ	G protein subunit alpha q	-8.0



HRas proto-oncogene, GTPase (HRAS)

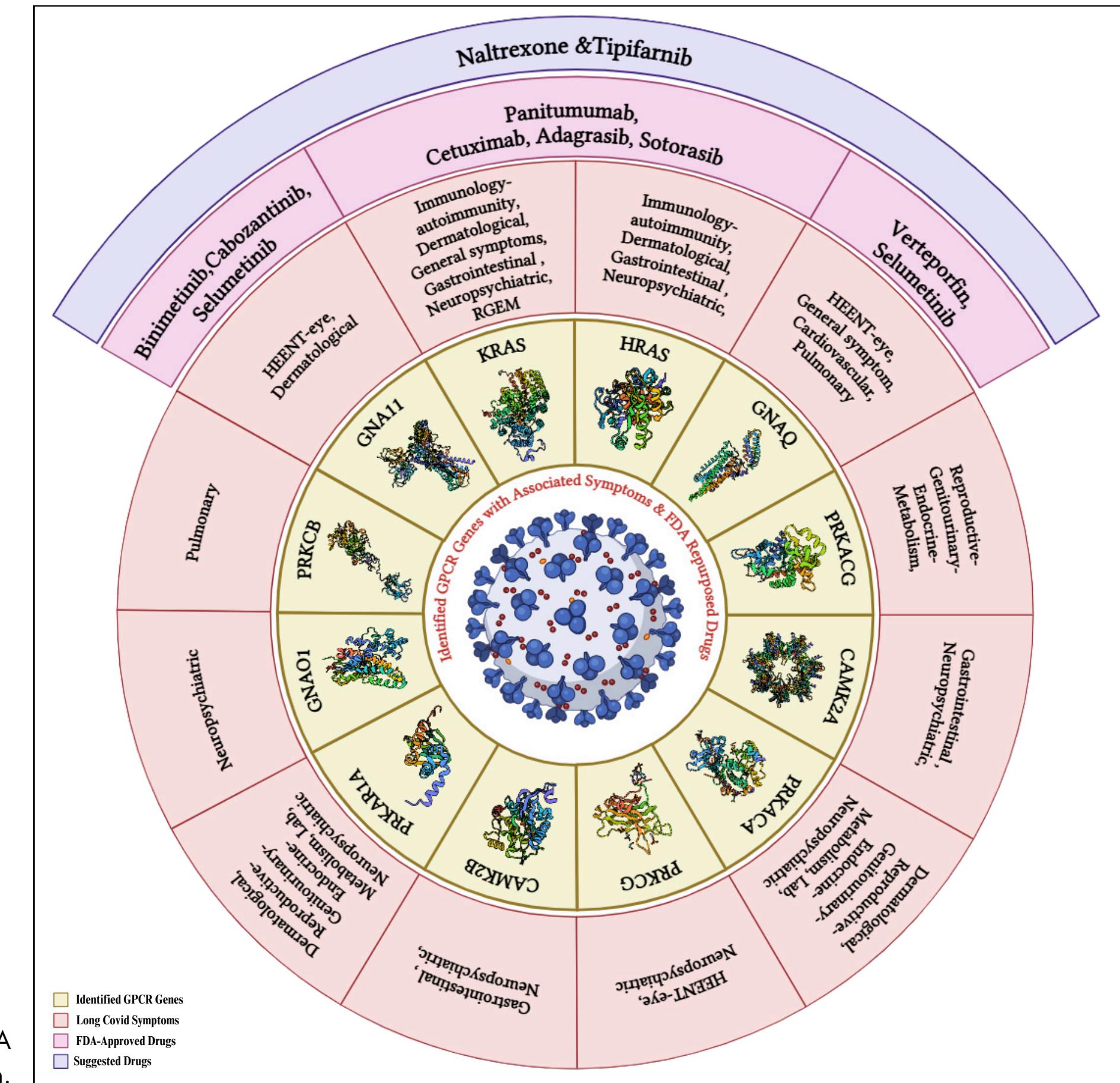
4.0 Results

Post-Docking Analysis:

Table 4.4: Common genes identified in more than one Long COVID symptoms.

Identified Genes	No of 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
PRKG	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

Figure 4.5: Identified GPCR genes with associated Long Covid Symptoms & FDA Repurposed Drugs, created with BioRender.com.



5.0 Discussion

5.1 Background of Identified Genes

- **RAS Family** (HRAS,KRAS..): involved in molecular switches controlling cell growth, differentiation, and survival activity (Killoran & Smith, 2019)
- **G protein** (GNAQ, GNA11..): bridge signal transduction from receptors to a large variety of effectors to influence processes from sensory perception to hormonal responses. (Hepler et al., 2019)
- **Protein Kinases** (PRKACA,PRKCB): modify proteins by phosphorylation, thus keying on and off important cellular functions, such as gene expression, secretion, growth, and immune responses (Taylor et al., 2021)
- **Calcium/calmodulin-dependent protein kinases** (CAMKK1..): regulating cell cycle, memory forming, and muscle contraction (Yasuda et al., 2022)

5.2 Association of GPCR with Long covid Symptoms

- only 12 among genes identified as pertinent to Long COVID symptoms since they participate in the receptor G protein-coupled cascade
- **Ras Family:** anaphylactic shock & alopecia (HRAS), chronic pain & Autoimmune probelems (KRAS)
- **G protein:** Ocular Pain (GNA11&GNAQ), neurotranmission problems (GNAO1)
- **Protein Kinases:** inflammation & synaptic signalling (PRKCB & PRKCG), celular signalling (PRKACA & PRKACG), metabolic and cognitive dysfunctions (PRKAR1A)
- **CAMKs:** neuropsychiatric & gastroesophageal disorders (CAMK2A & CAMK2B)

5.0 Discussion

5.3 Autoantibodies & Long Covid Symptoms Severity

- symptoms persist because of immune dysregulation, in which multiple proteins & tissue regions are targeted by autoantibodies, leading to gross inflammation of multiple tissues &, ultimately, organ failure.

5.6 Drug Candidates for GPCR Genes

- **Verteporfin & Selumetinib:** ocular and cellular proliferation (GNAQ)
- **Binimetinib & Cabozantinib:** dermatologic and ocular issues (GNA11)
- **Panitumumab, Cetuximab, Adagrasib, & Sotorasib:** autoimmunity & cellular growth (KRAS & HRAS)
- **Tipifarnib:** immune modulation & tissue repair (Lee, 2022; Odeniyide et al., 2022)
- **Naltrexone:** anti-inflammatory & immune regulatory (Choubey et al., 2022).

5.5 Roles of GPCR in Immune Modulation, Inflammation & Respiratory Functions

- **Ras Family:** involved in signalling pathway & regulate the proliferation & survival of immune (necessary for proper immune responses against viral infections)
- **G protein:** cellular signalling that translates to immune cell activities associated with cell mobility & cytokine production, promote the activation of phospholipase C, thereby initiating cascades that influence vascular and smooth muscle functions
- **Protein Kinases:** mediating cellular responses in immune activation and inflammation
- **CAMKs:** regulate calcium signaling in inflammation processes (central in the chronic inflammation)

5.0 Discussion

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Griffith University researchers to trial naltrexone on long COVID patients

By Janelle Miles and Emma Pollard
Posted Tue 7 May 2024 at 3:34am, updated Tue 7 May 2024 at 4:44am



Nineteen-year-old Jayden Donald is hoping to enter international dressage competitions once he fully recovers. (ABC News:

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Naltrexone

19-year-old talented equestrian, experienced severe Long covid symptoms such as severe exhaustion, brain fog, headaches, abnormally low blood pressure and a high heart rate.

After six months of these symptoms, researchers administered low-dose Naltrexone. Twelve months later, he showed a 70% improvement in his condition.

Limitations

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1. Reliance on Existing Databases:

The study's scope (long covid genes) is limited by its dependence on pre-existing databases and preprints, potentially missing or biased data from initial data cleaning.

2. Use of Parameters:

Default parameters in tools like ToppGene, EMBOSS Needle, and PA-Pred2 may affect reproducibility and specificity, with specific thresholds potentially excluding relevant genes.

3. Overlap of GPCR & Long Covid Genes:

The overlap may introduce confounders, complicating the understanding of genetic and biological mechanisms and interpretation of results.

6.0 Conclusion



Gene Identification

- identified 21 genes as potential druggable targets for Long COVID, with 12 of these genes associated with reported Long COVID symptoms.
- 4 key genes (HRAS, KRAS, GNAQ, GNA11) showed consistent identification across all steps suggesting their central role in Long COVID pathology and therapy.

Drug Repurposing

- identified 8 FDA-approved drugs and suggested 2 additional drugs for repurposing as new therapeutic avenues for Long COVID, highlighting the need to accelerate drug discovery using high-throughput screening and AI-guided predictive modeling.

Future Work

- focus on functional characterization, international collaboration, funding for comprehensive studies, and interdisciplinary efforts with patient advocacy involvement.

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