



IDENTIFICATION OF NATURAL REMEDIES FOR LONG COVID BASED ON
HUB GENE BIOMARKERS AND REPURPOSED DRUGS

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DECLARATION

I hereby declare that the thesis is based on my original work except for quotations and citations which have been duly acknowledged. I also declare that it has not been previously or concurrently submitted for any other degree at MSU or other institutions.

15 MAY 2023

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IDENTIFICATION OF NATURAL REMEDIES FOR LONG COVID BASED ON HUB GENE BIOMARKERS AND REPURPOSED DRUGS

ABSTRACT

Long COVID is a phenomenon in which individuals experience persistent symptoms after recovering from COVID-19. The symptoms are discovered to be unique for every individual and can affect multiple organs systems in the body. This study aims to identify effective natural remedies for long COVID by analyzing hub genes associated with the symptoms of the condition and evaluating the repurposed drugs catered and used for treating the symptoms of long COVID. The most common and prevalent symptoms of long COVID were identified; Fatigue, Shortness of Breath, Loss of Smell, Headache, Brain Fog, Chest Pain, Insomnia, Heart Palpitations, Dizziness, Joint Pain, Depression, Anxiety, Tinnitus, Loss of Appetite. Hub genes for each of the symptoms provided an insight on the key pathways and process of the symptom's biological system. The NF1 and RET genes were found to be associated with biological pathways of more than one symptom. Repurposed drugs identified for the symptoms, provided the template to identify the natural compounds with similar structure as a potential therapeutic drug. The natural compounds were retrieved using fingerprint search of the repurposed drugs from the Natural Product Activity and Species Source Database (NPASS). The findings of this study suggest several natural remedies for each symptom based on the molecular docking of the hub gene and natural compound using iGEMDOCK. Dehydroevidamine and Gefitinib were the natural compounds identified for the NF1 and RET genes respectively that could serve as a remedy for several symptoms. The identified natural remedies may hold promise in treating long COVID, but further research is required to explore the efficacy and effectiveness of the proposed natural compounds. The results of the study pose important implications for the development of effective treatments for long COVID.

**PENGENALAN PENAWAR SEMULAJADI UNTUK SINDROME POST COVID
BERDASARKAN BIOMARKER GEN HUB DAN UBAT-UBAT YANG DIGUNAKAN
SEMULA.**

ABSTRAK

Sindrom Post COVID adalah satu fenomena seseorang individu mengalami simptom berterusan selepas pulih daripada COVID-19. Gejala yang dialami berbeza bagi setiap individu dan ia boleh menjejaskan sistem organ dalam badan. Tujuan kajian ini adalah untuk mengenal pasti ubat dari produk semulajadi yang berkesan untuk sindrom post COVID dengan melakukan analisis gen hub dengan simptom yang dialami, dan menilai keberkesanan ubat yang sedia ada dan digunakan untuk merawat sindrom post COVID. Simptom yang lazim yang dialami bagi pesakit sindrom post COVID ialah keletihan, sesak nafas, hilang deria bau, sakit kepala, kabut otak, sakit dada, insomnia, jantung berdebar, pening, sakit sendi, kemurungan, keresahan, tinnitus dan hilang selera makan. Gen Hub untuk setiap simptom menggambarkan laluan dan proses sistem biologi simptom. Gen NF1 dan gen RET didapati mempunyai laluan biologi lebih daripada satu gejala. Ubat semula digunakan untuk mengenal pasti simptom, menyediakan templat bagi mengenal pasti sebatian semula jadi untuk menghasilkan sebatian semula jadi dengan struktur yang sama sebagai ubat terapi yang berpotensi. Sebatian semula jadi yang diperolehi daripada carian data ubat yang digunakan semula daripada Pangkalan Data Sumber Aktiviti dan Spesies Produk Asli (NPASS). Penemuan daripada kajian ini mencadangkan ubatan semula jadi bagi setiap gejala yang dialami berdasarkan molekul gen hub dan sebatian semula jadi dengan menggunakan iGEMDOCK. Dehydroevidiamine dan Gefitinib adalah sebatian semula jadi yang telah dikenal pasti untuk gen NF1 dan RET yang berfungsi sebagai ubat untuk beberapa gejala. Pemulihan semula jadi yang dikenal pasti mungkin menjanjikan untuk merawat sindrom post COVID tetapi kajian berpanjangan perlu dilakukan untuk mengkaji keberkesanannya dan keberkesanan sebatian semula jadi yang dicadangkan. Hasil kajian adalah titik terpenting dalam pembangunan rawatan berkesan untuk sindrom post COVID.

CONTENT

| | |
|------------------------------|------------|
| APPROVAL | i |
| DECLARATION | ii |
| ACKNOWLEDGEMENTS | iii |
| ABSTRACT | iv |
| ABSTRAK | v |
| CONTENT | vi |
| LIST OF TABLES | xi |
| LIST OF FIGURES | x |
| LIST OF ABBREVIATIONS | xi |

| | |
|-----------------------------------------|----------|
| CHAPTER I INTRODUCTION | 1 |
| 1.1 Overview | 1 |
| 1.2 Objectives | 4 |
| 1.2.1 General Objective | 4 |
| 1.2.2 Specific Objective | 4 |
| 1.3 Hypothesis | 4 |
| CHAPTER II LITERATURE REVIEW | 6 |
| 2.1 Introduction | 6 |
| 2.1.1 Epidemiology of Long COVID | 6 |
| 2.1.2 Understanding Long COVID | 6 |
| 2.2 Clinical Manifestations | 7 |
| 2.2.1 Symptoms of Long COVID | 7 |
| 2.2.2 Severity and Duration of Symptoms | 8 |
| 2.2.3 Risk Factors of Long COVID | 9 |
| 2.3 Underlying Mechanism of Long COVID | 9 |

| | | |
|--------------------|----------------------------------------------------------|-----------|
| 2.3.1 | Immunological Mechanisms | 10 |
| 2.3.2 | Viral Persistence and Latency | 11 |
| 2.3.3 | Organ Damage and Dysfunction | 12 |
| 2.3.4 | Psychological Factors | 12 |
| 2.4 | Diagnosis of Long COVID | 13 |
| 2.4.1 | Challenges in Diagnosing Long COVID | 13 |
| 2.4.2 | Diagnostic Criteria, Tests and Imaging | 14 |
| 2.5 | Treatment Strategies for Long COVID | 14 |
| 2.5.1 | Treatment Strategies | 15 |
| 2.6 | Prevention of Long COVID | 16 |
| 2.6.1 | COVID-19 Vaccines | 17 |
| 2.6.2 | Other Vaccines | 17 |
| 2.6.3 | Nutrition and Diet Management | 17 |
| 2.6.4 | Repurposed Medications | 18 |
| CHAPTER III | METHODOLOGY | 19 |
| 3.1 | Introduction | 19 |
| 3.2 | Data Retrieval | 21 |
| 3.3 | Identification of Natural Compounds | 21 |
| 3.4 | Hub Genes Data Collection | 22 |
| 3.5 | 2D Structure of Natural Compounds | 22 |
| 3.6 | Conversion of 2D Structures to 3D Structures | 22 |
| 3.7 | Molecular Docking of the Hub Genes and Natural Compounds | 23 |
| CHAPTER IV | RESULTS | 24 |
| 4.1 | Identification of Natural Compounds | 24 |
| 4.2 | Hub Gene Data Collection | 25 |
| 4.3 | Conversion of 2D Structures to 3D Structures | 25 |
| 4.4 | Molecular Docking of the Hub Genes and Natural Compounds | 26 |

| | | |
|-------------------|------------------------------------|------------|
| CHAPTER V | DISCUSSION | 34 |
| 5.1 | Fatigue | 34 |
| 5.2 | Shortness of Breath | 37 |
| 5.3 | Loss of Smell | 40 |
| 5.4 | Headaches | 42 |
| 5.5 | Brain Fog | 45 |
| 5.6 | Chest Pain | 47 |
| 5.7 | Insomnia | 48 |
| 5.8 | Heart Palpitations | 51 |
| 5.9 | Dizziness | 53 |
| 5.10 | Joint Pain | 55 |
| 5.11 | Anxiety and Depression | 57 |
| 5.12 | Tinnitus | 59 |
| 5.13 | Anorexia | 62 |
| | | |
| CHAPTER VI | CONCLUSION AND FUTURE WORKS | 64 |
| | | |
| REFERENCES | | 65 |
| | | |
| APPENDIX A | GANTT CHART | 85 |
| APPENDIX B | SUPPLEMENTARY TABLES | 86 |
| APPENDIX C | MANUSCRIPT | 108 |
| APPENDIX D | POSTER | 140 |

LIST OF TABLES

| Table No | Table Title | Page |
|------------|--------------------------------------------------------------------------------------------------------------------------------------|------|
| Table 4.1 | The top three molecular docking results for the symptom, Fatigue | 27 |
| Table 4.2 | The molecular docking result for Shortness of Breath with more than -100 binding energy | 27 |
| Table 4.3 | The molecular docking result for Loss of Smell with more than -100 binding energy | 28 |
| Table 4.4 | The molecular docking result for Headache with more than -90 binding energy | 28 |
| Table 4.5 | The molecular docking result for Brain Fog with more than -100 binding energy | 29 |
| Table 4.6 | The top two molecular docking results for Chest Pain and the top result of the SMAD4 gene complexes | 30 |
| Table 4.7 | The top two molecular docking results for Insomnia with the same natural compound | 30 |
| Table 4.8 | The molecular docking result for Heart Palpitations with more than -100 binding energy | 31 |
| Table 4.9 | The molecular docking result for Dizziness with more than -80 binding energy | 31 |
| Table 4.10 | The molecular docking result for Joint Pain with more than -100 binding energy, the lowest FAS gene complex and the CR2 gene complex | 32 |
| Table 4.11 | The molecular docking result for Depression and Anxiety | 33 |
| Table 4.12 | The top three molecular docking results for Tinnitus and the recurring gene, NF1 gene complex | 33 |
| Table 4.13 | The molecular docking result for Anorexia (Loss of Appetite) with more than -100 binding energy | 33 |

LIST OF FIGURES

| Figure No | Figure Title | Page |
|------------|---------------------------------------------------------------------------------------------------------------------------------------------|------|
| Figure 2.1 | The presence of hACE2 receptors in each organ and long COVID symptoms associated with each organ. Diagram obtained from Crook et al (2021). | 8 |
| Figure 3.1 | The overall workflow of the study | 20 |

LIST OF ABBREVIATIONS AND SYMBOLS

| | |
|------------|-----------------------------------------------------------------|
| ABCB11 | ATP-binding cassette sub-family B member 11 |
| ABCB4 | ATP Binding Cassette Subfamily B Member 4 |
| ACE2 | (human) Angiotensin-Converting Enzyme 2 |
| CACNA1B | Calcium Voltage-Gated Channel Subunit Alpha1 B |
| CACNA1D | Calcium Voltage-Gated Channel Subunit Alpha1 D |
| CACNA1G | Calcium Voltage-Gated Channel Subunit Alpha1 G |
| CDH23 | Cadherin-Related 23 |
| CHRNA1 | Cholinergic Receptor Nicotinic Beta 1 Subunit |
| CHRNA4 | Cholinergic Receptor Nicotinic Delta Subunit |
| CHRNA5 | Cholinergic Receptor Nicotinic Epsilon Subunit |
| COVID-19 | Coronavirus Disease 2019 |
| CR2 | Complement Receptor 2 |
| Elec | Electrostatic Interactions |
| ERBB4 | V-Erb-B2 Avian Erythroblastic Leukemia Viral Oncogene Homolog 4 |
| ESR1 | Estrogen Receptor 1 |
| FAS | Fatality-Associated Protein |
| FDA | Food and Drug Administration |
| GABRA1 | Gamma-aminobutyric acid receptor subunit alpha-1 |
| GATA4 | GATA binding protein 4 |
| GNRH1 | Gonadotropin-Releasing Hormone 1 |
| Hbond | Hydrogen Bonding |
| KCNT1 | Sodium-Activated Potassium Channel Subunit Alpha-1 |
| MEN1 | Multiple Endocrine Neoplasia Type 1 |
| NF1 | Neurofibromin 1 gene |
| NICE | National Institute for Health and Care Excellence |
| NPASS | Natural Product Activity and Species Source (Database) |
| NR1H4 | Nuclear Receptor Subfamily 1 Group H Member 4 |
| PALB2 | Partner and Localizer of BRCA2 |
| RET | Rearranged during Transfection |
| RYR2 | Ryanodine Receptor 2 |
| SARS-CoV-2 | Severe Acute Respiratory Syndrome Coronavirus 2 |
| SCN1B | Sodium Channel, Voltage-Gated, Type I, Beta Subunit |
| SCN5A | Sodium Voltage-Gated Channel Alpha Subunit 5 |
| SLC12A3 | Solute Carrier Family 12 Member 3 |
| SMAD4 | Mothers Against Decapentaplegic Homolog 4 |
| SNP | Single Nucleotide Polymorphism |
| TAC3 | Tachykinin 3 |
| TERT | Telomerase Reverse Transcriptase |
| VDW | Van der Waals Interactions |
| WHO | World Health Organization |
| >= | Greater than or equal to |

CHAPTER I

INTRODUCTION

1.1 OVERVIEW

The name, Coronavirus disease 2019 (COVID-19), is a disease that has spread like wildfire throughout the world in the year 2019 till the current date. As of early May 2023, a total of 688 million coronavirus cases caused by COVID-19 with over six million deaths worldwide (COVID-19 CORONAVIRUS PANDEMIC, n.d.). The World Health Organization (WHO) has identified COVID-19 as an infectious disease caused by the Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2) which causes respiratory illness to the infected individuals (World Health Organization: WHO, 2020). Efforts were conducted to curb the spreading of the virus but complete control of the virus was inevitable; thus, resulting in the rise of multiple variants of the virus which has exceeded the expectations of mankind. The major variants of concern include Alpha, Beta, Gamma, Delta and Omicron. Each variant was identified to possess unique and overlapping amino acid substitutions that influenced the transmissibility, disease severity and susceptibility to natural and vaccine-induced immune responses and monoclonal antibodies (J. L. Jacobs et al., 2022).

The primary mechanism of the COVID-19 infection revolves around the human angiotensin-converting enzyme 2 (ACE2), the specific surface receptor with which the virus binds to (Hoffmann et al., 2020). Upon entry to the cell, the virus replicates and matures, triggering an inflammatory response in the infected patient that involves the activation and the invasion of the immune cells by cytokines. As the ACE2 receptor is present in numerous types of cells throughout the human body, it provides a direct access to the biological pathways for the SARS-CoV-2 virus to inflict damage to multiple organs

at once (Crook et al., 2021). A new illness was identified where the initial damages induced by the virus to the organs appeared to persist even after a complete recovery of a patient from COVID-19.

Several clinical studies have identified that many COVID-19 survivors experience and undergo new or persistent symptoms that emerge and continue for weeks and months following the acute phase of COVID-19. These persistent symptoms were known as and went by with the names “Long COVID”, “Long Haulers” or “Post COVID syndrome” since October 2020 (Raveendran et al., 2021). Researchers, however, had identified this condition as long COVID, post-acute sequelae of COVID (PASC), or post-acute COVID-19 syndrome (PACS) because it characterizes the persistence of the prolonged symptoms that occurs after the acute phase of the SARS-CoV-2 infection (Deer et al., 2021).

Long COVID can affect anyone who has had COVID-19, regardless of the severity of the initial illness or whether they were hospitalized. The majority of patients with post-COVID syndrome have PCR results that are negative, which indicates microbiological recovery from acute-COVID. The delay between the microbiological recovery and the clinical recovery is, in other words, what is understood by Long COVID syndrome (Garg et al., 2021). According to a journal article, long-term COVID can be separated into two stages depending on how long a patient's symptoms last (Raveendran et al., 2021). Post-acute COVID refers to symptoms that last longer than three weeks but less than twelve weeks, while chronic COVID refers to symptoms that last longer than twelve weeks.

Several systematic reviews have documented patients suffering from a wide spectrum of persistent symptoms across the globe, including fatigue, headache, dyspnea, shortness of breath, and cognitive deficits (Akbarialiabad et al., 2021, Davis et al., 2023, Lopez-Leon et al., 2021, Crook et al., 2021). Among the many symptoms, chest pain, fatigue, dyspnea, and joint pain were reported as the most frequent symptom experienced by long COVID patients. Several studies have found a similar trend in the symptoms that are experienced by the patients (Sudre et al., 2021, Huang et al., 2021, Lopez-Leon et al., 2021, Carfi et al., 2020, Tenforde, 2020). Furthermore, it was identified that the symptoms caused by long COVID are spread across different organ systems including the cardiovascular, respiratory, neurological, musculoskeletal, and nervous systems, among others.

Symptoms such as fatigue, sleep disturbance, headaches, cognitive impairments and memory loss are examples that would impact the neurological health of the patient and are in direct relation with the central and peripheral nervous system (Umesh et al., 2022). Microvascular disorders and induced heart abnormalities, such as chest pain, are some symptoms that are related to the cardiovascular system (Castanares-Zapatero et al., 2022).

It has been identified that long COVID can cause an impact regardless of the demography of patients such as age groups and the severity of the symptoms which includes those who have very minor symptoms to those who have chronic or severe symptoms. In general, long COVID is not proven to be present in everyone who acquires COVID-19. But all individuals with a past history of COVID-19 infection are at risk. Interestingly, based on the results of numerous studies and the National Institute for Health and Care Excellence (NICE), the risk of long-term COVID appears to be more prevalent in certain categories; the female gender, people of color and Asian ancestry, people in poor pre-pandemic mental health, and people with certain underlying medical conditions such as obesity and heart disease.

Treatment for long COVID is a complex process as it is not well defined. No singular validated treatment or drug is effectively able to cover the wide range of symptoms that is presented in patients. The treatment of individuals with long COVID requires a multidisciplinary approach which involves assessment, symptomatic treatment, underlying problem treatment, physical therapy, occupational therapy, and psychological support. Several studies suggested the uses of different types of drugs such as Paxlovid (an antiviral) and Palmitoylethanolamide (reduces chronic inflammations) for long COVID symptoms (De Luca et al., 2022, Davis et al., 2023). Clinical trials are being conducted on various synthetic drugs, while some proposed drugs have not yet been utilized for treatment. Due to the complexity of long COVID and the multiple organs it affects, it poses a challenge to identify a single drug that can effectively treat it.

Similarly, the use of natural compounds as natural remedies for long COVID could provide promising therapeutic drugs. However, the exposure for the use of natural remedies are lower compared to synthetic and repurposed drugs. There have been multiple studies on

the use of natural remedies for COVID-19 but not for long COVID symptoms. Therefore, the study's aim is to explore the potential of natural phytochemical drugs as a treatment option. One hypothesis is that by studying the currently available repurposed drugs for long COVID, it can identify the natural compounds that can be used as ligands to bind with key genes acting as receptors. This approach could potentially lead to the development of effective natural treatments for long COVID.

1.2 OBJECTIVES

1.2.1 General Objective

The objective of the study is to identify the natural compounds that can be used as a therapeutic drug for long COVID based on the hub genes and the associated repurposed drugs using bioinformatics tools.

1.2.2 Specific Objectives

- i. To study the hub gene biomarkers for the most commonly presenting long COVID symptoms
- ii. To identify the natural compounds associated with hub genes of each individual long COVID symptoms
- iii. To examine the Molecular Docking between the hub gene biomarkers and natural compounds
- iv. To evaluate the binding affinity of the hub gene-natural compound complex

1.3 HYPOTHESIS

The hypothesis of the project is to identify the natural compound as a therapeutic drug using the repurposed drugs for each hub gene associated with the long COVID symptoms.

The hypothesis outcome for the project are:

Ho: No appropriate or suitable natural compounds were found as a therapeutic drug and natural remedy for the long COVID symptoms studied.

H1: Appropriate and suitable natural compounds were identified as a therapeutic drug and natural remedy for the long COVID symptoms studied.

CHAPTER II

LITERATURE REVIEW

2.1 INTRODUCTION

Long COVID, also known as post-acute sequelae of SARS-CoV-2 infection (PASC), is a term used to describe a collection of persistent symptoms that last longer than the standard two-week recovery period following acute COVID-19 infection. The symptoms of long COVID are varied for each individual based on the severity and may impact several organ systems. The purpose of this literature review is to summarize the current understanding of long COVID in terms of the symptoms, diagnosis, treatments, prevention and mechanism.

2.1.1 Epidemiology of Long COVID

The epidemiology of long COVID is still being studied, and several studies have found and provided valuable insights regarding its prevalence. A study of 25 observational studies, conducted by Martimbianco et al. (2021), discovered that among the 5440 participants, the prevalence of long Covid varied from 4.7 to 80%. According to a different study done in Jin Yin-tan Hospital in Wuhan, China, 76% of patients (1265 of 1655) have reported at least one symptom at the follow-up (Huang et al., 2021). Similar to this, a systematic study of the effect of long COVID found that 80% of SARS-CoV-2 infected patients experienced one or more long-term symptoms (Lopez-Leon et al., 2021). It is imperative to comprehend the mechanism of long COVID since recent data indicate that individuals who survive the acute phase of the illness are at risk for long-term sequelae with involvement of the skin, respiratory, cardiovascular, musculoskeletal, mental health, neurologic, and renal systems (Akbarialiabad et al., 2021).

2.1.2 Understanding Long COVID

Scientific studies show that there are several different types of symptoms that patients experience. The most frequently reported symptoms, according to a cohort study of 1733 patients in Wuhan, China, were muscle weakness and fatigue at 63%, followed by sleep issues with 26% and anxiety/depression at 23%. 76% of patients experienced at least one chronic symptom with a median follow-up of 186 days, with women reporting the highest rates (Huang et al., 2021). In another cohort analysis of 183 patients, fatigue, dyspnea, and muscle soreness persisted 35 days following hospitalization. These symptoms were also linked to lower assessments of physical and mental health, quality of life, and participation in active social activities (Jacobs et al., 2020). In a long-term trial of 180 non-hospitalized COVID-19 patients, 53% of patients experienced persisting symptoms, with exhaustion and loss of smell and taste and joint pain being the most common (Weihe et al., 2020). The symptoms of long COVID can range from joint pain, fatigue, headaches and more as identified by the previous studies. Therefore, Raveendran has clustered the symptoms into three categories which are respiratory symptom cluster, musculoskeletal symptom cluster and enteric symptom cluster. Each of these clusters specifies a set of symptoms (Raveendran et al., 2021). The respiratory symptom cluster includes symptoms such as cough, sputum, shortness of breath, and fever. The musculoskeletal symptom includes myalgia, joint pain, headache, and fatigue. Abdominal pain, vomiting, and diarrhea are the symptoms within the enteric symptom cluster.

2.2 CLINICAL MANIFESTATIONS

2.2.1 Symptoms of Long COVID

The most reported symptoms that were associated together with long COVID were researched and analyzed in many journals. Among all the studies, the most prominent symptom with the highest percentage among long COVID patients is Fatigue. Raveendran also had analyzed two primary symptom patterns in persons with long COVID based on the data obtained from a study that collected data via a COVID Symptoms Study App conducted by Sudre et al. (2021): 1) multi-system complaints, which include continuous fever and gastroenterological symptoms, and 2) exhaustion, headache, and upper

respiratory complaints (shortness of breath, sore throat, persistent cough, and loss of smell).

Several long-COVID symptoms have been documented and linked to different organs which have the hACE2 receptor. A study conducted in July 2021 had analyzed the different symptoms and categorized them accordingly (Crook et al., 2021). The image below (Figure 2.1) was retrieved from the article shows the organs and symptoms that are related to each organ.

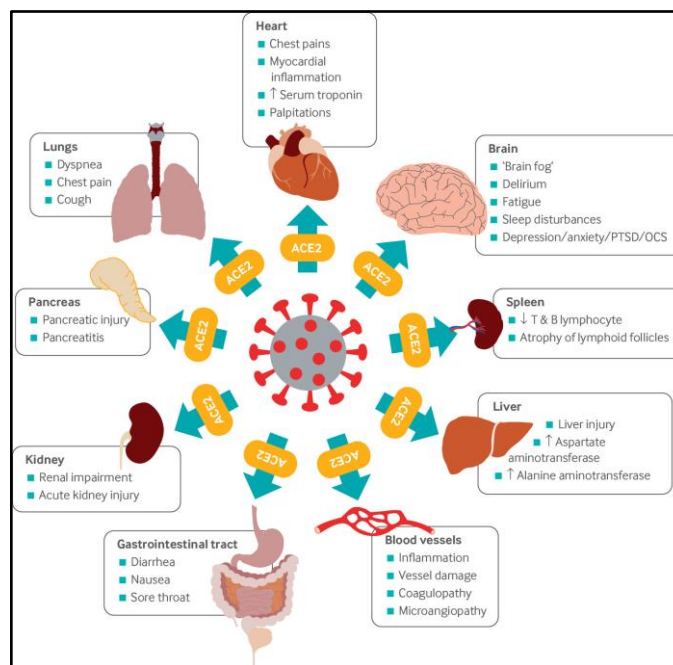


Figure 2.1: The presence of hACE2 receptors in each organ and long COVID symptoms associated with each organ. Diagram obtained from Crook et al (2021).

2.2.2 Severity and Duration of Symptom

Based on a systematic review of an article, it was found that there was a total of fifty-five long COVID symptoms that were identified. Among those fifty-five symptoms, the top five most prominent symptoms include fatigue (58%), headache (44%), attention disorder (27%), hair loss (25%), and dyspnea (24%) (Lopez-Leon et al., 2021). Fatigue, which is found to be the most common symptom of long COVID, was found to be present 100 days following the onset of the first acute COVID-19 symptom. The symptoms seen in post-COVID-19 patients share some similarities with those of chronic fatigue syndrome (CFS),

which is characterized by severe, incapacitating fatigue, pain, neurocognitive impairment, disrupted sleep, symptoms of autonomic dysfunction, and worsening of overall symptoms in response to slight increases in physical and/or mental activity. There have been reports of headache and attention problems among other neuropsychiatric symptoms. According to the report, the causes of COVID-19 individuals' neuropsychiatric symptoms are complicated and multifactorial. The direct impact of the infection, cerebrovascular disease (including hypercoagulation), physiological compromise (hypoxia), drug side effects, and social elements of having a potentially fatal illness are possible connections among them. After receiving a COVID-19 diagnosis, adults are twice as likely to receive a new diagnosis of a psychiatric disorder, and the most prevalent psychiatric illnesses reported were dementia, insomnia, and anxiety disorders. Dyspnea which is found in a significant number of patients presented around 35% of patients who continued to have abnormal CT lung scans 60 to 100 days after the initial presentation. In addition, follow-up research among non-critical COVID-19 hospitalized patients in China found that two-thirds of patients continued to have abnormal radiography 90 days after discharge. Interestingly, another review paper from nature.com, showed a survey where more than 1.3 million people who had COVID-19, identified mental health issues including anxiety and depression gradually returned to normal, but there was an increase in the chance of neurocognitive conditions like dementia, psychosis, seizures, and brain fog for at least two years (Davis et al., 2023).

2.2.3 Risk Factors of Long COVID

During the acute phase of COVID-19, long COVID can happen to people of various ages and with varying degrees of illness severity. There are a number of risk factors that have been found, such as advanced age, the female gender, and having certain underlying illnesses like diabetes, obesity, and other metabolic disorders. Among these risk factors, women, older people, and those with obesity are the three most common factors that are frequently mentioned in multiple articles (Mendelson et al., 2020). An increased risk of long COVID has also been linked to the severity of acute diseases and hospitalization.

2.3 UNDERLYING MECHANISM OF LONG COVID

The mechanism of long COVID is still being studied and is not yet fully understood. However, the studies have identified the wide range of symptoms that the patients experience, allowing us to identify and analyze the mechanism of long COVID based on the respective system that is affected. Therefore, the mechanisms of long COVID were studied and reviewed by several articles where they categorized the mechanism of long COVID according to the symptoms caused and organs system affected. Furthermore, the symptoms of long COVID could affect the quality of life and also cause multiple organ damages to the individuals who developed acute COVID-19 (Pierce et al., 2021). Thus, the mechanism of long COVID and its manifestation is crucial to be analyzed and studied with great scrutiny.

2.3.1 Immunological Mechanisms

As a field of study that is still ongoing, there is limited literature in regards to the immunological mechanism of long COVID. Some studies have suggested that in addition to vaccination, the active immune system which promotes the innate and active immunity in the body, can aid in the prevention of acute COVID-19, ultimately reducing the risk of long COVID symptom development (Sciscent et al., 2021). Patterson et al. (2022) identified and confirmed the cause of the presence of persistent SARS-CoV-2 protein being the monocytes, CD14^{lo} and CD16⁺, found in the body 65 weeks after the initial infection. It was also proposed that the intermediate monocytes remained in the circulation for a prolonged period due to the low presence of the CCL4 protein (regulates immune response and inflammation), which leads to an accumulation of non-classical monocytes (anti-inflammatory cells) that contain the S1 protein, identified to be associated with vascular inflammation. Patterson et al. (2022) proceeded to explain the presence of S1 protein in the non-classical monocytes due to the pre-existing CD14^{lo} CD16⁺ cells that engulfs the virally infected apoptotic endothelial cells via phagocytosis.

A study conducted in 2021 by Huang. L stated that “The chronic or late-onset psychological symptoms after COVID-19 could be driven by a direct effect of virus infection and might be explained by several hypotheses including aberrant immune response, hyperactivation of the immune system, or autoimmunity” (L. Huang et al., 2021). This suggests that some individuals who have recovered from COVID-19 may experience chronic or late-onset

psychological symptoms, and that there are several possible explanations for this phenomenon in relation to the immune response of the body. The first hypothesis is where the psychological symptoms are associated together with an aberrant immune response, where it leads to inflammation or other effects that occur in the brain or nervous system. The second hypothesis is due to the hyperactivation of the immune system. This event where the immune system is overactive despite the absence of the virus could be a possible explanation to the chronic and late-onset psychological symptoms. The final hypothesis is autoimmunity, where the healthy cells and tissues are mistaken for the virus and attacked by the body. This phenomenon may play a role in the development of long covid symptoms. Several other studies also support these hypotheses as a possible explanation to the underlying mechanism of the psychological symptoms of Long COVID (C. Huang et al., 2021).

Another proposed mechanism of long COVID for persistent in the body was identified where the viral RNA was found in the feces of long COVID patients (Wu et al., 2020). This goes to prove that the virus RNA persists in the human body including the gastrointestinal tract of the patient as exhibited from the study. A review had presented that a number of studies has demonstrated the SARS-CoV-2 virus in viral transmission via fecal-oral transmission (Cevik et al., 2020). However, these studies have focused on the viral transmission of the SARS-CoV-2 virus. The fact that the virus is able to survive in such an environment could provide a better insight to the underlying mechanism of the virus in regards to causing prolonged symptoms.

2.3.2 Viral Persistence and Latency

Long COVID's viral persistence and latency are still being studied and are not yet fully understood. After initial infection, some individuals continued to emit viral RNA from their respiratory tracts for a number of weeks or even months, according to a cohort study (L. Huang et al., 2021). In addition, a subsequent study reported in Nature in 2021 discovered that some people with COVID-19 who were mildly or asymptotically ill continued to have detectable levels of viral RNA in their respiratory tract weeks after the initial infection (Gaebler et al., 2021). In order to completely comprehend the viral persistence and latency

of long COVID and to develop efficient therapies and care techniques for patients who still have symptoms following their initial recovery, more study is required.

2.3.3 Organ Damage and Dysfunction

The clinical manifestation of long COVID has been identified to be associated with causing long term damages that causes impact to multiple organs systems at once. Not treating these prolonged symptoms can even lead to death. Pierce et al. (2021) determined the most common manifestations of long COVID and the organs that are usually affected by it where it causes disruption to the lungs, brain, heart and kidneys, ultimately affecting the quality of life. Since the clinical manifestations differ for each individual, it contributes to the difficulties of diagnosing the symptoms properly and identifying the organs affected. Fatigue, being one of the most common manifestations of long COVID, is a neurological dysfunction which was identified to cause dysfunction to the neuronal circuit in patients who experience fatigue for over six weeks (Astin et al., 2022). The acute phase of COVID infection, either mild or moderate, leads to inflammation which results in fatigue (Poenaru et al., 2021). The symptom fatigue, despite being a neurological dysfunction, is able to affect the cardiovascular system by causing high heart rates and lowered heart rates (Astin et al., 2022).

2.3.4 Psychological Factors

The psychological factors are primarily related to the neurological symptoms of long COVID including depressions, psychosis, and anxiety which is identified to persist amongst those who had contracted acute SARS-CoV-2 (Troyer et al., 2020). Uncertainty, loneliness, unnecessary fear, and depression are the common signs and psychological problems experienced after recovery of initial infection which induces neurological dysfunctions and fibrosis. These psychological signs are found to present in worse conditions for those who have a past history of psychiatric or psychological issues, developments disorders, domestic violence survivor, the elderly and children under the age of 12 years old (Ferrario et al., 2021). Other psychological symptoms were also reported; insomnia, acute stress disorder and intrusive thoughts and intense fear of dying. It was found by Ferrario et al. 2021 that without proper care, these psychological symptoms

caused by long COVID could result in life threatening conditions and even lead to cognitive impairments. Furthermore, it was analyzed and estimated that 30% of patients experience at least one underlying symptom that progresses into a medical condition affecting the individual's health after recovery from the acute phase (Buonsenso et al., 2022). This calls for a better understanding of long COVID's mechanism and identifies an accepted criterion in symptom duration where the manifestation of the illness takes place to help improve the healthcare system (Peluso et al., 2021).

An interesting case was identified where Mannarini and Rossi (2019) reported the occurrence of psychological problems caused by long COVID in patients that experienced the loss of a body part or limb due to the symptoms of the acute infection. They were found to express and feel shame, guilt, insecurity, and stigmatization and the fear of reactions by close family or friends. This goes to show that not only neurological related symptoms manifest psychological factors but external factors and stressors are to be included as potential causes.

2.4 DIAGNOSIS OF LONG COVID

2.4.1 Challenges in Diagnosing Long COVID

The diagnosis of long COVID poses as one of the greatest challenges for researchers in the fight against this illness. Medical professionals and researchers are working on the feasibility of diagnosing the symptoms of long COVID and its mechanisms. This was found to be conducted by identifying the indicators relating to the persistent responses (hyperinflammatory response, low antibody reaction, progressing viral load, and organ damage) caused by the acute infection (Vehar et al., 2021). However, the diagnostic criteria for long COVID posed a major challenge as studied by Raveendran et al. (2021). He had suggested that there are several factors that could affect proper diagnosis such as, effects of medications, COVID-19 complications, psychological issues, microbial infection and the reinfection of COVID-19, that could potentially exhibit similar symptoms of those from long COVID. Thus, it is important to rule out and filter the causes that are not related when conducting a diagnosis. Moreover, the unclear guidelines of long COVID also disrupts the diagnosis process, where it can affect the healthcare system in managing

long COVID. Hence, a multidisciplinary approach was undertaken for community-based and specialist healthcare centers in managing, examining and treating long COVID was provided by the National Institute for Health and Care Excellence (NICE) (NICE, 2020).

2.4.2 Diagnostic Criteria, Tests and Imaging

The diagnostic criteria for long COVID symptoms was categorized into four main criteria by Oronsky et al. (2021). The criteria include and involve laboratory investigation, radiologic pathology, deterioration in functional status, and subjective symptomatic and quality-of-life parameters. Apart from that, the tests and imaging conducted differs for each symptom as they can be determined via various methods. As an example, a study that identified the recurrence of neuropsychiatric symptoms such as loss of taste, loss of smell, fatigue and headache (Graham et al., 2021), had proposed Magnetic resonance imaging (MRI) imaging (Nuzzo et al., 2021). Nuzzo et al. (2021) further explained that the use of MRI in patients suspected with neurological symptoms from long COVID provides better diagnostic results. Thus, concluding that standardized and refined guidelines are crucial to diagnose and manage patient care of long COVID symptoms.

2.5 TREATMENT STRATEGIES FOR LONG COVID

Long COVID symptoms are found to manifest in several ways where patients must be vigilant with their health. Excellence (2020) suggested an examination where the symptoms experienced and the pre-existing problems of a patient must be established in order to propose a suitable treatment option. It is recommended that evidence-based diagnosis and management strategies are conducted before providing treatment and care to patients. The NICE guidelines also stated that the clinical evaluation of long COVID is to begin 4 weeks following the acute infection of SARS-CoV-2 virus. However, regardless of the established guidelines, Nurek et al. (2021) identified a significant gap in treatment where individual treatments are not well assessed. Therefore, the treatment development will have to take several assessment factors into consideration where new upcoming therapies are guided based on treating the long COVID symptoms related to organs-specific dysfunction. Before any form of treatment is provided, the medical history and medical exams of each individual patient is to be taken into consideration as each patient

presents with unique characteristics. Thus, conducting the treatment procedures based on the symptom-based strategy. Moreover, other underlying conditions of patients, not associated with long COVID symptoms, are to be analyzed to provide a proper treatment.

2.5.1 Treatment Strategies

The treatment strategies for long COVID are found to be under different aspects that cater to the different long COVID symptoms. The treatment options include immunomodulatory therapy, anti-viral therapy, rehabilitation and counseling. The type of therapy that goes for each symptom is determined by the diagnosis of the symptom using tests and imaging processes, where as an example, Nurek et al. (2021) suggested electrocardiography, chest imaging and pulmonary function testing for cardiopulmonary long COVID symptoms.

Different types of symptoms are connected to different types of biological pathways. Thus, each treatment strategy for each symptom would be unique. For example, it was revealed that mast cell activation syndrome (MCAS) is likely to present in long COVID patients; hypothesizing the mechanism is caused by the immunological dysfunctions caused by the SARS-CoV-2 virus (Weinstock et al., 2021). The MCAS induces inflammatory reactions in the body that results in allergy flare-ups (Glynne et al., 2021). To combat this, histamine antagonists or antihistamine due to their ability to reduce and regulate mast cells. The antihistamines were found to be used as a treatment option for COVID but Pinto et al. (2022) stated its application in long COVID remains unclear but is a potential therapeutic treatment. Immunomodulatory compounds such as Luteolin and quercetin, naturally occurring flavonoids, exhibit properties that may inhibit mast cells (Hagenlocher & Lorentz, 2015). These compounds may aid in reducing the systemic inflammation and boost the body immunity.

A treatment strategy or therapy for long COVID induced inflammation leading to multiple organ damage is a proper dietary supplement, as vitamins and minerals contain anti-inflammatory and anti-oxidative properties providing relief to the inflammations. Supporting this, a study has found that multi-vitamin supplements have helped to improve the conditions of long COVID symptoms (Naureen et al., 2021). Furthermore, natural compounds extracted from plant extracts of *Eleutherococcus senticosus* and *Panax*

ginseng used in a study, showed improvement in the health of the 201-long term COVID patients providing a relief from the long-term symptoms (Rossato et al., 2021). Different types of vitamins and minerals such as nicotinamide ribose and Omega-3 are currently being studied for their properties and potential role in long COVID symptoms.

Antibiotic and antiviral compounds are investigated in regards to long COVID as the viral infection causes the immune system to be weak increasing the probability of opportunistic infections. The efficacy of drugs such as azithromycin, remdesivir, and favipiravir in the management of long COVID is being studied (Koc et al., 2022). Hohberger et al. (2021) studied the BC007 medication which treats autoimmunity by reducing the G protein-coupled receptor autoantibodies and reduces the fatigue symptom within patients. A study by Wood et al. (2021) identified the Coenzyme Q10 and D-ribose supplements showed potential in treating chronic fatigue but requires additional study to determine the mechanisms. Even antidepressant drugs have shown improvement in the symptoms induced as it is able to restore immunological functions and lowers peripheral inflammatory indicators.

While certain symptoms are to be treated by drugs, several symptoms can be alleviated by non-drug therapies such as physical therapy. Schrimpf et al. (2022) encouraged medical practitioners to encourage patients to undergo physical therapies to get relieved from the symptoms and improve overall health. However, the physical therapy regime for a patient is to be personalized based on certain thresholds as physical activities are potentially able to cause relapse of certain symptoms such as joint pain, chest pain and dyspnea (Vance et al., 2021). Physical exercises with the right rehabilitation protocols are able to encourage patients and continue to improve their health as well as symptoms.

2.6 PREVENTION OF LONG COVID

Long COVID is to continue to cause a significant impact on society on a global range as long as individuals are contracting the COVID-19. The acute infection poses as the root cause of long COVID symptoms. Therefore, the preventative methods and care for long COVID would primarily involve methods to avoid contracting COVID-19 itself. As for long COVID itself, the risk factors are to be considered as the individuals within the risk factors

are more prone to experiencing a symptom and therefore are advised to increase their immunity.

2.6.1 COVID-19 Vaccines

The primary preventative care for long COVID would involve the prevention of contracting COVID-19 via vaccinations. Several vaccinations have been developed and approved by the FDA and CDC over the span of 2 years. The COVID-19 vaccination has greatly affected to reduce the morbidity and mortality of the virus (Moghadas et al., 2021). Not only by just reducing the number of cases and deaths, the vaccinations have been found to affect the long-term symptoms of COVID-19. Several studies have identified a substantial decrease in the long-term symptoms of COVID due to immunization (Byambasuren et al., 2023). It is speculated that immunization reduces the effects of the virus on the organs and pathways resulting in the reduction of long-term symptom effects. The number of COVID-19 related deaths in Switzerland reported that around 40% involved individuals that had not received immunization whereas a 10% of death was seen in individuals with at least one dose of vaccination. These reports and studies goes to imply vaccination as a method of prevention for not only COVID-19 infection symptoms but for long COVID symptoms too.

2.6.2 Other Vaccines

Apart from COVID-19 vaccines, other diseases vaccines have been reported to show protective effects towards long COVID symptoms. Healthcare workers have stated that the influenza vaccine has shown to reduce the possibility of the SARS-CoV-2 virus infection and influences the severity of the acute infection. Supporting the statement, Taghioff et al. (2021) conducted a retrospective analysis of the influenza vaccination and presented a reduction in the risk of sepsis and deep vein thrombosis related to long COVID.

2.6.3 Nutrition and Diet Management

Nutrition and proper diet may not seem like a serious factor but it has been proven that with appropriate nutritional management, the symptoms of viral infections are reduced and are also used in the treatment of chronic illness (De Araújo Morais et al., 2021). The difference in the occurrence of COVID-19 infection between populations is speculated to have epigenetic polymorphism connected to the population's nutrition by Cao and Li (2020). Vitamin D, an important nutrient for the body, has been revealed by Liu et al. (2022) to exhibit prospective properties against SARS-CoV-2 virus. Vitamin D has therapeutic effects where it may reduce the pathogenicity of the SARS-CoV-2 virus by influencing the renin-angiotensin-aldosterone system (RAAS). The vitamin D supplements were identified to reduce respiratory infections and are being studied in clinical trials to provide evidence of its effects against COVID-19 (Rastogi et al., 2020).

2.6.4 Repurposed Medications

The use of repurposed medications for other diseases is common and the same goes for COVID-19 infections. Repurposed drugs aimed for COVID-19 include hydroxychloroquine and ivermectin, candidate drugs used widely during the pandemic. The repurposed medications can be used to prevent the infection of SARS-CoV-2 virus, thus, preventing the occurrence of the long COVID symptoms. However, this theory is not proven to be true. There might be cases where the repurposed drug causes adverse effects towards the virus as these drugs are not developed for COVID-19 infections. The use of the repurpose drugs instead of established drugs for COVID-19 and vaccination may enhance the development of long-term symptoms. Regardless, the use of repurposed drugs is a wide research field that has high potential to develop more therapeutic agents against long COVID symptoms. This can be determined by using drugs specified for the various long COVID symptoms not only as preventative care but also as a treatment for long COVID patients.

CHAPTER III

METHODOLOGY

3.1 Introduction

The Identification of natural remedies for long COVID based on hub gene biomarkers and repurposed drugs involved various bioinformatics tools and databases; PubChem, NCBI, NPASS, Open Babel, AlphaFold database, UniProt, iGEMDOCK. The overall flow of the methodology is simplified and represented in a flowchart in Figure 3.1.

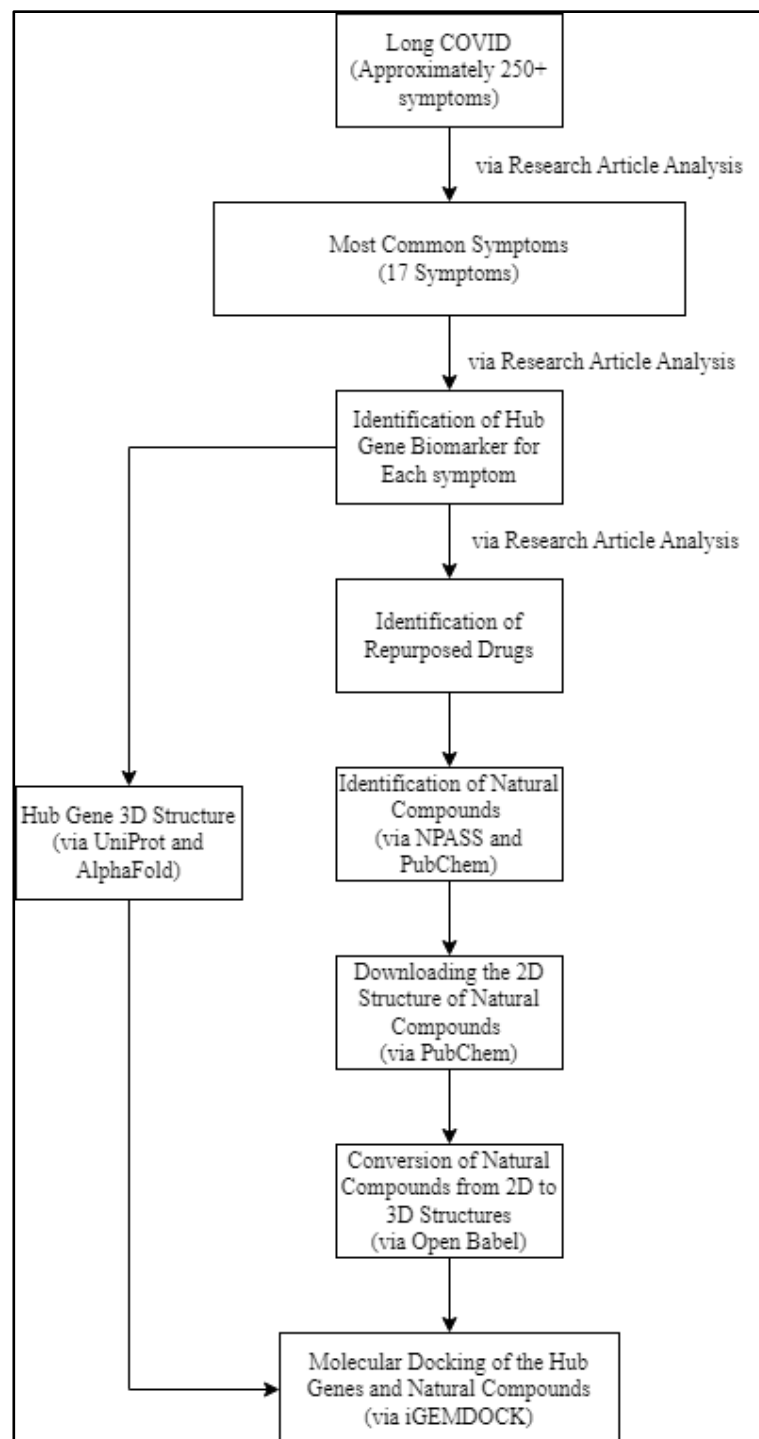


Figure 3.1: The overall workflow of the study

3.2 Data Retrieval

The data regarding the common symptoms of Long COVID were obtained based on publications that reported for long COVID symptoms- systematic reviews, meta-analyses, and other publications. Based on a journal, several peer-reviewed journals and databases have shown the presence of over 250 symptoms related to long COVID found in patients. Among the 250 symptoms, the most frequently appearing symptoms were subjected for the study. As a total, there were seventeen symptoms that were abundantly found in patients compared to the other symptoms. The seventeen symptoms include fatigue, shortness of breath, loss of smell, muscle pain, headache, brain fog, chest pain, difficulty sleeping, heart palpitations, dizziness, joint pain, depression, anxiety, tinnitus, diarrhea, loss of appetite and skin rash. The top ten hub genes related and associated with these seventeen symptoms were obtained from a study on long COVID G Protein-Coupled Receptors (GPCRs) by Sanisha Das (Das & Kumar, 2022). The hub genes for each symptom was identified as a biomarker for the symptom and the FDA approved repurposed drugs for each hub genes were also found and obtained from the study.

3.3 Identification of Natural Compounds

The presence of natural remedies for long COVID was to be determined by analyzing the FDA approved repurposed drugs that are currently in use as treatments for long COVID. This approach was utilized as over 50% of drugs that have been approved are either derived from natural products or are natural products themselves (Atanasov et al., 2021). Therefore, by using the Natural Product Activity and Species Source Database (NPASS) which has served as one of a leading data source for the Natural Product research community since its first version release in 2017, the natural compounds or their derivatives can be found from the repurposed drugs. For each symptom, there are a total of 10 hub genes and each gene has its respective repurposed drug. However, not all hub genes may have an FDA-approved drug. The FDA-approved drugs for each gene were taken individually and searched in the PubChem database to identify their canonical SMILES, the line notation used to represent the structure of the molecules. The canonical SMILES were required to identify the natural compounds using the NPASS database. The queries were searched by structure and the fingerprint type was set as PubChem-881 fp

and with a threshold of ≥ 0.80 . The natural compounds identified were recorded. In cases where there are more than 10 natural compounds, the top ten compounds that were found were taken into consideration. Hub genes and drugs that resulted in no natural compounds or natural products were found to be redundant. The remainder that resulted in the presence of natural compounds were advanced to the next stage.

3.4 Hub Genes Data Collection (3D Structure)

The UniProt database was used to retrieve and download the 3D structure of the filtered list of hub genes based on the presence of natural compounds. Each hub gene was searched in the database individually and the following filters were applied in the search query; Organism: Homo sapiens and Status: Reviewed (Swiss-Prot). The AlphaFold structure was downloaded to obtain the 3D structure of the hub genes. As not all AlphaFold 3D structures are available, for hub genes that do not have the AlphaFold structure, the SMR structure was obtained.

3.5 2D Structure of Natural Compounds

The resulting natural compounds that were found from the structure of the FDA-approved drugs (repurposed drugs) were recorded from the NPASS database. To further study the natural compounds, the PubChem database, which is a public database containing information on millions of chemical compounds, was utilized. Using this database, the 2D structures of each natural compound under its respective hub genes can be identified. The secondary structures of each natural compound were downloaded in the SDF (Structure Data File) format from the PubChem database which would allow the analysis of the molecular structure of each compound in detail and to determine its properties.

3.6 Conversion of 2D Structures to 3D Structures (Natural Compounds)

The 2D structures of the natural compounds were converted from a 2D SDF file format to 3D PDB, Mol or Mol2 formats. The OpenBabel cheminformatics online conversion tool (<http://www.cheminfo.org/Chemistry/Cheminformatics/FormatConverter/index.html>) was utilized for this process. The conversion process used the SDF file and some utilized the

canonical SMILES for the 2D structures of the natural compounds to produce the 3D structures in either one of the applicable 3D structure formats; PDB, Mol and Mol2. The settings used in the conversion tools were set to convert the 2D structure into 3D structure and the rest were set to default.

3.7 Molecular Docking of the Hub Genes and Natural Compounds

Molecular docking is an in-silico structure-based method well established in the field of drug discovery (Pinzi & Rastelli, 2019). The molecular docking process utilized the iGEMDOCK docking tool to identify the binding affinity of each of the hub genes with their respective natural compounds. This tool is a Generic Evolutionary Method for molecular DOCKing. Thus, GEMDOCK. It is a software program that is commonly used for computing and analyzing the ligand conformation and orientation relative to the active site of a particular target protein. The tool was downloaded from the official iGEMDOCK webpage (<http://gemdock.life.nctu.edu.tw/dock/igemdock.php>). The hub genes were uploaded into the tool as the binding site and all the natural compounds for a specific gene were uploaded at the same time under compounds. Multiple compounds can be docking against a single binding site as the tool will complete each docking individually and move on to the next. This makes it more efficient to complete the docking process for hub genes that have multiple natural compounds. The results produced were saved in a text file format where the binding energy, VDW, Hbond and Elec are recorded. The natural compounds were then analyzed to identify the best natural compound to be used as a therapeutic natural remedy/drug for symptoms of long COVID based on the binding affinity of the complexes.

CHAPTER IV

RESULTS

4.1 Identification of Natural Compounds

The natural compounds for only fourteen among the seventeen symptoms were identified and tabulated in Supplementary Tables 1.1 to 1.14. The FDA approved drugs were used to identify the natural compounds/compounds for each hub gene biomarker. The canonical SMILES of the drugs were searched and retrieved from the PubChem database where it was used to search similar structured natural compounds in the NPASS database. For the symptoms, fatigue, shortness of breath, loss of smell, headache, brain fog, chest pain, insomnia, heart palpitations, dizziness, joint pain, anxiety, depression, tinnitus and loss of appetite (anorexia), at least one natural compound was identified and present. However, for muscle pain, diarrhea and skin rash symptoms resulted in no natural compounds. This is mainly due to either the lack of canonical SMILES of the FDA approved drugs or there were no identical or similar structured natural compounds for the particular drug.

For each symptom, different hub genes had provided a series of natural products/compounds. Fatigue presented with four hub genes; NF1, SMAD4, RET and ERBB4 genes. Shortness of breath symptoms resulted in three genes; CHRNE, CHRND and CHRNA1 genes. Loss of smell with two genes, GNRH1 and TAC3. Headaches presented with five genes where some of the genes were of those from fatigue; ESR1, NF1, SMAD4, RET and TERT. Brain fog includes KCNT1, GABRA1 and CACNA1B genes. Chest pain resulted in NF1, SMAD4 and RET genes, which are frequently seen amongst different symptoms. Insomnia was found with four genes; NR1H4, ABCB4, ABCB11 and SLC12A3. Heart palpitations showed two genes called SCN5A and GATA4. Dizziness shares the SCN5A with heart palpitation as well as CACNA1G, RYR2, SCN1A, NF1 and

RET genes. Joint pain resulted in CR2 and FAS genes. Depression and anxiety presented with the same gene, CDH23. Tinnitus with NF1, RET genes again and TERT and CACNA1D genes. Finally, loss of appetite (anorexia) resulted with SMAD4, MEN1 and PALB2 genes.

Among all genes, NF1, RET and SMAD4 were found to be associated with more than two symptoms. This shows that they contribute and play a major role in the biological pathway related to multiple symptoms of long COVID.

4.2 Hub Gene Data Collection

The AlphaFold 3D structure was used for the majority of the hub genes as it is a powerful computational tool that predicts the 3-dimensional structures of proteins and genes with high accuracy. It utilizes the deep neural network algorithm to predict structures that are yet to be determined as well (Jumper et al., 2021). However, for the genes NF1, RYR2 and MEN1, the AlphaFold structures were not available in the UniProt database, so the SWISS-MODEL Repository (SMR) structure was downloaded instead. The SMR contains non-experimental structures and is based on the SWISS-MODEL homology modeling pipeline, which utilizes a protein structure as a template to predict the structure of a target molecule/protein (Bienert et al., 2016). The crystal structures were selected and downloaded to obtain the SMR 3D structure of the 3 respective genes.

4.3 Conversion of 2D to 3D Structure of Natural Compounds

The 2D structures of the natural compounds obtained from NPASS and PubChem databases were used as a template to obtain the 3D structure of the compounds. The 3D structure is crucial for the molecular docking process as it provides a detailed representation of the shape and chemical properties of the target molecule and ligands in the binding process. The OpenBabel cheminformatics online conversion tool was able to convert the majority of the natural compounds from the 2D structure (SDF) to 3D structures in either PDB, MOL or MOL2 formats. However, some compounds resulted in error where the SDF structure of the compound was not able to be converted into the 3D structure. The canonical SMILES were then used to overcome this problem. Yet, some compounds

persisted to cause an issue. These compounds were able to be converted into the 2D PDB format. But they cannot undergo molecular docking. Therefore, the natural compounds that were not able to be converted into any of the 3 3D structures, were excluded from the following stages. The hub genes that contained natural compounds that were rejected are presented in Table 2.1.

4.4 Molecular Docking of Hub Genes and Natural Compounds

The molecular docking process provided the binding energy (Energy), Van Der Waals interaction (VDW), hydrogen bonds (HBond) and electron interactions (Elec). The energy shows the total energy of the interaction between the hub gene and natural compound. It is the total sum of energy that is the result of the calculated sum of the VDW interaction, HBond interaction and Elec interactions. The VDW interaction is produced due to the attractive and repulsive forces between the electrons of the molecules binding and the short the distance between, the higher and stronger the interactions. The HBond is an interaction that is crucial for the stabilization of the complex where the hydrogen atoms are bonded with an electron negative atom. The Elec interaction, finally, is similar to the VDW interaction which involves the attraction and repulsion of the charged particle in the two molecules. Together the three interactions, VDW, HBond and Elec interactions, result in the binding energy of the complexes.

The docking energy, or binding energy for each hub gene and natural compound were tabulated based on the symptoms that the genes are associated with. The data was categorized based on complexes with the highest negative binding energy to the lowest energy where the complete data for all symptoms are seen in Supplementary Tables 2.1 to 2.13.

For the symptom Fatigue, a total of 13 complexes were docked with the 4 hub genes. Among all the complexes, the RET-NPC56271, ERBB4-NPC56271 and NF1-NPC117032 resulted with the highest binding energy with -94.65, -94.29 and -83.94 respectively (Table 4.1). All the SMAD4 complexes all resulted with binding energy lower than -80 where the highest value was at -73.85 and the lower SMAD4 complex at -54.80. The shortness of breath symptom resulted in the highest number of complexes docked, 36 complexes of

hub gene-natural compound. 9 complexes were excluded from the results as they failed to dock with their respective genes, CHRNE, CHRNA1 and CHRNA2. However, among the docking results of the remaining complexes, only 5 complexes had a binding energy of -100 and more (Table 4.2); CHRNE-NPC108434 (-106.33), CHRNA1-NPC10908 (-104.70), CHRNA2-NPC11296 (-103.00), CHRNA1-NPC115284 (-102.98) and CHRNA2-NPC10871 (-101.86). Although they were complexes with high binding energy at -90 and -80, the complexes with -100 binding energy were selected as they have a higher affinity to bind and potential to act as a therapeutic drug.

Table 4.1: The top three molecular docking results for the symptom, Fatigue

| Hub Gene & Natural compound | Energy | VDW | HBond | Elec |
|-----------------------------|--------|--------|-------|-------|
| RET-NPC56271 | -94.65 | -85.16 | -9.49 | 0.00 |
| ERBB4-NPC56271 | -94.29 | -85.58 | -8.71 | 0.00 |
| NF1-NPC117032 | -83.94 | -80.65 | -2.74 | -0.55 |

Table 4.2: The molecular docking result for Shortness of Breath with more than -100 binding energy

| Hub Gene & Natural compound | Energy | VDW | HBond | Elec |
|-----------------------------|---------|--------|--------|------|
| CHRNE-NPC108434 | -106.33 | -93.35 | -12.99 | 0.00 |
| CHRNA1-NPC10908 | -104.70 | -92.98 | -11.72 | 0.00 |
| CHRNA2-NPC11296 | -103.00 | -83.34 | -19.66 | 0.00 |
| CHRNA1-NPC115284 | -102.98 | -94.69 | -8.29 | 0.00 |
| CHRNA2-NPC10871 | -101.86 | -78.73 | -23.43 | 0.29 |

The loss of smell symptom involves two hub genes, GNRH1 and TAC3, but the docking results showed most of the GNRH1 gene complexes to have a higher binding energy

compared to the TAC3 complexes. 3 complexes from the GNRH1 gene had failed to complete the docking protocol due to incorrect docking. The process was repeated for the 3 complexes separately but was in vain as it resulted in error again and was excluded from the results in Table 4.3. The top two complexes with more than -100 binding energy were GNRH1-NPC69843 and GNRH1-NPC322594 with -105.95 and -100.52 energy. This suggests that the GNRH1 complexes have higher potential as compared to the TAC3 complexes. A total of 31 complexes resulted from the 5 hub genes for headaches. The NF1, RET and SMAD4 genes were present in this symptom together with TERT and ESR1. But among the 3 recurring genes in different symptoms, only the RET gene complex had a high energy range that is considered for the symptom (Table 4.4). Energy value of -90 and higher were taken into consideration, therefore, resulting in 6 complexes, TERT-NPC230098, ESR1-NPC136948, RET-NPC56271, TERT-NPC474324, ESR1-NPC123319 and TERT-NPC319549, in descending negative energy order.

Table 4.3: The molecular docking result for Loss of Smell with more than -100 binding energy

| Hub Gene & Natural compound | Energy | VDW | HBond | Elec |
|-----------------------------|---------|--------|--------|-------|
| GNRH1-NPC69843 | -105.95 | -91.90 | -12.60 | -1.46 |
| GNRH1-NPC322594 | -100.52 | -82.39 | -18.12 | 0.00 |

Table 4.4: The molecular docking result for Headache with more than -90 binding energy

| Hub Gene & Natural compound | Energy | VDW | HBond | Elec |
|-----------------------------|--------|--------|--------|------|
| TERT-NPC230098 | -97.62 | -85.53 | -12.09 | 0.00 |
| ESR1-NPC136948 | -96.97 | -78.32 | -18.65 | 0.00 |
| RET-NPC56271 | -94.65 | -85.16 | -9.49 | 0.00 |
| TERT-NPC474324 | -92.79 | -82.29 | -10.50 | 0.00 |

Continued Table 4.4

| | | | | |
|----------------|--------|--------|--------|------|
| ESR1-NPC123319 | -92.16 | -77.78 | -14.38 | 0.00 |
| TERT-NPC319549 | -91.83 | -71.07 | -20.76 | 0.00 |

The brain fog symptom involves the KCNT1, GABRA1 and CACNA1B genes and among these 3 genes (Table 4.5), the CACNA1B gene was identified as the gene to produce the highest energy complexes. All the complexes by the CACNA1B gene produced binding energy of more than -100, CACNA1B-NPC198254 (-119.29), CACNA1B-NPC473404 (-115.68), CACNA1B-NPC153554 (-109.71), CACNA1B-NPC240130 (-104.61) and CACNA1B-NPC274198 (-100.59). This implies that the CACNA1B gene has more influence on the biological pathway associated with brain fog. All except for one natural compound, NPC472111 from the KCNT1 gene failed to produce docking energy due to the incompatible file format. The 2D structure of the natural compound is unable to undergo molecular docking in iGEMDOCK and since the 3D structure of the natural compound is not available, it was disregarded. Chest pain, in Table 4.6, is one of the symptoms that involve the repetitive genes, NF1, RET and SMAD4. The results were similar to fatigue where RET complex (-94.65) and NF1 complex (-83.94) resulted with the top complexes whereas SMAD4 complexes had energies below -75.

Table 4.5: The molecular docking result for Brain Fog with more than -100 binding energy

| Hub Gene & Natural compound | Energy | VDW | HBond | Elec |
|-----------------------------|---------|--------|--------|-------|
| CACNA1B-NPC198254 | -119.29 | -90.52 | -28.77 | 0.00 |
| CACNA1B-NPC473404 | -115.68 | -86.02 | -33.71 | 4.05 |
| CACNA1B-NPC153554 | -109.71 | -85.83 | -23.88 | 0.00 |
| CACNA1B-NPC240130 | -104.61 | -70.44 | -31.19 | -2.99 |
| CACNA1B-NPC274198 | -100.59 | -76.19 | -24.98 | 0.58 |

Table 4.6: The top two molecular docking results for Chest Pain and the top result of the SMAD4 gene complexes

| Hub Gene & Natural compound | Energy | VDW | HBond | Elec |
|-----------------------------|--------|--------|--------|-------|
| RET-NPC56271 | -94.65 | -85.16 | -9.49 | 0.00 |
| NF1-NPC117032 | -83.94 | -80.65 | -2.74 | -0.55 |
| SMAD4-NPC189301 | -73.85 | -53.31 | -21.34 | 0.80 |

As for Insomnia, unfortunately, not all the natural compounds were able to undergo molecular docking with their respective genes. Similar to the natural compound in Brain Fog, there were over 21 natural compounds that failed the docking due to incompatible file format. Regardless, with the remaining 11 natural compounds, a suitable candidate with high binding affinity was identified (Table 4.7), ABCB11-NPC100366 with -107.98 energy. Ironically, this single natural compound that had not only undergone docking with the ABCB11 gene, but also the NR1H4 gene that produced the second-best binding energy, NR1H4-NPC100366. Heart palpitations comes under the category of symptoms with the greatest number of complexes produced (29 complexes) (Table 4.8). There were two natural compounds, one from each gene that did not produce a docking result due to the incompatible file formats. Amongst the docked results, two complexes from the GATA4 gene resulted in over -100 binding energy leading them to be potential targets against heart palpitations, GATA4-NPC101636 (-107.59) and GATA4-NPC100818 (-103.25). Despite having the most number of complexes produced, the SCN5A gene appears to have lower binding energy compared to the GATA4 gene complex.

Table 4.7: The top two molecular docking results for Insomnia with the same natural compound

| Hub Gene & Natural compound | Energy | VDW | HBond | Elec |
|-----------------------------|---------|--------|--------|------|
| ABCB11-NPC100366 | -107.98 | -85.27 | -22.71 | 0 |
| NR1H4-NPC100366 | -97.79 | -87.37 | -10.42 | 0 |

Table 4.8: The molecular docking result for Heart Palpitations with more than -100 binding energy

| Hub Gene & Natural compound | Energy | VDW | HBond | Elec |
|-----------------------------|---------|--------|--------|------|
| GATA4-NPC101636 | -107.59 | -90.62 | -16.97 | 0.00 |
| GATA4-NPC100818 | -103.25 | -67.84 | -35.40 | 0.00 |

Dizziness is accompanied by 6 hub genes, SCN5A, CACNA1G, RYR2, SCN1B and includes the RET and NF1 genes (Table 4.9). 31 complexes were produced with the RET complex being the highest with -94.65 energy. Aside from a RYR2 gene complex (RYR2-NPC122235), several SCN5A complexes (SCN5A-NPC162417, SCN5A-NPC64436 and SCN5A-NPC470971) and the NF1 complex (NF1-NPC117032), the remainder of complexes resulted in binding energy of lower than -80. Joint pain (Table 4.10) on the other hand, with the FAS gene, resulted in almost all 10 FAS complexes to be suitable and potential therapeutic drug. However, the CR2 gene which is also associated with joint pain, only one complex (NPC139397) was obtained as the remaining nine natural compounds were only available in 2D formats. All 10 FAS complexes were between -115 and -91 range of binding energy, the highest complexes being FAS-NPC115624 and FAS-NPC116759 with the same binding energy (-115.52) and the lowest complex being FAS-NPC163527 with -91.19 energy. The CR2 gene complex came below all FAS complexes with the lowest binding energy for joint pain, -70.48. The affinity between the 10 natural compounds and the FAS gene is high, showing promise as a suitable candidate when compared to the singular CR2 gene complex.

Table 4.9: The molecular docking result for Dizziness with more than -80 binding energy

| Hub Gene & Natural compound | Energy | VDW | HBond | Elec |
|-----------------------------|--------|--------|-------|-------|
| RET-NPC56271 | -94.65 | -85.16 | -9.49 | 0.00 |
| RYR2-NPC122235 | -85.94 | -78.79 | -7.15 | 0.00 |
| NF1-NPC117032 | -83.94 | -80.65 | -2.74 | -0.55 |

Continued Table 4.9

| | | | | |
|-----------------|--------|--------|--------|------|
| SCN5A-NPC162417 | -83.58 | -73.08 | -10.50 | 0.00 |
| SCN5A-NPC64436 | -82.21 | -74.38 | -7.83 | 0.00 |
| SCN5A-NPC470971 | -81.67 | -71.48 | -10.19 | 0.00 |

Table 4.10: The molecular docking result for Joint Pain with more than -100 binding energy, the lowest FAS gene complex and the CR2 gene complex

| Hub Gene & Natural compound | Energy | VDW | HBond | Elec |
|-----------------------------|---------|--------|--------|------|
| FAS-NPC115624 | -115.52 | -84.27 | -31.25 | 0.00 |
| FAS-NPC116759 | -115.52 | -77.79 | -37.72 | 0.00 |
| FAS-NPC163527 | -91.19 | -70.10 | -21.08 | 0.00 |
| CR2-NPC139397 | -70.48 | -63.87 | -6.61 | 0.00 |

Anxiety and depression associated with the same hub gene biomarker, CDH23 resulted in a single hub gene-natural compound complex (Table 4.11). As compared to the previous symptoms results being from -80 to -100 in energy value, the CDH23 complex has an average/low value of -53.12. As there are no other potential complexes for the symptom, the CDH23-NPC67043 is considered by default as a candidate and the lack of a comparison makes it the to have the best binding affinity towards the hub gene. Tinnitus produced 15 complexes where only one complex has a binding energy of -109.75 (Table 4.12), from the CACNA1D (CACNA1D-NPC36836). Tinnitus is also associated with the NF1 and RET genes where the NF1 gene complex is found in 9th place with -83.94 and the RET gene complex in 3rd place with -94.65 energy respectively. In second place, the TERT-NPC230098 complex with -97.62 energy also shows considerable potential as a natural remedy candidate for tinnitus. The final symptom, loss of appetite (anorexia), presented with one complex by the MEN1 gene complex (MEN1-NPC474814) with -114.41 binding energy (Table 4.13). Among the 9 MEN1 natural compounds, only 8

proceeded to molecular docking as an error was present in one of the natural compounds (NPC475983). Therefore, it was ruled out from the docking phase and the PALB2 gene's natural compound which was only available in 2D format failed the docking process resulting in its exclusion from the final result. Nonetheless, the majority of the MEN1 gene complexes resulted in high binding energy as compared to the SMAD4 gene which produced complexes with lower binding energy.

Table 4.11: The molecular docking result for Depression and Anxiety

| Hub Gene & Natural compound | Energy | VDW | HBond | Elec |
|-----------------------------|--------|--------|--------|------|
| CDH23-NPC67043 | -53.12 | -42.68 | -10.44 | 0.00 |

Table 4.12: The top three molecular docking results for Tinnitus and the recurring gene, NF1 gene complex

| Hub Gene & Natural compound | Energy | VDW | HBond | Elec |
|-----------------------------|---------|--------|--------|-------|
| CACNA1D-NPC36836 | -109.75 | -94.82 | -16.40 | 1.47 |
| TERT-NPC230098 | -97.62 | -85.53 | -12.09 | 0.00 |
| RET-NPC56271 | -94.65 | -85.16 | -9.49 | 0.00 |
| NF1-NPC117032 | -83.94 | -80.65 | -2.74 | -0.55 |

Table 4.13: The molecular docking result for Anorexia (Loss of Appetite) with more than -100 binding energy

| Hub Gene & Natural compound | Energy | VDW | HBond | Elec |
|-----------------------------|---------|--------|--------|------|
| MEN1-NPC474814 | -114.41 | -96.24 | -18.17 | 0.00 |

CHAPTER V

DISCUSSION

5.1 Fatigue

The decreased ability to perform physical and mental labor, the overwhelming experiences of exhaustion and weakness is commonly referred to and characterized as fatigue. It is a complex phenomenon that involves multiple factors, including central regulation, psychological factors and physiological factors (Heine et al., 2023). Fatigue is identified as one of the most prevalent symptoms of long COVID, affecting over 64% of the cases (Joli et al., 2022). It was found to be associated with more than one organ system such as the nervous and cardiovascular system where it causes inflammation, psychological and psychiatric disorders, cognitive dysfunction and many more (Joli et al., 2022, Castanares-Zapatero et al., 2022). Fatigue is a symptom that is not attributed to a single source, making identifying the pathogenic mechanism of fatigue in long COVID patients a challenge and it remains unknown due to the lack of significant research. Regardless, it can be stated that, Post-COVID-19 fatigue can be defined as the decrease in physical and mental abilities that resulted due to the changes presented in central regulation, psychological and physiological factors caused by the COVID-19 disease (Rudroff et al., 2020).

In a study investigating the long COVID symptoms, several genes were identified as potential biomarker addressing fatigue (Das & Kumar, 2022). NF1 gene (Neurofibromin 1 gene), SMAD4 gene, RET gene and the ERBB4 gene are the four genes that were identified by our study to be associated with the relevant FDA-approved/repurposed drugs enabling the identification of potential natural remedies for fatigue. The NF1 gene (Neurofibromin 1 gene) is a tumor suppressor gene that produces the neurofibromin

protein (NF1 Neurofibromin 1 [Homo Sapiens (Human)] - Gene - NCBI, n.d.). The NF1 gene is responsible for playing a part in the formation and operation of the nervous system. The neurofibromin protein has a significant role in the growth and differentiation of neurons, and mutations in the NF1 gene can cause neurological symptoms including brain complications (Bergoug et al., 2020). The SMAD4 gene produces a protein called SMAD4, which plays a vital function in the TGF- β signaling pathway. This pathway is responsible for regulating cell growth and development. The signaling pathway is initiated when a TGF- β protein binds to a receptor protein on the cell surface. This binding causes a group of related SMAD proteins to become activated and bind to the SMAD4 protein to form a protein complex (McCarthy & Chetty, 2018). The RET gene belongs to the receptor tyrosine kinase (RTK) family, which is composed of a collection of membrane-bound receptors that participate in signaling networks that control cell growth, differentiation, migration, and survival. In contrast to other RTKs, the RET gene is unique because it has a distinct method of RET aberrant activation where it binds with GDNF (glial cell-line derived neurotrophic factor) to promote receptor dimerization and activation of downstream signaling pathways (Zhao et al., 2023, RET Ret Proto-oncogene [Homo Sapiens (Human)] - Gene - NCBI, n.d.). The RET gene encodes a protein that is necessary for the development of the neurological and renal systems as well as for cellular signaling. The ERBB family of tyrosine kinase receptors consists of four individuals: HER-1/ERBB1/EGFR, HER-2/ERBB2, HER-3/ERBB3, and HER-4/ERBB4. The expression of only the ERBB4 gene, has been shown to be downregulated in various forms of aggressive cancers, is capable of suppressing growth and promoting differentiation (Hu et al., 2021). The ErbB4 protein is activated by the binding of particular ligands, such as neuregulins, which causes the activation of downstream signaling pathways that control many cellular functions (Bouyain et al., 2005).

Each of the hub genes were associated with several natural components. Based on the natural components or products identified for each gene, molecular docking was conducted to identify the binding affinity between the two complexes. This is to determine the best potential natural component to be used as a therapeutic drug against fatigue. Supplementary Table 2.1 presents the results of the molecular docking conducted, where the RET gene complex and ERBB4 gene complexes produced the highest results followed

by the NF1 gene. The docking energy of the SMAD4 is found to be relatively high, but in contrast to the other genes, the SMAD4 complexes are weaker.

The natural compound Gefitinib (NPC56271) was identified based on the FDA-approved drug (Vandetanib) which was utilized and targeted against the RET gene. Similar to the RET gene, the ERBB4 gene has the same natural component, Gefitinib. However, unlike the RET gene, the natural component was not identified based on the FDA-approved drug (Vandetanib) but via another FDA-approved drug that was meant to address the ERBB4 gene, Dacomitinib. Gefitinib is a tyrosine kinase inhibitor, a drug used to treat non-small cell lung carcinoma (NSCLC) (Gefitinib: Uses, Interactions, Mechanism of Action | DrugBank Online, n.d.). It functions to inhibit the epidermal growth factor receptor (EGFR) tyrosine kinase to block the signal transduction pathways in cancer growth (Lee et al., 2016). The binding energy between the receptor (ERBB4) and ligand (Gefitinib) resulted as -94.29. This implies that Gefitinib has a strong affinity towards the ERBB4 gene and has high probability to bind together. It also indicated that Gefitinib could potentially cause therapeutic effects to the symptoms caused by the ERBB4 gene. As for the RET gene, the molecular docking between the gene and natural component resulted in -94.65. This suggests that the RET gene also has a strong affinity towards the Gefitinib compound. Based on previous studies, it was identified that the Gefitinib natural compound was used as a licenced drug by the FDA for diseases such as non-small cell lung cancer and lung cancer (Shang et al., 2020, Costanzo et al., 2011). Both these diseases were found to influence the RET gene mutation. Though, the mutation of the RET gene (tyrosine kinase receptor) is comparatively lesser than the epidermal growth factor receptor (tyrosine kinase inhibitor) which plays a more significant role (Shang et al., 2020, Costanzo et al., 2011) it shows that the treatment of the Gefitinib natural product singularly for the RET gene is not yet well established. Nevertheless, the docking results from our study has provided significant evidence that Gefitinib can in fact be used as a drug for the RET gene in hopes to reduce the significance of the neurologic long COVID symptom, fatigue.

Dehydroevidiamine (NPC117032), a natural compound was identified in our study as a potential therapeutic target against the dysregulation of NF1 gene. The molecular docking between the hub gene and natural component using the iGEMDOCK tool resulted with a negative value of 83.94. It suggests that the binding energy is considerably high. The

properties of Dehydroevdiamine are still unclear as there is a lack of scientific studies on the compound. It is predicted that Dehydroevdiamine is to have anti-neoplastic, antioxidant, and anti-inflammatory properties as a prior research study on the potential natural components against neurofibromatosis syndromes, had mentioned that the selected natural components presented with the following properties were considered as primary or adjuvant therapy (Amaravathi et al., 2021). Regardless, as the binding affinity between the NF1 gene and Dehydroevdiamine is found to be strong, it can be used as a potential natural remedy to silence the symptoms that are caused.

5.2 Shortness of Breath

Shortness of breath or also known as dyspnoea, is a common distress symptom that causes breathing discomfort that varies in intensity depending on the individual and the disease. This symptom is usually associated together with diseases such as lung disease, neurodegenerative diseases and chronic heart failure (Hentsch et al., 2021). The SARS-CoV-2 virus is no exception, as this virus targets and attacks the respiratory system. The virus targets the lungs and attacks the organ via three mechanisms. They include the acute respiratory distress syndrome (ARDS), diffuse thrombotic alveolar microvascular occlusion and inflammatory mediator-associated airway inflammation (Wang et al., 2020). The consequences of these damages despite recovery cause symptoms that are associated with the respiratory system such as shortness of breath. This is because there is still the presence of inflammation, scarring of lung tissue and damaged blood vessels in the lungs which is predicted to be caused by ARDS and may persist from six months to a year after infection (Vijayakumar et al., 2022). The exact mechanism of this symptom is yet to be determined as a meta-analysis study identified that the range of potential causes for shortness of breath is too broad and involves multiple factors (Zheng et al., 2022).

To combat this symptom, therapeutic drugs are identified by analyzing the hub genes that are associated with dyspnea. Three genes from the same family, CHRNE, CHRNA1 and CHRNA2, were identified as the hub gene biomarker for shortness of breath symptom. The CHRNE gene generates the acetylcholine receptor's epsilon subunit, which is essential for signal transmission at the neuromuscular junction between muscle and nerve cells. The beta-1 subunit of the acetylcholine receptor, an essential part of the neuromuscular

junction, is encoded by the CHRNA1 gene. This subunit is essential for the development and operation of the receptor because it binds acetylcholine and starts the signaling pathways that results in muscle contraction (CHRNA1 Cholinergic Receptor Nicotinic Alpha 1 Subunit [Homo Sapiens (Human)] - Gene - NCBI, n.d.). The CHRND gene encodes a protein called nicotinic acetylcholine receptor subunit delta (CHRND Cholinergic Receptor Nicotinic Delta Subunit [Homo Sapiens (Human)] - Gene - NCBI, n.d.). This protein is a receptor subunit that plays a role in the signaling process between nerve cells. The CHRND gene is mostly expressed in the neuromuscular junction, a specialized synapse between a nerve cell and a muscle cell.

All these three genes are associated together with the same family, the nicotinic acetylcholine receptor (nAChR) gene family. They all also play important roles in the muscular and nerve cells signaling pathways. Mutation in any of these genes results in illness related to neuromuscular illness, where an example of illness that all three genes are associated with would be the Congenital Myasthenic Syndrome (CMS) (Yang et al., 2018, Freed et al., 2021).

The genes were docked against their respective natural components and the results are seen in Supplementary Table 2.2. CHRNA1-NPC108434 (-106.33), CHRNA1-NPC10908 (-104.70), CHRNA1-NPC11296 (-103.00), CHRNA1-NPC115284 (-102.98) and CHRNA1-NPC10871 (-101.86) are the top five complexes that produced high results. Lindoldhamine (NPC108434), Isotetrandrine (NPC10908), Daphnandrine (NPC11296), Fanchinin (NPC115284) and Tetrandrine (NPC10871) are known as Alkaloids. Alkaloids are naturally occurring chemical compounds that have significant psychological and pharmacological effects on organisms. The Lindoldhamine compound is a bisbenzylisoquinoline alkaloid that is extracted from the plant leaves of *Laurus nobilis L* (Osmakov et al., 2019). Bisbenzylisoquinoline alkaloids, which are commonly found within the plants of the Lauraceae family, exhibit a broad range of pharmacological effects and medicinal properties (Atanasov et al., 2015). These natural compounds are anti-cancer agents that possess anti-inflammatory, antiplasmodial and antiviral properties. Isotetrandrine, Daphnandrine, Fanchinin and Tetrandrine are also a part of the bisbenzylisoquinoline alkaloid family. The Lindoldhamine compound is found to be an inhibitor for Acid-sensing ion channels (ASICs) as a study in 2019 identified Lindoldhamine

to activate the ASIC3 channel which plays a role in sensory perception (Osmakov et al., 2019). This compound was also found to have the ability to block and prevent acetylcholinesterase, inhibit trypanothione reductase and prevent platelet aggregation. Fanchinin, Tetrandrine and Isotetrandrine are natural compounds that are derived and extracted from the same plant species, *Stephania tetrandra* (Efficacy of Traditional Medicine in Cardiovascular Diseases in the People's Republic of China, 1982, T. Liu et al., 2016, Schütz et al., 2020). Despite being originated from the same plant species, they each have different functions. Fanchinin was identified as an anti-hypertensive drug capable of treating cerebral vascular accidents, cardiac arrests and hypertension (Efficacy of Traditional Medicine in Cardiovascular Diseases in the People's Republic of China, 1982). Furthermore, it is predicted that it functions to cause reflexive vasodilation and influences the sympathetic nervous system. Tetrandrine is a traditional Chinese herb which is used in both Chinese and Japanese medicine to treat various diseases including tuberculosis, hypertension, asthma, cancer and more (T. Liu et al., 2016). It is found to be a clinical agent for autoimmune disorders and inflammatory pulmonary diseases as well. Being associated with the biological activities and regulation of cancer cells makes it a potential candidate for a cancer chemotherapeutic. Isotetrandrine is a diastereomer of tetrandrine which possesses anti-bacterial, anti-viral and anti-inflammatory properties (Zhang et al., 2016). Daphnandrine is a natural compound that is a part of the Daphnandra alkaloids species and is found in organisms such as *Stephania erecta* and *Stephania pierrei* (PubChem, n.d.-b). Due to the lack of information and access to research articles, the function of Daphnandrine and the Daphnandra alkaloids species is uncertain.

All four natural compounds, Lindoldhamine, Isotetrandrine, Fanchinin and Tetrandrine have shown anti-inflammatory properties which can aid in relieving the inflammation caused by the SARS-CoV-2 virus. Moreover, they all have a high binding affinity towards the genes, CHRND, CHRNE and CHRNB1 genes which are primarily involved in the nerve and muscle cells. This suggests that the probability for the formation of ligand-receptor complexes is remarkably high. A study in 2010, revealed the neural mechanisms of dyspnea where an increase in reflex afferent information produces the shortness of breath sensation (Burki & Lee, 2010). The dysregulation of the nicotinic acetylcholine receptor (nAChR) genes can pose a significant threat as they are associated with the nerve signaling pathway and can cause neuromuscular diseases. Studies have shown that

dyspnea is found to be present in advanced stages of neuromuscular disorders and also as symptoms for underlying neuromuscular disorders (Pavletic & Hnatiuk, 2012).

Thus, concluding that the natural components, Lindoldhamine, Isotetrandrine, Fanchinin and Tetrandrine, can be considered as candidates of therapeutic drugs for the long COVID symptom, dyspnea or shortness of breath. Daphnandrine would also be included and taken into consideration but it is advised to conduct more studies to identify and gain more knowledge on the natural compound.

5.3 Loss of Smell

Loss of smell, or anosmia, is a common symptom of COVID-19 and is also one of the persistent symptoms of long COVID. This symptom is typically not harmful by itself but may affect the quality of life in a negative manner. Based on statistics, around 40% of COVID-19 patients experienced anosmia as an early indicator and over 70% reported a decrease in the quality of life due to the loss of an important sense. Furthermore, more than 10% of the patients suffered from prolonged anosmia for over a year since the onset of the disease (Park et al., 2022). Anosmia falls under the neurological symptoms of long COVID, specifically, the dysfunction of the olfactory system (Davis et al., 2023, Castanares-Zapatero et al., 2022). It was reported that the olfactory dysfunction was caused by the invasion of the virus in the olfactory epithelium and resulting in inflammation (Park et al., 2022, Castanares-Zapatero et al., 2022). Anosmia becomes persistent when the viral infection and inflammatory environment in the olfactory epithelium affects the regeneration potential and prolongs the effects and recovery rate of the tissues (Park et al., 2022).

The hub gene biomarkers associated with anosmia were identified as TAC3 and GNRH1. TAC3 gene, commonly referred to as tachykinin3, encodes for the Neurokinin B protein. The TAC3 gene belongs to the tachykinin family of neuropeptides that is known to regulate and control a number of physiological processes such as inflammation, pain and cardiovascular functions (Z. Zhang et al., 2019). The neurokinin B protein encoded by the TAC3 gene was identified to be an essential component in the regulation of the hypothalamic-pituitary-gonadal (HPG) axis making it to be associated with the

reproductive system/activity (Debeljuk & Lasaga, 1999). The gonadotropin-releasing hormone (GnRH) is a protein that is produced by the GNRH1 gene in the brain's hypothalamus. The GNRH stimulation stimulates the release of the follicle-stimulating hormone (FSH) and luteinizing hormone (LH) from the pituitary glands to regulate the reproductive functions in the body (Orlowski, 2022).

The isolated gonadotropin-releasing hormone (GnRH) deficit (IGD), of which there are two types—normosmic IGD and Kallmann Syndrome—was found to be correlated with both the TAC3 and GNRH1 genes. In the normosmic IGD, there is no loss of smell, whereas anosmia is found to manifest in the Kallmann Syndrome (Balasubramanian, 2022). GNRH1 and TAC3 are solely associated with normosmic IHH, not Kallmann Syndrome (Valdes-Socin et al., 2014). As a result, it is unclear and poorly understood how the genes, TAC3 and GNRH1, and anosmia relate to one another.

Despite the lack of evidence of the association of the TAC3 and GNRH1 genes to the long COVID symptom, they have been identified as a hub gene biomarker with their respective natural components. The docking of the natural components to their respective genes resulted in two complexes that have a binding energy more than -100. The natural compounds, Tunicyclin D (NPC69843) and Deoxyuridine triphosphate or 2'-Deoxy-Utp (NPC322594) exhibited great binding affinity towards their gene, GNRH1. Tunicyclin D is a cycloocta-peptide that is derived from the root of *Psammosilene tunicoides* (Guo et al., 2012). It was identified as an antibiotic for fungal infections as its mechanism involves targeting the fungal membrane, mainly the lipid components disrupting the membrane's integrity (Q. Zhao et al., 2014). The GNRH1-NPC69843 (Tunicyclin D) complex with a docking energy of -105.95 suggests that the natural compound has a high affinity towards the gene despite not having a direct relationship with each other. Deoxyuridine triphosphate (NPC322594), on the other hand, also known as dUTP, is a natural component found in humans. Based on the PubChem entry, it is an uracil nucleotide composed of three phosphate groups esterified to the deoxyribose sugar moiety. (PubChem, n.d.-c). It plays a crucial role in DNA synthesis and repair and is similar to the deoxythymidine triphosphate (dTTP) which has a thymine base and not an uracil base like dUTP. dUTPs are crucial and essential for regular cellular functions but high levels of dUTP can be toxic for the body and damage the DNA (Ladner, 2001). No evidence is yet

discovered on the use of deoxyuridine triphosphate as a natural remedy for a specific health condition. Furthermore, similar to the GNRH1-NPC69843 complex, the GNRH1-PC322594 complex also has a high energy (-100.52), but has no direct relationship with the gene and the symptom to the current date.

Regardless of the lack of direct association with each other, our study has shown and hypothesizes that the natural compound has an unknown property that enables it to bind together with the GNRH1 gene aiding to suppress the long COVID symptom, anosmia (shortness of breath).

5.4 Headache

Headaches are a common symptom of COVID-19 and long COVID. Long COVID headache can present itself as a worse form of preexisting headache or the development of new headaches in the form of during or after the initial infection (Tana et al., 2022). Headaches were identified as one of the earliest symptoms to appear during the acute infection where 13 to 60% of patients reported experiencing pain in the upper and frontal parts of the head (Martelletti et al., 2020). In long COVID, headache has a prevalence of 113 to 74% and persists for over twelve weeks (Membrilla et al., 2021). The mechanism behind the headaches experienced by people after recovery is not yet fully understood but is speculated to be caused by the damages caused during the acute infection and is found to be associated with different organs systems, such as the nervous and reproductive systems. The main pathophysiology mechanism of headache proposed is caused by the activation of the peripheral trigeminal nerve endings by the SARS-CoV-2 virus (Bolay et al., 2020). The inflammation of the trigeminal nerves caused by the initial infection is thought to be the cause of persistent headaches and general pain in the body (Castanares-Zapatero et al., 2022). Other proposed mechanisms suggest that the alteration of the white matter axonal, persistent activation of the immune system and prior medical history of a patient having headaches could be involved in causing long COVID headaches (Tana et al., 2022). Headache is also associated with the reproductive system where it was identified that female individuals who experience COVID-19 infection and undergo menstrual changes have a higher probability of experiencing headaches as well (Davis et al., 2023).

Potential natural remedies for long COVID headaches were identified in our study revolving around five hub genes; ESR1, RET, SMAD4, NF1 and TERT. The ESR1 gene produces the estrogen receptor alpha protein, a nuclear hormone receptor that binds to estrogen and controls the expression of genes. This gene produces a protein that is produced in many organs other than those used for reproduction and that controls the transcription of numerous estrogen-inducible genes involved in growth, metabolism, sexual development, pregnancy, and other reproductive processes (*ESR1 Estrogen Receptor 1 [Homo Sapiens (Human)] - Gene - NCBI, n.d.*). Although there was no direct association of the ESR1 gene with headaches, a study in 2017 found that the sex hormones play a crucial role in the pathogenesis of migraine, a specific form of headache, where the polymorphism of the ESR1 gene was a candidate in migraine susceptibility (An et al., 2017, Kumar et al., 2022). In humans, the TERT gene encodes the catalytic subunit of the telomerase enzyme, which is responsible for attaching telomeric DNA to the ends of chromosomes. Telomeres play a significant role in chromosomal senescence and chromosome aging by shielding them from deterioration (TERT Telomerase Reverse Transcriptase [Homo Sapiens (Human)] - Gene - NCBI, n.d.). In order to preserve telomere length and continue to proliferate, cancer cells exhibit high levels of TERT expression in both adult and embryonic stem cells as well as in many other types of cells. There have been reports of the length of the telomeres associated with migraine; individuals with migraine were found to have shorter telomere length (Ren et al., 2010). The TERT gene which plays a crucial role in maintaining the telomere length may play an indirect role in causing migraine. However, the association and mechanism of TERT gene and migraine as well as headache is limited and not well established. The NF1 gene (Neurofibromin 1 gene), SMAD4 gene, RET gene also were identified by our study that were associated with headache. The NF1 gene (Neurofibromin 1 gene) is a tumor suppressor gene, the SMAD4 gene which is involved in the TGF- β signaling pathway and is responsible for regulating cell growth and development while the RET gene which influences the signaling networks that control cell growth, differentiation, migration, and survival, as mentioned under fatigue. Headaches are a common neurological manifestation in the NF1 gene where one in every three to four NF1 patients experience it (Gao et al., 2022). While there is no direct relation between headaches and the SMAD4 gene, studies have proposed that the TGF- β signaling pathway regulated by the gene plays a role in the development of

migraines (Wain et al., 2014). Similarly, the RET gene also has no evidence linking it to headaches. The gene was however found to be associated with several other genetic disorders that potentially causes headaches (Hazimeh et al., 2014).

A total of thirty-one natural components were identified in the study based on the docking energy of the natural compounds with their respective genes. The top three results were deemed as the potential candidates as a therapeutic drug for headache; TERT-NPC230098 (unidentified gene based from PubChem and ChEMBL), ESR1-NPC136948 (Norselic Acid D) and RET-NPC56271 (Gefitinib). The NPC230098 is a compound with no proper name and an IUPAC name of [(1S,17S,18S,19S)-17-hydroxy-5,7-dioxo-12-azapentacyclo[10.6.1.0²,10.0⁴,8.0¹⁵,19]nonadeca-2,4(8),9,15-tetraen-18-yl] hexadecanoate. No information was available on the natural compound in the PubChem database and NPASS database. The properties of the compound remain unknown but it is identified as the compound that has the highest binding affinity towards the TERT gene as a therapeutic candidate for headache. Hence, the compound is accepted as valid data but is advised to establish the properties and the therapeutic nature of the compound. Norselic Acids are a series of asymmetric dimeric steroids that are found and isolated from the marine natural product, *Crella* sp (Antarctic Sponge), found in the waters of Antarctica (Sheung et al., 2009). A study on the five different Norselic acids, including norselic acid D, suggests that this natural compound presents antimicrobial activity against certain microorganisms (Sheung, n.d.). However, further research is needed to understand the properties and characteristics of the compound as it is not fully understood, especially in terms of the long COVID symptom, headache. Nonetheless, the binding energy of the natural compound and ESR1 gene could propose that Norselic acid D affects the biological pathway of the gene. As for the RET-Gefitinib complex, the association between the RET gene and Gefitinib has already been established under the fatigue symptom, but in relation to headache, a case study had revealed that the natural compound, Gefitinib, is a potential treatment option for erlotinib-induced liver injury where it relieves headache and nausea (Nakatomi et al., 2011). This goes to show that Gefitinib may contain the ability to relieve headache which can be integrated into the long COVID symptom's treatment regime. This is supported by the high docking energy obtained by the complex, -94.65, resulting in a prospective therapeutic drug for headache.

5.5 Brain Fog

Brain fog is a cognitive impairment that affects an individual by causing mental confusion, forgetfulness, concentration difficulties, intellectual functions and short-term memory loss. Due to the effects of the symptom, it is bound to negatively affect the quality of life and day-to-day aspects of life. The occurrence of this symptom within long COVID patients was found to be within the range of 31 to 69% after mild or severe initial infection (Nouraeinejad, 2022). The cause of brain fog was predicted to be caused by the SARS-CoV-2 virus directly affecting the brain causing damage. A study presented a case where a long COVID patient's brain, who suffers from brain fog, was studied and identified hypermetabolic activities in the brain's metabolism (Castanares-Zapatero et al., 2022). There are several other proposed mechanisms where it potentially causes brain fog to an individual. They include the hippocampal distribution of microglial activation as it was identified to be interrelated with the virus-induced cognitive impairment (Vasek et al., 2016), vascular damages and hypoxia due to inadequate oxygen leading to cognitive dysfunction as well as organ inflammations (Alonso-Lana et al., 2020) and ischemic injury of the cerebral white matter affecting its integrity and the maintenance of cognitive functions (Miners et al., 2020, Nouraeinejad, 2022).

Addressing this symptom, KCNT1, GABRA1 and CACNA1B were identified to be associated with the biological process of the occurrence of brain fog. The KCNT1 gene is a member of a large gene family that codes for potassium channel production. These channels are crucial for a cell's capacity to produce and send electrical impulses because they carry positively charged potassium atoms (ions) into and out of cells (Encyclopedia Scholarly Community Encyclopedia, 2020). The gene is highly expressed in the nervous system. The encoded ion channel is thought to control hyperpolarization during repeated action potential firing (Lim et al., 2016). Epilepsy, intellectual disability, and developmental delay are just a few neurological conditions that have been linked to mutations in the KCNT1 gene. The alpha-1 subunit of the Gamma-aminobutyric acid (GABA) type A receptor is encoded by the GABRA1 gene. GABA is the most abundant inhibitory neurotransmitter in the mammalian brain, where it binds on GABA-A receptors, which are ligand-gated chloride channels that are important for mediating inhibitory neurotransmission in the central nervous system (GABRA1 Gamma-aminobutyric Acid

Type a Receptor Subunit Alpha1 [Homo Sapiens (Human)] - Gene - NCBI, n.d.). Childhood absence epilepsy type 4 and epilepsy have both been linked to mutations in the GABRA1 gene. Furthermore, several studies have also demonstrated that the mutation of the GABRA1 gene significantly contributes to Dravet syndrome and the genetic etiology of both benign and severe epileptic syndromes (Carvill et al., 2014, Johannesen et al., 2016). Whereas the CACNA1B gene encodes the CaV α 1 pore-forming subunit of an N-type voltage-dependent calcium channel (all CaV2.2 channels). CACNA1B exons that are alternatively spliced affect the channel's functionality and sensitivity to G-protein coupled receptors (GPCRs) (Bunda et al., 2019). The hippocampus, basal ganglia, cerebral cortex and white matter, as well as the cerebellum and basal ganglia, are just a few of the areas in the human brain where CACNA1B is expressed (Gorman et al., 2019). Hence, the release of neurotransmitters from brain neurons depends on this gene. Several neurological conditions, such as familial hemiplegic migraine, episodic ataxia type 2, and autism spectrum disorder have all been linked to mutations in the CACNA1B gene.

The three hub gene biomarkers are all associated with neurological pathways in the human body which are proposed to be associated with the brain fog symptom. The docking between the hub genes and their respective natural components have provided the study of the probable therapeutic drugs for brain fog. CACNA1B-NPC198254 and CACNA1B-NPC473404 complexes were present with high docking energy of -119.29 and -115.68 respectively. The NPC198254 is also referred to as Micropeptin B and NPC473404 as Anabaenopeptin F. Micropeptin B, resulting in the highest docking energy, is a cyclic peptide that is cultured from a form of cyanobacterium called *Microcystis aeruginosa* (Ishida et al., 1997). Similarly, the Anabaenopeptin F is a cyclic peptide that is extracted from the cyanobacterium, *Oscillatoria agardhii* (Shin et al., 1997). Both peptides were found to be cyanobacterium mined from marine and freshwater blooms. A study revealed that both peptides are effective in reducing neuroinflammation (Kirk et al., 2021). Neuroinflammation is one of the proposed mechanisms to cause brain fog as it is able to impact the cognitive abilities of an individual (Kavanagh, 2022). This goes to show a possible relationship between the natural component as a drug reducing neuroinflammation and the long COVID symptom, brain fog. Both Micropeptin B and Anabaenopeptin F can be argued to be a potential drug candidate for the CACNA1B gene

despite showing no signs of direct relation with each other, except for the common association between the mechanism of the symptom.

5.6 Chest Pain

Underlying cardiac sequelae after recovery from the acute infection of COVID-19 is found to be the cause of persistent cardiac symptoms such as chest pain. A study identified that persistent cardiac abnormalities were experienced by 58% of patients for a duration of over twelve months (Roca-Fernandez et al., 2022). The chest pain experienced by patients can be due to and related to either the cardiovascular system or the respiratory system. It is hypothesized that chest pain is related to the cardiac system as underlying pulmonary embolism, caused by the virus attacking the ACE2 receptors in the myocardial cells, where it is found to cause chest irritations (Fiala et al., 2022). Inflammation of the myocardium or myopericarditis is also suspected to present chest pain as a symptom (Raman et al., 2022). According to research studies on 2 long COVID patients, chest pains were found to be associated with residual pneumonia from the acute infection. Furthermore, the inflammation caused due to the immune response of the body affects a range of organ systems, including the lungs where the damage results in chest pains and accompanied by cough, difficulty breathing and more (Daines et al., 2022). The diagnosis of chest pain in long COVID patients can be identified via a chest x-ray or a CT scan which can aid to identify the predicted cause of the pain.

The genes that are found to be associated with chest pain are similar to those found in fatigue; NF1, SMAD4 and RET genes. Some of the common symptoms of Neurofibromin 1 in patients are headaches, fatigue, pain, and seizures and many more based on the National Institute of Neurological Disorders and Stroke (NIH). The symptoms caused by the NF1 gene are similar to those that arise from long COVID, including chest pains. Chest pain was found to be indirectly associated with the NF1 gene as it causes cardiac abnormalities and manifestations that would cause chest pains to the affected individual (Nguyen et al., 2013). Unlike the NF1 gene, the SMAD4 gene is directly associated with cardiac abnormalities like chest pains. The transforming growth factor (TGF)- β pathway regulated by the SMAD4 gene plays an important role in the myocardial fibrosis and the modulation of the TGF- β pathway is bound to cause cardiac symptoms such as chest

pains (Umbarkar et al., 2019). As for the RET gene, since it is mainly associated with the nervous system, it is found to be linked to neurological long COVID symptoms such as fatigue, brain fog and headache (Stefanou et al., 2022). The connection between the gene and chest pain remains unclear and unknown due to lack of evidence. Regardless, as a hub gene biomarker, the RET gene is hypothesized to have a link with the symptom. Thus, docking was conducted for all three genes with their respective natural components.

The NF1 and RET genes posed to have higher binding energy compared to the SMAD4 gene similar to fatigue and headache. The natural compounds Gefitinib and Dehydroevdiamine were considered as potential candidates due to the high binding affinity compared to the N2-Acetyl-L-Lysine (NPC189301) compound with SMAD4 gene. The Supplementary Table 2.6 shows the binding energy for each complex where all the SMAD4 gene complexes are found to be under both NF1 and RET gene complexes. A study identified the natural component, Gefitinib, as a drug providing relief to symptoms such as fatigue, shortness of breath and chest pains despite primarily being used for non-small cell lung cancer (Natale, 2004). The anti-inflammatory effects of Gefitinib are assumed to play a role in aiding to improve conditions such as myocardial fibrosis and preventing cardiac symptoms. The NF1 gene complex with Dehydroevdiamine is speculated to have anti-neoplastic, antioxidant, and anti-inflammatory properties (Amaravathi et al., 2021). This raises questions on the mechanism of the natural compound in regards to chest pain but due to lack of research it remains unsolved. Even so, due to the elevated binding affinity of the natural compound to the gene, it gives a probable cause to study the drug further as a potential therapeutic drug.

5.7 Insomnia

The pandemic faced by the world had a significant negative impact worldwide. Leading to stress of all types including social isolation and loneliness, unemployment, grief, and fear. These factors have been found to negatively impact a person's sleep cycle leading to insomnia (Vargas et al., 2022). Chronic insomnia is caused mainly by environmental factors, such as stress, anxiety and social isolation, and also by persistent inflammatory response of the body. However, these are not the only cause of insomnia in COVID patients and it can also be induced due to the virus itself. A cohort study identified

persistent sleep disorders among long COVID patients that would last up to 18 months post infection (Moura et al., 2022). A possible mechanism for this symptom could be related to the dysfunction of brainstem nuclei. The brainstem nuclei have been found to contain numerous distinct nuclei that are responsible for the sleep and waking regulation of the body (Yong, 2021, Moura et al., 2022). The study further explains that the neurotransmitters found in the midbrain of the brainstem (ventral tegmental area and substantia nigra) are associated with neurological symptoms, including insomnia (Yong, 2021). The dysregulation of the neurotransmitter system due to the virus can not only cause insomnia but a broad range of neurological disorders.

Insomnia is a symptom that is not easy to live with as it affects the daily aspects of life where it may disrupt work life, social interactions and reduce the overall quality of life. Studying the following genes that were identified as a hub gene biomarker may provide a potential therapeutic drug to combat insomnia; ABCB4, ABCB11, NR1H4 and SLC12A3. ABCB4 encodes ATP-binding cassette (ABC) subfamily B member 4 (ABCB4), also known as multidrug resistance protein 3 (MDR3), which is involved in biliary phospholipid production and shields the hepatobiliary system against harmful detergent and lithogenic qualities of bile. The MDR3 protein is involved in removing chemicals, such as phospholipids, bile acids, and cholesterol, from liver cells into bile for excretion from the body (Sticova & Jirsa, 2020). The ATP binding cassette subfamily B (ABCB11) is the gene that encodes for the bile salt export pump protein (BSEP). The primary function of this protein is to transfer bile acid from the cell cavity to the bile duct. It is also involved in assisting the breakdown of cholesterol and the prevention of bile supersaturated crystallization. The NR1H4 gene is also known as the Farnesoid X receptor (FXR), a nuclear receptor that is expressed in several organs including the liver, intestine, kidneys and adrenal gland. This gene is essential for the control of glucose homeostasis, lipid metabolism, inflammation and the synthesis and transport of bile acids (Chiang & Ferrell, 2022). Finally, the SLC12A3 gene encodes the thiazide-sensitive sodium-chloride cotransporter (NCC) protein. This protein is predominantly expressed in the kidneys where the thiazide-sensitive NCC carries the sodium and chloride ions from the environment into cells of the nephron distal convoluted tubule (C. An et al., 2016). This process of maintaining the Na⁺ and Cl⁻ ion balance in the body is essential for maintaining normal blood pressure and fluid balance.

All four genes are identified to be intertwined with the renal system via KEGG Pathway and not the nervous system which is proposed to be associated with insomnia. However, several studies have presented evidence of the implications of the renal system towards causing insomnia. The risk of insomnia is more prevalent to individuals who are suffering from end-stage renal disease due to several reasons including increase in physical stress, high levels of parathyroid hormones, dialysis short, chronic pain and more (Maung et al., 2016). Therefore, it can be hypothesized that the ABCB4, ABCB11, NR1H4 and SLC12A3 may affect the renal system in a manner which causes insomnia to long COVID patients. Although the identified hub genes have no relation towards the proposed mechanism on how long COVID affects and causes the symptom, the natural components associated with the genes may potentially alleviate the symptom caused.

Among the eleven hub gene-natural compound complexes obtained from the docking process, one single complex stood out with the highest energy value (Supplementary Table 2.7); ABCB11-NPC100366 with -107.98. The NPC100366 is commonly known as Ethyl(4R,20S,24R)-Epoxy-4,25,28-Trihydroxy-3,4-Secodammar-3-Oate. Interestingly, the same natural compound was docked against the NR1H4 gene and resulted with the second highest binding energy value (-97.79). This shows that the Ethyl(4R,20S,24R)-Epoxy-4,25,28-Trihydroxy-3,4-Secodammar-3-Oate has a high binding affinity and influence towards both NR1H4 and ABCB11 genes. The natural compound is found to be isolated from the stem bark of the *dysoxylum binecteriferum*, a plant species primarily found in mainland China and India. The properties and functions of the natural compound are inaccessible as no information is available, however, it was identified that the *dysoxylum binecteriferum* species presents medicinal properties including cytotoxic and anti-inflammatory activity (Huijiao et al., 2014). Thus, it is hypothesized that the natural compound would exhibit similar properties. Under the assumption, the natural compound, an alkaloid of *dysoxylum binecteriferum*, would be a potential chronic kidney disease inhibitor and anti-cancer properties (Varun et al., 2023, Tungmunthum et al., 2018).

In regards to insomnia, neither the hub genes nor the natural remedy components showed evidence of a link between each other. The functions and pathways of the genes and natural components differ from the proposed mechanism of insomnia caused by long

COVID. Hence, additional studies are required to understand the implications of the hub genes and the natural remedies proposed for long COVID insomnia.

5.8 Heart Palpitations

Heart palpitations is a feeling where a person experiences fluttering and pounding in the chest which is caused by an irregular and abnormal heartbeat. It is one of the common long COVID symptoms which is associated with the cardiovascular organs and neuro cardio systems and is present in 20% of patients (DePace & DePace, 2022). These lingering cardiac symptoms are known as cardiac sequelae as mentioned under chest pain. The underlying mechanism causing the persistent heart palpitations were identified to be associated with myocardial injuries that occurred during the acute infection (DePace & DePace, 2022, Gluckman et al., 2022). Studies have proven the presence of mild yet persistent inflammation of the hearts of those who recovered from COVID-19 (“Lingering Cardiac Involvement in Previously Well People After Mild COVID-19,” 2022). This inflammation typically results in myocarditis, however, despite not causing much structural damage to the organ, the inflammation may result in persistent symptoms such as irregular heartbeats. Similarly, another research proposed that the inflammation that is caused by the virus is caused by autonomic dysfunction where it forms an imbalance between the parasympathetic and sympathetic nervous systems leading to symptoms such as depression, diarrhea, palpitations, tachycardia, cognitive dysfunction, headache, dizziness, and tinnitus (S. S. Yong, 2021, DePace & DePace, 2022). The exact cause and mechanism is yet to be analyzed and understood, requiring additional research to identify its relationship with the SARS-CoV-2 virus to design drugs against the symptoms.

The SCN5A gene and GATA4 genes were found to have a main role in the biological pathway related to heart palpitations. The SCN5A gene is a member of a group of genes that is responsible for producing sodium channels. To regulate the entry of positively charged sodium atoms (sodium ions) into cells, these channels open and close at predetermined intervals (Encyclopedia, 2020c). The SCN5A gene encodes the alpha subunit of the major cardiac sodium channel Nav1.5, which is known to be responsible for the inward sodium current function (INa). Recent studies have discovered that this gene is expressed primarily in cardiomyocytes and cardiac tissue and other tissues, including

the brain, the GI tract, and cancer tissues, where it plays essential roles (Li et al., 2018). Whereas, the GATA4 gene codes for a transcription factor that is required for the development of the heart. It belongs to the GATA family of (zinc fingers) transcription factors (GATA4 GATA Binding Protein 4 [Homo Sapiens (Human)] - Gene - NCBI, n.d.). GATA4 is a transcription factor that is crucial to numerous biological functions. Heart, liver, pancreas, stomach, small intestines, gallbladder, ovary, and testicles are the organs in humans which exhibit the GATA4 gene. GATA4 is essential for controlling the expression of genes involved in cardiac differentiation, proliferation, and morphogenesis (Chen et al., 2019).

Both SCN5A and GATA4 genes are associated with the organ that is responsible for causing heart palpitations. Thus, molecular docking was conducted to identify the best therapeutic drug among the natural remedies for the symptom. The docking results of the GATA4 gene and SNC5A genes (Supplementary Table 2.8) resulted in a total of twenty-nine complexes. Only two complexes were deemed significant for having a high binding affinity towards the gene. Both natural compounds were from the GATA4 gene; GATA4-NPC101636 (Apigenin 7-O-Alpha-L-3-O-Acetyl Rhamnopyranosyl-(1->6)-Beta-D-Glucopyranoside) and GATA4-NPC100818 (asphodelin A-4'-O-beta-glucoside) with -107.59 and -103.25 energy respectively. Apigenin 7-O-Alpha-L-3-O-Acetyl Rhamnopyranosyl-(1->6)-Beta-D-Glucopyranoside is a class of flavonoids and is isolated from the methanol extract of the *Scoparia dulcis* L. (*S. dulcis*) plant species (Jiang et al., 2021). The compound was shown and experimentally confirmed to promote neurite outgrowth in PC12D cells (mouse cells) stimulated with nerve growth factors (NFG). However, no link was identified for the

Apigenin 7-O-Alpha-L-3-O-Acetyl Rhamnopyranosyl-(1->6)-Beta-D-Glucopyranoside compound in relation to the GATA4 gene and heart palpitations. The mechanism of the natural compound remains unknown but additional studies may provide insights to the properties of the compound. The asphodelin A-4'-O-beta-glucoside is a complex chemical compound that is found to be isolated from the roots of *Asphodelus microcarpus* (El-Seedi, 2007). Based on the compound's entry in the European Bioinformatics Institute, asphodelin A-4'-O-beta-glucoside is involved in biological activities such as antimicrobial activity against certain bacteria and fungi species (Team, n.d.). Furthermore, a study in 2007 identified asphodelin A-4'-O-beta-glucoside to express moderate antimicrobial

activity against several bacteria such as *Staphylococcus aureus*, *Escherichia coli* and *Pseudomonas aeruginosa* and also low antifungal activity against *Candida albicans* and *Botrytis cinerea* (El-Seedi, 2007). This particular property facilitates the natural compound to be used as traditional medicine for infections, wounds and inflammations. Hence, it can be deemed that the asphodelin A-4'-O-beta-glucoside has the potential to reduce and provide relief for the inflammation of the heart caused by SARS-CoV-2 virus.

5.9 Dizziness

Dizziness is one of the common long COVID symptoms that is experienced by some individuals. The severity of the symptom varies for each person ranging from mild to severe cases. Dizziness is the disturbed impaired spatial orientation with a distorted sense of motion. This symptom causes considerable changes in life which reduces a person's well-being and the quality of life (Rodríguez-Pérez et al., 2022). A cohort study on 1082 long COVID patients identified that neurological symptoms are common where 60% reported experiencing dizziness (Degen et al., 2022). The study was found to have a higher percentage compared to other studies where the prevalence was within the range of 27 to 38%. This was due to the sensitivity of the study being able to recognise even mild dizziness in patients. It can be determined that the pathophysiology and the mechanism of dizziness are caused due to multiple factors. Initially it was thought that dizziness is caused mainly due to a neurological manifestation of COVID-19 but can be caused by other factors such as inner ear problems, low blood sugar, low blood pressure, side effects of medications and other nonspecific common neurological symptoms (Korres et al., 2022).

The RYR2, SCN1B, SCN5A, NF1, RET and CACNA1G genes were found to be associated with the biological pathway related to the symptom. The analysis of these six genes would provide a solution for dizziness. The RYR2 gene codes for the production of the ryanodine receptor 2 protein. This protein is a member of the ryanodine receptor family, which creates channels for the movement of calcium ions, or positively charged calcium atoms, inside of cells (Encyclopedia, 2020b). The endoplasmic reticulum membrane is where RYR2 is primarily found, and it also functions to control the level of intracellular calcium. Both the heart and the brain have significant levels of RYR-2 protein expression. RYR2 mutations

have been linked to the etiology of catecholaminergic polymorphic ventricular tachycardia (CPVT), arrhythmogenic right ventricular dysplasia 2, and a potential gene causative agent for epilepsy (Ma et al., 2021). The RYR2 being a causative agent for CPVT, a ventricular tachycardia induced by stress, causes dizziness, one of the minor symptoms that arises (Vemireddy et al., 2021). The SCN1B gene codes for the beta-1 subunit of the voltage-gated sodium channel protein. This protein contributes to the development of sodium channels, which are necessary for action potential production and transmission in neurons and muscle cells. Epilepsy, arrhythmias, and sudden infant death syndrome (SIDS) are just a few of the neurological and cardiac conditions that have been linked to mutations in the SCN1B gene (Martinez-Moreno et al., 2020, Ricci et al., 2015). Dizziness is not a symptom caused by the SCN1B gene but it could have an indirect relationship where epilepsy caused by the gene is accompanied by dizziness. The CACNA1G gene encodes Cav3.1, a protein that belongs to the voltage-gated calcium channel family. This protein controls the calcium ion influx into cells, which is important for numerous biological activities. The Cav3.1 protein, produced by the gene is found in significant amounts in the brain, and is categorized as a low-threshold voltage-dependent calcium channel protein. This protein is primarily involved in the control of calcium signaling pathways as well as the regulation of membrane potential (X. Li et al., 2018). A study identified a specific mutation in the CACNA1G gene (heterozygous missense substitution in exon 38), results in episodic vestibulocerebellar ataxia (Gazulla et al., 2021). The episodic vestibulocerebellar ataxia is a rare disease associated with ion channels and often presents with episodes of dizziness. Thus, forming a hypothesized link between the gene and symptom. As previously discussed, the SCN5A gene is responsible for the inward sodium current function (INa) regulated by the alpha subunit of the major cardiac sodium channel Nav1.5. While dizziness is not a direct symptom to the SCN5A gene mutation, it is a common symptom that presents with cardiac conditions which are associated with SCN5A mutations. A patient who initially experienced dizziness and then severe cardiac symptoms was diagnosed with SCN5A overlap syndrome, a rare genetic condition (Ahn et al., 2021). This clinical presentation hence, may be potentially consistent with the relationship between the dizziness and SCN5A gene. As for the NF1 gene, a case study identified a patient with NF1 mutation experiencing dizziness (Zhou et al., 2020). But the mechanism revolving the cause of dizziness in NF1 patients was not described. Furthermore, impairment in coordination and balance is the neurological manifestation of

NF1 which potentially causes dizziness (Friedman, 2022). The RET gene on the other hand, the association between both symptom and gene is unclear and not understood. Several studies on RET mutations had dizziness as the clinical symptoms of the patients which provides a faint and potential relationship. However, further research is required to identify the specific mechanism of the genes in the biological pathways of dizziness.

The natural remedies for dizziness in regards to the 6 hub genes underwent molecular docking (Supplementary Table 2.9). The RET-NPC56271 produced the highest binding energy, -94.65, with the natural component, Gefitinib. Gefitinib was identified to be a potential therapeutic candidate for dizziness. This is supported by a study where elderly patients were treated with Gefitinib for advanced non-small cell lung cancer resulting in a reduction of a number of symptoms which included dizziness (Z. Gao et al., 2012). The RYR2-NPC122235 (Linalyl anthranilate) complex came in second with a binding affinity of -85.94. Linalyl anthranilate is a natural compound that is commonly found in plants such as thyme, lavender, marjoram and Mexican giant hyssop (S. Yang et al., 2021). This natural compound exhibits antimicrobial properties and is found to be a major component in lavender essential oils that presents with antimicrobial activity. The study also identified that Linalyl anthranilate is able to induce oxidative stress by generating reactive oxygen species causing damage in bacterial membranes (S. Yang et al., 2021). Similarly, with the context of oxidative stress, another study stated dizziness to be related to inflammation and oxidative stress (Kao et al., 2014). Both, unfortunately, this does not indicate a direct relationship between Linalyl anthranilate and dizziness in humans, as the study of Linalyl anthranilate inducing oxidative stress was specified for bacteria cells and not animal cells. Thus, the effects of Linalyl anthranilate must be extensively studied to understand the potential association between dizziness and Linalyl anthranilate.

5.10 Joint Pain

Joint pain is a part of rheumatic and musculoskeletal diseases which is known to affect the joints as well as the organs of the body. It was found that joint pain occurs approximately one in five patients and also reported that the female gender is more prevalent to experience joint pain as compared to males (Mills et al., 2022). There is increasing data on the effects of long COVID and musculoskeletal structures where pain is present in

bones, muscles, joints, ligaments and tendons (Khoja et al., 2022). Several possible causes were identified to determine the pathophysiology of the symptom. One of the hypothesized factors is the excessive and uncontrolled release of cytokines such as interleukin (IL) 17 and tumor necrosis factor, in response to COVID-19 (Cui et al., 2022). The elevated levels of the cytokines (IL-4, IL-9, and IL-13) have shown to play a role in the joints and contribute to the development of rheumatic symptoms. Another proposed hypothesis is the inflammatory responses caused by the invasion of the SARS-CoV-2 virus inducing persistent inflammation and tissue damages that lead to rheumatic/musculoskeletal symptoms (Karaarslan et al., 2021). Despite the cause, it is recommended by the National Institute for Healthcare and Care Excellence (NICE) to conduct regular physical activities to reduce the effects and the pain caused by the chronic joint pains.

By understanding the biological mechanism that contributes to joint pain in long COVID, potential hub gene biomarkers can be identified. As such, two potential biomarkers have been found; CR2 and FAS. The CR2 gene encodes the complement receptor type 2 protein, commonly known as CD21. This protein is found on the surface of B cells, follicular dendritic cells, and certain epithelial cells (Kovács et al., 2021). It has been shown in a study that CR2 can bind C3 degradation products during complement activation, indicating that CR2 may be crucial for immunity. As a gene that codes for a protein involved in the immune response, CR2 gene is speculated to be involved in the pathophysiology of joint pain. The FAS gene, commonly known as CD95, belongs to the tumor necrosis factor (TNF) receptor superfamily. It encodes the cell surface receptor protein that controls apoptosis, which is the programmed cell death function. When FAS attaches to its ligand, a signaling cascade is triggered, activating the caspase enzymes, which then cause the fragmentation of cellular components (Yamada et al., 2017). The FAS gene interacts with various cytokines involved in the immune response such as IL-8 and IL-1 β (D. S. Park et al., 2003). Unfortunately, the FAS gene was not found to be associated with the cytokines present in the joint pain. Regardless, both genes were found to be linked with joint pain as they both are a causative agent of the autoimmune disorder, systemic lupus erythematosus (SLE) where joint pain is a common musculoskeletal manifestation (Cojocar, 2011).

The presence of a link between the hub genes and the symptom allows us to identify potential therapeutic drugs for the symptoms derived from natural components. Both FAS and CR2 genes and their respective natural components were docked together to identify the binding affinity to suppress the symptom. Two components from the FAS genes resulted with the same highest binding energy value (Supplementary Table 2.10); FAS-NPC115624 (4'-Demethyl Deoxypodophyllotoxin beta-D-glucopyranoside) and FAS-NPC116759 (4-Demethyl-Epipodophyllotoxin-7'-O-Beta-D-Glucopyranoside). Both natural components are identified to be isolated from the plant species, *Podophyllum hexandrum*. The *Podophyllum hexandrum* is a plant species to be a potential source of lead bioactive metabolites with anticancer activity (Zilla et al., 2014, Shah et al., 2021). Thus, the natural compounds derived from the *Podophyllum hexandrum* plant species also exhibit anti-cancer properties. In addition, the NPC115624 compound was proven to exhibit cytotoxic lignan metabolite inducing cancer cell death (Zilla et al., 2014). Alas, there is no direct relationship between both natural components to neither the FAS gene and joint pain. As a natural component with anti-cancer properties, its function in the biological pathway of the FAS gene, which is involved in apoptosis, and the exact mechanism in relation to joint pain is not fully understood.

5.11 Anxiety and Depression

Mental health and psychological issues are still being reported after the recovery of the initial COVID infection. This is mainly hypothesized due to the impact of the COVID-19 virus on the central nervous system (CNS), increasing the risk of neurological disorders such as depression and anxiety to be more prevalent post infection. Chronic low-grade inflammation and the activation of the microglial caused by COVID in the CNS is found to be one the causes of the presence of mental health disorders (S. Wang et al., 2022). A study revealed a strong positive association between SAR-CoV-2 infection and depression/anxiety within 30 days after infection, suggesting that the symptoms are short-term symptoms and may disappear as time passes (Klaser et al., 2021). However, external factors such as the lockdown and other stressors may influence the duration of the mental health disorders. Various drugs such as Vortioxetine, a multi-modelled antidepressant with anti-inflammatory, anti-thrombotic and/or anti-viral properties, can be used to manage mental disorders (Fenton & Arnold, 2022). They can aid in reducing the effects of

depression, stress and anxiety but many are still undergoing the clinical trials and are not readily available.

Our study identified a potential hub gene biomarker for both depression and anxiety symptoms. The CDH23 gene (cadherin-related 23 gene) belongs to the cadherin superfamily, whose genes produce cell-cell adhesion glycoproteins that are calcium dependent. The creation of hair bundles and the arrangement of stereocilia are considered to be regulated by the encoded protein (CDH23 Cadherin Related 23 [Homo Sapiens (Human)] - Gene - NCBI, n.d.). It plays a crucial role in the normal functioning of the auditory system by mediating the tip links, which connect adjacent stereocilia and are involved in the mechanotransduction process that converts sound vibrations into electrical signals (Encyclopedia, 2020a). According to a large-cohort study, mutations in CDH23 have been linked to a wide range of hearing loss, including non-syndromic and syndromic hearing loss, congenital hearing loss, and hearing loss associated with aging (Usami et al., 2022).

The CDH23 gene is associated with long-term conditions such as hearing impairment and balance disorders where both are symptoms found in long COVID patients. However, the CDH23 gene is found to be a hub gene biomarker for two symptoms of long COVID, depression and anxiety. Studies have shown that among all the cadherin genes, the CDH23 gene was found to be implicated in some of the five major psychiatric disorders, such as ADHD and schizophrenia (Hawi et al., 2018). Moreover, it was reported that anxiety symptoms may occur to schizophrenic individuals around 65% of the time (Temmingh & Stein, 2015). Hence, the CDH23 gene may play a role in the causing of schizophrenia and anxiety disorders but it may not be the sole reason for the causation of the mental health disorders. Another study has proven a significant association between the CDH23 gene and the Major Depressive Disorder (MDD). The rs11592462, a specific SNP variation in CDH23, is involved in the thinning of the right anterior cingulate cortex (ACC) (Han et al., 2020), a region of the brain that involves and regulates emotion-processings. The relation of this variant of the CDH23 gene and the MDD disorder is potentially able to cause depression within individuals. Regardless of the major psychiatric disorders, the cognitive decline in hearing caused by the gene is bound to cause

depression and anxiety disorders due to the withdrawal in individual experience in situations where they are unable to hear and communicate (Rutherford et al., 2017).

Studies have shown that low-doses of methylphenidate have shown an appropriate effect on medically ill patients with depression and anxiety (Hardy, 2009). With the aid of methylphenidate, a FDA-approved drug used for depression and anxiety, serving as a reference, we were able to identify the natural compound that has a similar structure as the potential therapeutic drug. In order to combat both symptoms of depression and anxiety, the natural compound, (2S)-2-(methylazaniumyl)-3-phenylpropanoate (NPC67043) was identified. The (2S)-2-(methylazaniumyl)-3-phenylpropanoate, also known as N-methyl-L-phenylalanine zwitterion, is the zwitterionic form of N-methyl-L-phenylalanine (PubChem, n.d.-a). This natural compound was analyzed using molecular docking where it was docked against the CDH23 gene as the receptor. The docking analysis resulted in a value of -53.12 which is considered to be a good binding affinity and interaction between the two molecules. The strength and the binding affinity of both molecules are said to be strong to form stable complexes as there is no presence of another complex to make a comparison. Further information regarding the natural compound was not available due to insufficient data. But having a similar structure to the FDA-approved drug, methylphenidate, could infer the natural component to have similar properties as well to manage against anxiety and depression.

5.12 Tinnitus

Tinnitus is a condition where a person is hearing a buzzing or a ringing in the ears, phantom perception, without an external source creating the sound. This condition is usually experienced when an individual is exposed to loud sounds, having an ear infection or a side effect of a medication. Evidence has shown that tinnitus is a symptom of long COVID where its prevalence among the general population ranges between 16 to 26% (Degen et al., 2022). The exact mechanism revolving tinnitus in long COVID patients is proposed to be one of the theories that are proposed. A study showed the prevalence of tinnitus being associated with hearing loss caused by COVID-19 (Figueiredo et al., 2022). The data can be interpreted that the hearing loss is found to be higher in patients with chronic tinnitus and long COVID tinnitus. Research on the pathophysiology of tinnitus

suggests that abnormal neural activity can lead to the development of tinnitus and since the SARS-CoV-2 virus is known to cause inflammation in the neural cells (Haider et al., 2018). It is found to cause a person to perceive a sound even though there is no longer underlying damage from the acute infection. The duration of the symptom varies between each individual where acute tinnitus can last for a week to six months whereas chronic tinnitus can range for more than six months (Haider et al., 2018). Emotional distress, such as anxiety and depression, caused by the acute infection is found to increase the duration of tinnitus (Degen et al., 2022). People affected with anxiety and depression have been found to contribute to the increase in prevalence of tinnitus.

To overcome this manifestation in patients, four hub gene biomarkers were identified to be correlated to the symptom; CACNA1D, NF1, RET and TERT genes. CACNA1D is a gene that encodes for a subunit of the voltage-gated calcium channel CaV1.3, commonly present in the inner ear, brain, heart and other tissues. This channel is essential for several physiological functions, including hormone release, neural communication, and muscle contraction. CaV1.3, which controls insulin secretion, is the main L-type voltage-gated calcium channel in pancreatic beta cells (Flanagan et al., 2017). The gene CACNA1D is found to be associated with neurodevelopmental disorders. Although not directly connected to tinnitus, the CACNA1D gene is associated with Non-Syndromic Auditory Synaptopathies which may present tinnitus as a symptom (Saidia et al., 2023). The mutation of the gene leads to the loss of function of the CaV1.3L-type calcium channel causing a disruption in the transmission signals to the auditory nerves. The NF1 gene is involved in the biological pathways of tinnitus as a recent study has revealed NF1 patients experiencing auditory pathway abnormalities (Rance et al., 2021). Hearing loss is one of the mild complications of the NF1 gene mutation and tinnitus may arise as an underlying condition for the hearing loss caused. The RET gene encodes a protein that is necessary for the development of the neurological and renal systems as well as for cellular signaling. Hirschsprung disease is an example of an aggressive disease caused by mutations inhibiting the activation of the RET gene (Mahato & Sidorova, 2020). A study in 2012 identified a direct link between the RET gene and deafness or hearing impairment (Ohgami, 2012). It was further suggested that the impairments occur depending on the site or location of mutation present in the gene. Moreover, in regards to Hirschsprung disease, an observational study identified hearing impairment as an associated anomaly

to the disease (Prato et al., 2013). A complex and indirect link can be formed between the RET gene and the occurrence of tinnitus through the findings of the study. TERT is a gene that is an essential component and is responsible for the maintenance of telomeres. Various diseases and conditions are found to be associated with the length of the telomeres including hearing loss. The risk of hearing loss was determined to be more prevalent in individuals with shorter telomeres, females in particular (H. Zhang et al., 2020). Hence, creating a probable link that may connect the TERT gene to tinnitus.

Even though the identified hub genes may not have a direct link to tinnitus, they all show an association related to hearing loss. Hearing loss and tinnitus are closely related symptoms that a patient may experience. But, several studies have observed that patients with hearing loss are reported to experience tinnitus as well (Hong et al., 2023). Thus, enabling us to relate the genes associated more towards hearing loss to contribute a certain amount towards the causation of tinnitus.

Docking results of the hub genes and the natural components provide the prospective therapeutic targets candidates for tinnitus (Supplementary Table 2.12). The CACNA1D-NPC36836 complex resulted with the highest energy, -109.75, where the natural compound is referred to as Nicardipine. According to the NCBI database, Nicardipine is a calcium channel blocker from the dihydropyridine class and utilized in the treatment of angina pectoris and hypertension. As a calcium channel blocker, it is able to block L-type calcium channels causing vasodilation and a decrease in blood pressure. This natural component is usually employed in vascular disorders and as an anti-inflammatory agent as it is found to be more selective for the blood vessels in the nervous and cardiac systems (B. Huang et al., 2014). However, nicardipine showed no evidence to have direct effect on the voltage-gated calcium channel CaV1.3 encoded by the CACNA1D gene. There is limited research of the interaction between nicardipine and CACNA1D in relation to the long COVID symptom, tinnitus. As an alternative, the TERT-NPC230098 complex can be proposed as a potential candidate as the next best choice. However, as examined in the headache symptom, the NPC230098 is an unknown natural compound with unknown properties in relation to its hub gene, TERT. Thus, further studies are recommended to investigate the properties and therapeutic benefits of both natural compounds as they are determined viable targets for managing tinnitus.

5.13 Anorexia (Loss of Appetite)

Loss of appetite, or also known as anorexia, is an eating disorder which is a symptom of long COVID. Anorexia has an overall negative impact in life where it causes weight-loss and malnutrition. An Australian study has shown an increase of 104% of anorexia in children ever since the COVID-19 pandemic (Haripersad et al., 2021). Although it is curious on how anorexia is related to the virus and causes long COVID symptoms. It is found, during the acute infection phase, the SARS-CoV-2 virus damages the gastrointestinal system by binding to the ACE2 receptors present causing complications leading to anorexia (Sikaroudi et al., 2021). The underlying mechanism is proposed that during the initial infection, when the ACE2 receptor is bound to the virus in fat tissues, it induces an increased production and release of leptin (Van Der Voort et al., 2020). Leptin is a hormone produced to provide the sensation of satiety and over production of this hormone not only leads to anorexia but also respiratory failure. Psychological impacts are also found to be associated with anorexia. A study revealed that the fear and stress caused by the disease as well as the isolation caused by the pandemic produces negative emotions that can induce anorexia (Sikaroudi et al., 2021, Touyz et al., 2020). Furthermore, supporting this study, positive correlations have been identified between eating disorders and mental health issues such as depression. In terms of the distribution gender and anorexia, an equal distribution was identified implying that the long COVID symptom affects both genders equally unlike the usual sex difference present in eating disorders (Brasseler et al., 2022).

As anorexia causes severe malnutrition and weight loss, it is crucial to treat the symptom preventing further damage. Thus, the SMAD4 and MEN1 genes were identified as potential hub gene biomarkers for anorexia. SMAD4 gene and anorexia do not share many similarities to determine the direct association of the gene and symptom. A study on lung cancer patients with anorexia found the major pathways that are involved and suggested to cause loss of appetite, including the TGF- β signaling pathway (Muscaritoli et al., 2022). The presence of the TGF- β signaling pathways in relation to anorexia results in an indirect relationship between the SMAD4 gene and the long COVID symptom. The MEN1 gene, commonly known as multiple endocrine neoplasia type 1, produces the tumor

suppressor protein menin. This protein regulates the action of other genes and proteins involved in cell development and division. This gene is known as a rare autosomal dominant hereditary disease that shortens life expectancy and is associated with considerable morbidity (De Laat et al., 2018). This is because the mutations in the MEN1 gene can cause tumors to grow in a number of endocrine organs, including the pituitary gland, pancreas, and parathyroid glands. Depending on which organs are damaged, these tumors can produce a variety of symptoms. The role of anorexia in the MEN1 gene is not well understood. Several case studies have found a faint link where the MEN1 gene causes hyperparathyroidism, which then induces hypoglycemia, leading to a range of symptoms including anorexia (Giusti, 2022, Gupta et al., 2022). While the only relation found between the gene and condition is through the indirect symptoms and conditions of the gene such as hyperparathyroidism, the exact mechanism behind it remains a mystery.

Based on the genes, MEN1 and SMAD4, and their respective natural components (Supplementary Table 2.13), a single therapeutic candidate was identified due to its high binding affinity towards its gene; MEN1-NPC474814 (-114.41). The compound from the complex is known as 5-[bis[(2,5-dihydroxyphenyl)methyl]amino]-2-hydroxybenzoic acid. The properties of the compound and its origin remains unknown due to the lack of knowledge of the compound. However, a similarity search in the database identified the compound to be related or possibly a derivative to the Lavendustin family, a class of molecules commonly referred to as epidermal growth factor receptor tyrosine kinase inhibitors. Compounds from this family are known to interfere with the tyrosine kinase enzyme function affecting several biological pathways, including cell growth, differentiation and cell division. Interestingly, the second potential candidate for anorexia is the MEN1-NPC199737 (-95.91) complex where the natural compound is known as Lavendustin B. Lavendustin B is identified as a weak inhibitor and is majorly used as a negative control analogue among other compounds of the Lavendustin family. A singular study on the derivatives of Lavendustin B had mentioned that the compound, Lavendustin B, was previously identified as an inhibitor of HIV-1 integrase (IN) interaction (Agharbaoui et al., 2016). The relationship of both compounds to anorexia is not well understood as no evidence is present linking both together. Further research is required to analyze the properties of these natural components as both are found to be associated with the biological pathway of anorexia based on our study.

CHAPTER VI

CONCLUSION

In a nutshell, the identification of natural compounds for long COVID as natural remedies based on hub gene biomarkers and repurposed drugs shows promising potential for improving the symptoms caused by the condition. The analysis of the hub genes associated with each long COVID symptoms, we were able to gain further insights on the possible underlying biological mechanisms of the illness. Moreover, this information aided in the identification of potential targets (natural compounds) for therapeutic interventions. By using the repurposed drugs as a template to identify the natural compounds, it potentially offers a faster and cost-effective approach to develop treatments as compounds with similar structures are likely to exhibit similar functions. However, the complete mechanism of long COVID remains not fully understood and is a field where studies are still ongoing. Thus, additional studies are required by many of the natural compounds to determine its properties and understand its functions in relation to the mechanism of long COVID. This serves as the limitation to the study; despite the study providing promising results. Overall, the study provides a stable foundation for further research of the natural compounds being used as natural remedies for the management of long COVID. The identified hub gene biomarkers and repurposed drugs also serve as a medium to further understand the potential cause of long COVID and its mechanisms. As this study is a computational study, further research is required in this area in order to fully understand the efficacy and safety of the proposed treatment candidates and to develop more effective therapeutic interventions for individuals affected by long COVID.

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APPENDIX A

GANTT CHART

| Activity | 2023 | | | | | |
|---------------------------------|------|-----|-------|-----|-----|------|
| | Jan | Feb | March | Apr | May | June |
| Journal Reading | | | | | | |
| Progress Report (Logbook) | | | | | | |
| Literature Review | | | | | | |
| Data Collection | | | | | | |
| Data Processing | | | | | | |
| Results Tabulation | | | | | | |
| Data Analysis | | | | | | |
| Viva Preparation & Presentation | | | | | | |
| Thesis Writing & Submission | | | | | | |

APPENDIX B

SUPPLEMENTARY TABLES

Table 1.1: The Hub Genes, Repurposed Drugs, Canonical SMILES and Natural Compounds associated with Fatigue

| Hub Genes | FDA Approved Drugs | SMILES | Natural Compound |
|-----------|--------------------|----------------------------------------------------------------------------------------------|--------------------------------------------------------------------------------------------------------------------------------|
| NF1 | TRAMETINIB | <chem>CC1=C2C(=C(N(C1=O)C)NC3=C(C=C(C=C3)I)F)C(=O)N(C(=O)N2C4=CC=CC(=C4)NC(=O)C)C5CC5</chem> | NPC117032 |
| SMAD4 | LYSINE | <chem>C(CCN)CC(C(=O)O)N</chem> | NPC112890 NPC118459 NPC140872 NPC162620 NPC174246 NPC176164 NPC183845 NPC189301 NPC226027 NPC245027 |
| RET | VANDETANIB | <chem>CN1CCC(CC1)COC2=C(C=C3C(=C2)N=CN=C3NC4=C(C=C(C=C4)Br)F)OC</chem> | NPC56271 |
| ERBB4 | DACOMITINIB | <chem>COC1=C(C=C2C(=C1)N=CN=C2NC3=CC(=C(C=C3)F)Cl)NC(=O)C=CCN4CCCCC4</chem> | NPC56271 |

Table 1.2: The Hub Genes, Repurposed Drugs, Canonical SMILES and Natural Compounds associated with Shortness of Breath

| Hub Genes | FDA Approved Drugs | SMILES | Natural Compounds |
|-----------------------------|------------------------|------------------------------------------------------------------------------------------------------------------------------------------------------------------|----------------------------------------------------------------------------------------------------------------------------|
| CHRNE, CHRNA1, CHRNA2 | MIVACURIUM CHLORIDE | <chem>C[N+](CCCC2=CC(=C(C=C2C1CC3=CC(=C(C(=C3)OC)OC)OC)OC)OC)CCCOC(=O)CCC=CCCC(=O)OCCC[N+](CCCC5=C(C=C(C=C5C4CC6=CC(=C(C(=C6)OC)OC)OC)OC)OC)C.[Cl-].[Cl-]</chem> | NPC104196 NPC106295 NPC108434 NPC10871 NPC10908 NPC11296 NPC115284 NPC116465 NPC123323 NPC12424 |

| | | | |
|--|---------------------|------------------------------------------------------------------------------------------------------------------------------------------------------------------------|-----------------------------------------------------------------------------------------------------------------------------|
| | METOCURINE IODIDE | <chem>C[N+](CCC2=CC(=C3C=C2C1CC4=CC=C(C=C4)OC5=C6C(CC7=CC(=C(C=C7)OC)O3)[N+](CCC6=CC(=C5OC)OC)(C)C)OC.[I-].[I-]</chem> | NPC103379 NPC104196 NPC106295 NPC108434 NPC10871 NPC10908 NPC110416 NPC11296 NPC114124 NPC115284 |
| | DOXACURIUM CHLORIDE | <chem>C[N+](CCC2=CC(=C(C(=C2C1CC3=CC(=C(C(=C3)OC)OC)OC)OC)OC)OC)CCCCO C(=O)CCC(=O)OCCC[N+](CCC5=CC(=C(C(=C5C4CC6=CC(=C(C(=C6)OC)OC)OC)OC)OC)OC)OC)C.[Cl-].[Cl-]</chem> | NPC104196 NPC106295 NPC10871 NPC10908 NPC11296 NPC115284 NPC116465 NPC12424 NPC129518 NPC13916 |

Table 1.3: The Hub Genes, Repurposed Drugs, Canonical SMILES and Natural Compounds associated with Loss of Smell

| Hub Genes | FDA Approved Drugs | SMILES | Natural Compounds |
|-----------|--------------------|-------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|-------------------------------------------------------------------------------------------------------------------------------|
| GNRH1 | ZALCITABINE | <chem>C1CC(OC1CO)N2C=CC(=NC2=O)N</chem> | NPC106780 NPC120887 NPC17892 NPC226769 NPC229249 NPC280946 NPC320249 NPC322594 NPC324390 NPC328779 |
| | GOSERELIN | <chem>CC(C)CC(C(=O)NC(CCCN=C(N)N)C(=O)N1CCCC1C(=O)NNC(=O)N)NC(=O)C(CO C(C)(C)C)NC(=O)C(CC2=CC=C(C=C2)O)NC(=O)C(CO)NC(=O)C(CC3=CNC4=CC=CC=C43)NC(=O)C(CC5=CN=CN5)NC(=O)C6CCCC(=O)N6</chem> | NPC477633 NPC477634 NPC69843 |

| | | | |
|------|--------------------------|--------------------------------------------------------------------|-------------------------------------------------------------------------------------------------------------------------------|
| | AMINOGLUTET HIMIDE | <chem>CCC1(CCC(=O)NC1=O)C2=CC=C(C=C2)N</chem> | NPC474926 |
| | DAPSONE | <chem>C1=CC(=CC=C1N)S(=O)(=O)C2=CC=C(C=C2)N</chem> | NPC107135 NPC43655 |
| TAC3 | LIOETHYRONIN E SODIUM | <chem>C1=CC(=C(C=C1OC2=C(C=C(C=C2I)CC(C(=O)[O-])N)I)O.[Na+]</chem> | NPC106551 NPC142638 NPC17760 NPC188867 NPC197239 NPC239697 NPC241086 NPC259800 NPC281686 NPC282087 |

Table 1.4: The Hub Genes, Repurposed Drugs, Canonical SMILES and Natural Compounds associated with Headache

| Hub Genes | FDA Approved Drugs | SMILES | Natural Compounds |
|-----------|--------------------|------------------------------------------------------------------------|------------------------------------------------------------------------------------------------------------------------------|
| ESR1 | CLOMIPHENE | <chem>CCN(CC)CCOC1=CC=C(C=C1)C(=C(C2=CC=CC=C2)Cl)C3=CC=CC=C3</chem> | NPC255253 |
| | LEVONORGES TREL | <chem>CCC12CCC3C(C1CCC2(C#C)O)CCC4=C(C(=O)CCC34</chem> | NPC1015 NPC111015 NPC123319 NPC126993 NPC129913 NPC133391 NPC136548 NPC136948 NPC139397 NPC144258 |
| RET | VANDETANIB | <chem>CN1CCC(CC1)COC2=C(C=C3C(=C2)N=CN=C3NC4=C(C=C(C=C4)Br)F)OC</chem> | NPC56271 |

| | | | |
|-------|------------------------------|----------------------------------------------------------------------------------------------|--------------------------------------------------------------------------------------------------------------------------------|
| SMAD4 | LYSINE | <chem>C(CCN)CC(C(=O)O)N</chem> | NPC112890 NPC118459 NPC140872 NPC162620 NPC174246 NPC176164 NPC183845 NPC189301 NPC226027 NPC245027 |
| NF1 | TRAMETINIB | <chem>CC1=C2C(=C(N(C1=O)C)NC3=C(C=C(C=C3)I)F)C(=O)N(C(=O)N2C4=CC=CC(=C4)NC(=O)C)C5CC5</chem> | NPC117032 |
| TERT | OMACETAXINE MEPESUCCINATE | <chem>CC(C)(CCCC(CC(=O)OC)(C(=O)OC1C2C3=CC4=C(C=C3CCN5C2(CCC5)C=C1OC)OCO4)O)O</chem> | NPC165797 NPC230098 NPC237044 NPC298186 NPC301189 NPC304675 NPC319549 NPC33256 NPC474324 NPC474325 |

Table 1.5: The Hub Genes, Repurposed Drugs, Canonical SMILES and Natural Compounds associated with Brain Fog

| Hub Genes | FDA Approved Drugs | SMILES | Natural Compounds |
|-----------|--------------------|--------------------------------------------------------------|-------------------------------------------------------------------------------------------------------------------------------|
| KCNT1 | CLOFILIMUM | <chem>CCCCCCC[N+](CC)(CC)CCCC1=CC=C(C=C1)Cl</chem> | NPC231986 |
| | QUINIDINE | <chem>COC1=CC2=C(C=CN=C2C=C1)C(C3CC4CCN3CC4C=C)O</chem> | NPC165349 NPC47059 NPC193238 NPC203754 NPC150048 NPC274291 NPC472111 NPC264166 NPC118832 NPC329708 |
| GABRA1 | ZOLPIDEM | <chem>CC1=CC=C(C=C1)C2=C(N3C=C(C=CC3=N2)C)CC(=O)N(C)C</chem> | NPC317054 |

| | | | |
|---------|--------------------|--------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|--------------------------------------------------------------------------------------------------------------------------------|
| CACNA1B | ZICONOTIDE | <chem>CC1C(=O)NC(C(=O)NC2CSSCC3C(=O)NC(C(=O)NC(C(=O)NCC(=O)NC(C(=O)NC(CSSCC(C(=O)NC(CSSCC(C(=O)NC(C(=O)NCC(=O)NC(C(=O)NCC(=O)N1)CCCCN)CCCCN)N)C(=O)NC(C(=O)NCC(=O)NC(C(=O)N3)CO)C(C)O)NC(=O)C(NC(=O)C(NC(=O)C(NC(=O)C(NC(=O)C(NC2=O)CO)CCCNC(=N)N)CC(C)C)CCSC)CC4=CC=C(C=C4)O)CC(=O)O)C(=O)N)C(CCCN)CO)CCCNC(=N)N)CCCCN</chem> | NPC153554 NPC198254 NPC240130 NPC274198 NPC473404 NPC477631 NPC477632 NPC477636 NPC477638 NPC477639 |
| | ZICONOTIDE ACETATE | <chem>CC1C(=O)NC(C(=O)NC2CSSCC3C(=O)NC(C(=O)NC(C(=O)NCC(=O)NC(C(=O)NC(CSSCC(C(=O)NC(CSSCC(C(=O)NC(C(=O)NCC(=O)NC(C(=O)NCC(=O)N1)CCCCN)CCCCN)N)C(=O)NC(C(=O)NCC(=O)NC(C(=O)N3)CO)C(C)O)NC(=O)C(NC(=O)C(NC(=O)C(NC(=O)C(NC(=O)C(NC2=O)CO)CCCNC(=N)N)CC(C)C)CCSC)CC4=CC=C(C=C4)O)CC(=O)O)C(=O)N)C(CCCN)CO)CCCNC(=N)N)CCCCN</chem> | NPC153554 NPC198254 NPC240130 NPC274198 NPC473404 NPC477631 NPC477632 NPC477636 NPC477638 NPC477639 |

Table 1.6: The Hub Genes, Repurposed Drugs, Canonical SMILES and Natural Compounds associated with Chest Pain

| Hub Genes | FDA Approved Drugs | SMILES | Natural Compounds |
|-----------|--------------------|----------------------------------------------------------------------------------------------|--------------------------------------------------------------------------------------------------------------------------------|
| NF1 | TRAMETINIB | <chem>CC1=C2C(=C(N(C1=O)C)NC3=C(C=C(C=C3)I)F)C(=O)N(C(=O)N2C4=CC=CC(=C4)NC(=O)C)C5CC5</chem> | NPC117032 |
| SMAD4 | LYSINE | <chem>C(CCN)CC(C(=O)O)N</chem> | NPC112890 NPC118459 NPC140872 NPC162620 NPC174246 NPC176164 NPC183845 NPC189301 NPC226027 NPC245027 |
| RET | VANDETANIB | <chem>CN1CCC(CC1)COC2=C(C=C3C(=C2)N=CN=C3NC4=C(C=C(C=C4)Br)F)OC</chem> | NPC56271 |

Table 1.7: The Hub Genes, Repurposed Drugs, Canonical SMILES and Natural Compounds associated with Insomnia (Difficulty Sleeping)

| Hub Genes | FDA Approved Drugs | SMILES | Natural Compounds |
|-----------|--------------------|-------------------------------------------------------------------|--------------------------------------------------------------------------------------------------------------------------------|
| NR1H4 | OBETICHOLIC ACID | <chem>CCC1C2CC(CCC2(C3CCC4(C(C3C1O)C CC4C(C)CCC(=O)O)C)C)O</chem> | NPC100366 NPC102156 NPC102414 NPC108078 NPC108131 NPC111582 NPC114891 NPC116683 NPC119036 NPC121121 |
| | CHENODIOL | <chem>CC(CCC(=O)O)C1CCC2C1(CCC3C2C(C4C3(CCC(C4)O)C)O)C</chem> | NPC100366 NPC102156 NPC102414 NPC108078 NPC108131 NPC111582 NPC114891 NPC116683 NPC119036 NPC121121 |
| ABCB4 | BENZQUINAMIDE | <chem>CCN(CC)C(=O)C1CN2CCC3=CC(=C(C=C3C2CC1OC(=O)C)OC)OC</chem> | NPC102760 NPC128019 NPC136860 NPC169387 NPC187022 NPC188163 NPC208890 NPC213206 NPC222524 NPC230098 |
| ABCB11 | CHOLIC ACID | <chem>CC(CCC(=O)O)C1CCC2C1(C(CC3C2C(C4C3(CCC(C4)O)C)O)O)C</chem> | NPC100366 NPC102156 NPC102414 NPC108078 NPC108131 NPC111582 NPC114891 NPC116683 NPC119036 NPC121121 |

| | | | |
|-------|--------------|----------------------------------------------------------------|------------------------------------------------------------------------------------------------------------------------------|
| | PROCAINAMIDE | <chem>CCN(CC)CCNC(=O)C1=CC=C(C=C1)N</chem> | NPC162417 NPC226143 NPC322433 NPC57051 |
| | MEXILETINE | <chem>CC1=C(C(=CC=C1)C)OCC(C)N</chem> | NPC136112 NPC141739 NPC21890 NPC26524 NPC42383 NPC97811 |
| | DISOPYRAMIDE | <chem>CC(C)N(CCC(C1=CC=CC=C1)(C2=CC=C(C=N2)C(=O)N)C(C)C</chem> | NPC248462 NPC265100 NPC64436 NPC65408 NPC70406 |
| GATA4 | WARFARIN | <chem>CC(=O)CC(C1=CC=CC=C1)C2=C(C3=CC=CC=C3OC2=O)O</chem> | NPC10027 NPC100818 NPC100887 NPC10097 NPC100985 NPC100986 NPC101255 NPC101294 NPC101366 NPC101636 |

Table 1.9: The Hub Genes, Repurposed Drugs, Canonical SMILES and Natural Compounds associated with Dizziness

| Hub Genes | FDA Approved Drugs | SMILES | Natural Compounds |
|------------------|---------------------------|-------------------------------------------------------------------------|--------------------------------------------------|
| SCN5A | BENZONATATE | <chem>CCCCNC1=CC=C(C=C1)C(=O)OCCOCCOCCOCCOCCOCCOCCOCCOCCOCCOCCOC</chem> | NPC173295 NPC242933 NPC319645 NPC470971 |
| | HEXYLCAINE | <chem>CC(CNC1CCCCC1)OC(=O)C2=CC=CC=C2</chem> | NPC24777 NPC26285 |
| | PROCAINAMIDE | <chem>CCN(CC)CCNC(=O)C1=CC=C(C=C1)N</chem> | NPC162417 NPC226143 NPC322433 NPC57051 |

| | | | |
|-------------|------------------|----------------------------------------------------------------------------------------------|------------------------------------------------------------------------|
| | MEXILETINE | <chem>CC1=C(C(=CC=C1)C)OCC(C)N</chem> | NPC136112 NPC141739 NPC21890 NPC26524 NPC42383 NPC97811 |
| | DISOPYRAMID E | <chem>CC(C)N(CCC(C1=CC=CC=C1)(C2=CC=C C=N2)C(=O)N)C(C)C</chem> | NPC248462 NPC265100 NPC64436 NPC65408 NPC70406 |
| CACNA1 G | ZONISAMIDE | <chem>C1=CC=C2C(=C1)C(=NO2)CS(=O)(=O)N</chem> | NPC264400 |
| | METHSUXIMID E | <chem>CC1(CC(=O)N(C1=O)C)C2=CC=CC=C2</chem> | NPC256452 NPC55529 NPC71140 |
| | FLUNARIZINE | <chem>C1CN(CCN1CC=CC2=CC=CC=C2)C(C3=CC=C(C=C3)F)C4=CC=C(C=C4)F</chem> | NPC470926 |
| | PHENSUXIMID E | <chem>CN1C(=O)CC(C1=O)C2=CC=CC=C2</chem> | NPC256452 NPC55529 NPC71140 |
| RYR2 | PROCAINE | <chem>CCN(CC)CCOC(=O)C1=CC=C(C=C1)N</chem> | NPC122235 NPC150323 NPC226794 NPC319645 |
| SCN1B | ZONISAMIDE | <chem>C1=CC=C2C(=C1)C(=NO2)CS(=O)(=O)N</chem> | NPC264400 |
| NF1 | TRAMETINIB | <chem>CC1=C2C(=C(N(C1=O)C)NC3=C(C=C(C=C3)I)F)C(=O)N(C(=O)N2C4=CC=CC(=C4)NC(=O)C)C5CC5</chem> | NPC117032 |
| RET | VANDETANIB | <chem>CN1CCC(CC1)COC2=C(C=C3C(=C2)N=CN=C3NC4=C(C=C(C=C4)Br)F)OC</chem> | NPC56271 |

Table 1.10: The Hub Genes, Repurposed Drugs, Canonical SMILES and Natural Compounds associated with Joint Pain

| Hub Genes | FDA Approved Drugs | SMILES | Natural Compounds |
|-----------|--------------------|-------------------------------------------------------|-------------------------------------------------|
| CR2 | PROGESTERONE | <chem>CC(=O)C1CCC2C1(CCC3C2CCC4=CC(=O)CCC34C)C</chem> | NPC100297 NPC107704 NPC115023 NPC11711 |

| | | | |
|-----|------------|--------------------------------------------------------------------------------------------------------|-------------------------------------------------------------------------------------------------------------------------------|
| | | | NPC127582 NPC136548 NPC136948 NPC139397 NPC142253 NPC142754 |
| FAS | TENIPOSIDE | <chem>COC1=CC(=CC(=C1O)OC)C2C3C(COC3=O)C(C4=CC5=C(C=C24)OCO5)OC6C(C(C7C(O6)COC(O7)C8=CC=CS8)O)O</chem> | NPC100465 NPC103197 NPC115281 NPC115624 NPC116759 NPC119910 NPC14294 NPC150943 NPC152424 NPC163527 |

Table 1.11: The Hub Genes, Repurposed Drugs, Canonical SMILES and Natural Compounds associated with Depression

| Hub Genes | FDA Approved Drugs | SMILES | Natural Compounds |
|-----------|--------------------|--------------------------------------------|-------------------|
| CDH23 | METHYLPHENIDATE | <chem>COC(=O)C(C1CCCCN1)C2=CC=CC=C2</chem> | NPC67043 |

Table 1.12: The Hub Genes, Repurposed Drugs, Canonical SMILES and Natural Compounds associated with Anxiety

| Hub Genes | FDA Approved Drugs | SMILES | Natural Compounds |
|-----------|--------------------|--------------------------------------------|-------------------|
| CDH23 | METHYLPHENIDATE | <chem>COC(=O)C(C1CCCCN1)C2=CC=CC=C2</chem> | NPC67043 |

Table 1.13: The Hub Genes, Repurposed Drugs, Canonical SMILES and Natural Compounds associated with Tinnitus

| Hub Genes | FDA Approved Drugs | SMILES | Natural Compounds |
|-----------|--------------------|----------------------------------------------------------------------------------------------|-------------------|
| NF1 | TRAMETINIB | <chem>CC1=C2C(=C(N(C1=O)C)NC3=C(C=C(C=C3)I)F)C(=O)N(C(=O)N2C4=CC=CC(=C4)NC(=O)C)C5CC5</chem> | NPC117032 |
| RET | VANDETANIB | <chem>CN1CCC(CC1)COC2=C(C=C3C(=C2)N=CN=C3NC4=C(C=C(C=C4)Br)F)OC</chem> | NPC56271 |

| | | | |
|---------|------------------------------|--------------------------------------------------------------------------------------|-------------------------------------------------------------------------------------------------------------------------------|
| TERT | OMACETAXINE MEPESUCCINATE | <chem>CC(C)(CCCC(CC(=O)OC)(C(=O)OC1C2C3=CC4=C(C=C3CCN5C2(CCC5)C=C1OC)OCO4)O)O</chem> | NPC165797 NPC230098 NPC237044 NPC298186 NPC301189 NPC304675 NPC319549 NPC33256 NPC474324 NPC474325 |
| CACNA1D | NIMODIPINE | <chem>CC1=C(C(C(=C(N1)C)C(=O)OC(C)C)C2=CC(=CC=C2)[N+](=O)[O-])C(=O)OCCOC</chem> | NPC190945 NPC36836 NPC63370 |
| | NITRENDIPINE | <chem>CCOC(=O)C1=C(NC(=C(C1C2=CC(=CC=C2)[N+](=O)[O-])C(=O)OC)C)C</chem> | NPC190945 NPC36836 NPC63370 |

Table 1.14: The Hub Genes, Repurposed Drugs, Canonical SMILES and Natural Compounds associated with Anorexia (Loss of Appetite)

| Hub Genes | FDA Approved Drugs | SMILES | Natural Compounds |
|-----------|--------------------|----------------------------------------------------------------|--------------------------------------------------------------------------------------------------------------------------------|
| SMAD4 | LYSINE | <chem>C(CCN)CC(C(=O)O)N</chem> | NPC112890 NPC118459 NPC140872 NPC162620 NPC174246 NPC176164 NPC183845 NPC189301 NPC226027 NPC245027 |
| MEN1 | OLSALAZINE | <chem>C1=CC(=C(C=C1N=NC2=CC(=C(C=C2)O)C(=O)O)C(=O)O)O</chem> | NPC112336 NPC181526 NPC199737 NPC301702 NPC316574 NPC323798 NPC471778 NPC474814 NPC475983 |
| PALB2 | MITOMYCIN | <chem>CC1=C(C(=O)C2=C(C1=O)N3CC4C(C3(C2COC(=O)N)OC)N4)N</chem> | NPC139867 |

Table 2.1: Molecular Docking results for Fatigue

| Hub Gene & Natural Component | Energy | VDW | HBond | Elec |
|------------------------------|--------|--------|--------|-------|
| RET-NPC56271 | -94.65 | -85.16 | -9.49 | 0.00 |
| ERBB4-NPC56271 | -94.29 | -85.58 | -8.71 | 0.00 |
| NF1-NPC117032 | -83.94 | -80.65 | -2.74 | -0.55 |
| SMAD4-NPC189301 | -73.85 | -53.31 | -21.34 | 0.80 |
| SMAD4-NPC176164 | -73.26 | -47.26 | -26.00 | 0.00 |
| SMAD4-NPC226027 | -70.85 | -56.50 | -13.59 | -0.75 |
| SMAD4-NPC174246 | -69.07 | -55.26 | -15.09 | 1.28 |
| SMAD4-NPC183845 | -67.90 | -31.13 | -36.77 | 0.00 |
| SMAD4-NPC112890 | -65.48 | -31.35 | -32.29 | -1.84 |
| SMAD4-NPC140872 | -64.27 | -46.55 | -17.72 | 0.00 |
| SMAD4-NPC118459 | -59.74 | -38.78 | -20.96 | 0.00 |
| SMAD4-NPC245027 | -55.96 | -44.00 | -10.50 | -1.46 |
| SMAD4-NPC162620 | -54.80 | -47.63 | -7.17 | 0.00 |

Table 2.2: Molecular Docking results for Shortness of Breath

| Hub Gene & Natural Component | Energy | VDW | HBond | Elec |
|------------------------------|---------|--------|--------|------|
| CHRNE-NPC108434 | -106.33 | -93.35 | -12.99 | 0.00 |
| CHRNA1-NPC10908 | -104.70 | -92.98 | -11.72 | 0.00 |
| CHNRD-NPC11296 | -103.00 | -83.34 | -19.66 | 0.00 |
| CHRNA1-NPC115284 | -102.98 | -94.69 | -8.29 | 0.00 |
| CHNRD-NPC10871 | -101.86 | -78.73 | -23.43 | 0.29 |
| CHRNA1-NPC108434 | -98.10 | -89.39 | -8.72 | 0.00 |
| CHRNA1-NPC108434 | -97.35 | -78.84 | -18.50 | 0.00 |
| CHRNE-NPC10871 | -96.64 | -84.54 | -12.10 | 0.00 |
| CHRNA1-NPC116465 | -94.42 | -81.36 | -13.06 | 0.00 |
| CHNRD-NPC115284 | -94.09 | -93.60 | -0.49 | 0.00 |

| | | | | |
|------------------|--------|--------|--------|------|
| CHRNE-NPC11296 | -93.10 | -74.49 | -18.61 | 0.00 |
| CHRNB1-NPC10871 | -93.05 | -90.87 | -2.90 | 0.73 |
| CHRNB1-NPC11296 | -93.02 | -88.30 | -4.72 | 0.00 |
| CHNRD-NPC13916 | -92.25 | -74.21 | -18.04 | 0.00 |
| CHRNB1-NPC13916 | -92.22 | -81.32 | -10.89 | 0.00 |
| CHRNB1-NPC104196 | -91.30 | -87.10 | -4.20 | 0.00 |
| CHNRD-NPC104196 | -88.75 | -71.78 | -16.98 | 0.00 |
| CHRNE-NPC106295 | -86.51 | -84.75 | -1.76 | 0.00 |
| CHNRD-NPC106295 | -84.77 | -83.12 | -1.65 | 0.00 |
| CHRNE-NPC10908 | -84.70 | -74.68 | -10.02 | 0.00 |
| CHNRD-NPC116465 | -83.01 | -68.36 | -14.65 | 0.00 |
| CHRNE-NPC115284 | -82.07 | -79.57 | -2.50 | 0.00 |
| CHRNB1-NPC106295 | -81.97 | -81.97 | 0.00 | 0.00 |
| CHRNB1-NPC114124 | -81.78 | -65.48 | -16.30 | 0.00 |
| CHRNB1-NPC103379 | -81.66 | -60.92 | -20.74 | 0.00 |
| CHRNE-NPC13916 | -81.21 | -65.30 | -15.91 | 0.00 |
| CHNRD-NPC10908 | -79.41 | -79.41 | 0.00 | 0.00 |
| CHRNE-NPC116465 | -79.33 | -73.68 | -5.65 | 0.00 |
| CHRNE-NPC114124 | -78.43 | -60.67 | -17.77 | 0.00 |
| CHNRD-NPC103379 | -78.37 | -70.02 | -8.35 | 0.00 |
| CHRNE-NPC104196 | -78.19 | -73.19 | -5.00 | 0.00 |
| CHNRD-NPC114124 | -77.32 | -65.32 | -12.00 | 0.00 |
| CHNRD-NPC123323 | -76.01 | -73.71 | -2.30 | 0.00 |
| CHRNB1-NPC123323 | -74.20 | -66.93 | -7.27 | 0.00 |
| CHRNE-NPC123323 | -73.07 | -73.07 | 0.00 | 0.00 |
| CHRNE-NPC103379 | -72.46 | -59.26 | -13.20 | 0.00 |

Table 2.3: Molecular Docking results for Loss of Smell

| Hub Gene & Natural Component | Energy | VDW | HBond | Elec |
|------------------------------|---------|--------|--------|-------|
| GNRH1-NPC69843 | -105.95 | -91.90 | -12.60 | -1.46 |
| GNRH1-NPC322594 | -100.52 | -82.39 | -18.12 | 0.00 |
| GNRH1-NPC328779 | -95.88 | -46.03 | -49.85 | 0.00 |
| GNRH1-NPC120887 | -92.41 | -70.14 | -22.27 | 0.00 |
| GNRH1-NPC17892 | -88.11 | -63.65 | -24.47 | 0.00 |
| GNRH1-NPC320249 | -82.83 | -55.55 | -27.28 | 0.00 |
| GNRH1-NPC324390 | -77.60 | -56.91 | -20.69 | 0.00 |
| GNRH1-NPC226769 | -73.68 | -44.94 | -28.74 | 0.00 |
| TAC3-NPC17760 | -70.87 | -64.87 | -6.00 | 0.00 |
| GNRH1-NPC474926 | -70.68 | -48.60 | -22.08 | 0.00 |
| GNRH1-NPC229249 | -70.02 | -70.02 | 0.00 | 0.00 |
| TAC3-NPC241086 | -69.42 | -59.93 | -9.49 | 0.00 |
| TAC3-NPC197239 | -68.37 | -61.77 | -6.61 | 0.00 |
| TAC3-NPC282087 | -67.22 | -51.77 | -15.45 | 0.00 |
| GNRH1-NPC106780 | -66.35 | -66.35 | 0.00 | 0.00 |
| TAC3-NPC259800 | -66.01 | -50.62 | -15.39 | 0.00 |
| GNRH1-4-NPC107135 | -58.84 | -36.30 | -22.54 | 0.00 |
| TAC3-NPC142638 | -58.49 | -38.96 | -19.54 | 0.00 |
| TAC3-NPC106551 | -57.96 | -45.84 | -12.78 | 0.66 |
| TAC3-NPC281686 | -57.96 | -44.77 | -13.83 | 0.64 |
| GNRH1-NPC43655 | -55.76 | -47.40 | -8.36 | 0.00 |
| TAC3-NPC188867 | -54.67 | -33.17 | -21.51 | 0.00 |
| TAC3-NPC239697 | -52.34 | -39.72 | -12.62 | 0.00 |

Table 2.4: Molecular Docking results for Headache

| Hub Gene & Natural Component | Energy | VDW | HBond | Elec |
|------------------------------|--------|-----|-------|------|
|------------------------------|--------|-----|-------|------|

| | | | | |
|-----------------|--------|--------|--------|-------|
| TERT-NPC230098 | -97.62 | -85.53 | -12.09 | 0.00 |
| ESR1-NPC136948 | -96.97 | -78.32 | -18.65 | 0.00 |
| RET-NPC56271 | -94.65 | -85.16 | -9.49 | 0.00 |
| TERT-NPC474324 | -92.79 | -82.29 | -10.50 | 0.00 |
| ESR1-NPC123319 | -92.16 | -77.78 | -14.38 | 0.00 |
| TERT-NPC319549 | -91.83 | -71.07 | -20.76 | 0.00 |
| TERT-NPC33256 | -85.58 | -69.20 | -16.38 | 0.00 |
| ESR1-NPC1015 | -85.42 | -70.77 | -14.64 | 0.00 |
| TERT-NPC237044 | -85.10 | -75.60 | -9.50 | 0.00 |
| ESR1-NPC139397 | -84.00 | -80.50 | -3.50 | 0.00 |
| NF1-NPC117032 | -83.94 | -80.65 | -2.74 | -0.55 |
| TERT-NPC474325 | -83.26 | -62.39 | -20.87 | 0.00 |
| TERT-NPC298186 | -83.22 | -72.58 | -10.94 | 0.30 |
| ESR1-NPC255253 | -82.47 | -71.97 | -10.50 | 0.00 |
| TERT-NPC304675 | -81.06 | -65.27 | -15.79 | 0.00 |
| ESR1-NPC144258 | -80.38 | -74.17 | -6.21 | 0.00 |
| TERT-NPC301189 | -80.12 | -69.50 | -10.61 | 0.00 |
| TERT-NPC165797 | -78.17 | -69.69 | -8.47 | 0.00 |
| ESR1-NPC126993 | -76.15 | -73.83 | -2.33 | 0.00 |
| ESR1-NPC136548 | -74.01 | -61.01 | -13.00 | 0.00 |
| SMAD4-NPC189301 | -73.85 | -53.31 | -21.34 | 0.80 |
| SMAD4-NPC176164 | -73.26 | -47.26 | -26.00 | 0.00 |
| ESR1-NPC129913 | -73.01 | -64.51 | -8.50 | 0.00 |
| SMAD4-NPC226027 | -70.85 | -56.50 | -13.59 | -0.75 |
| SMAD4-NPC174246 | -69.07 | -55.26 | -15.09 | 1.28 |
| SMAD4-NPC183845 | -67.90 | -31.13 | -36.77 | 0.00 |

| | | | | |
|-----------------|--------|--------|--------|-------|
| SMAD4-NPC112890 | -65.48 | -31.35 | -32.29 | -1.84 |
| SMAD4-NPC140872 | -64.27 | -46.55 | -17.72 | 0.00 |
| SMAD4-NPC118459 | -59.74 | -38.78 | -20.96 | 0.00 |
| SMAD4-NPC245027 | -55.96 | -44.00 | -10.50 | -1.46 |
| SMAD4-NPC162620 | -54.80 | -47.63 | -7.17 | 0.00 |

Table 2.5: Molecular Docking results for Brain Fog

| Hub Gene & Natural Component | Energy | VDW | HBond | Elec |
|------------------------------|---------|--------|--------|-------|
| CACNA1B-NPC198254 | -119.29 | -90.52 | -28.77 | 0.00 |
| CACNA1B-NPC473404 | -115.68 | -86.02 | -33.71 | 4.05 |
| CACNA1B-NPC153554 | -109.71 | -85.83 | -23.88 | 0.00 |
| CACNA1B-NPC240130 | -104.61 | -70.44 | -31.19 | -2.99 |
| CACNA1B-NPC274198 | -100.59 | -76.19 | -24.98 | 0.58 |
| KCNT1-NPC329708 | -85.81 | -77.31 | -8.50 | 0.00 |
| KCNT1-NPC193238 | -85.17 | -70.08 | -15.09 | 0.00 |
| KCNT1-NPC274291 | -84.64 | -70.82 | -13.82 | 0.00 |
| GABRA1-NPC317054 | -83.96 | -77.67 | -6.30 | 0.00 |
| KCNT1-NPC47059 | -80.67 | -73.67 | -7.00 | 0.00 |
| KCNT1-NPC203754 | -78.36 | -72.36 | -6.00 | 0.00 |
| KCNT1-NPC165349 | -76.72 | -72.59 | -4.13 | 0.00 |
| KCNT1-NPC264166 | -74.93 | -72.43 | -2.50 | 0.00 |
| KCNT1-NPC118832 | -70.83 | -62.29 | -8.54 | 0.00 |
| KCNT1-NPC150048 | -70.42 | -65.85 | -4.57 | 0.00 |
| KCNT1-NPC231986 | -69.20 | -65.70 | -3.50 | 0.00 |

Table 2.6: Molecular Docking results for Chest Pain

| Hub Gene & Natural Component | Energy | VDW | HBond | Elec |
|------------------------------|--------|--------|-------|------|
| RET-NPC56271 | -94.65 | -85.16 | -9.49 | 0.00 |

| | | | | |
|-----------------|--------|--------|--------|-------|
| NF1-NPC117032 | -83.94 | -80.65 | -2.74 | -0.55 |
| SMAD4-NPC189301 | -73.85 | -53.31 | -21.34 | 0.80 |
| SMAD4-NPC176164 | -73.26 | -47.26 | -26.00 | 0.00 |
| SMAD4-NPC226027 | -70.85 | -56.50 | -13.59 | -0.75 |
| SMAD4-NPC174246 | -69.07 | -55.26 | -15.09 | 1.28 |
| SMAD4-NPC183845 | -67.90 | -31.13 | -36.77 | 0.00 |
| SMAD4-NPC112890 | -65.48 | -31.35 | -32.29 | -1.84 |
| SMAD4-NPC140872 | -64.27 | -46.55 | -17.72 | 0.00 |
| SMAD4-NPC118459 | -59.74 | -38.78 | -20.96 | 0.00 |
| SMAD4-NPC245027 | -55.96 | -44.00 | -10.50 | -1.46 |
| SMAD4-NPC162620 | -54.80 | -47.63 | -7.17 | 0.00 |

Table 2.7: Molecular Docking results for Insomnia

| Hub Gene & Natural Component | Energy | VDW | HBond | Elec |
|------------------------------|---------|--------|--------|------|
| ABCB11-NPC100366 | -107.98 | -85.27 | -22.72 | 0.00 |
| NR1H4-NPC100366 | -97.79 | -87.37 | -10.42 | 0.00 |
| ABCB4-NPC213206 | -96.30 | -79.94 | -16.35 | 0.00 |
| ABCB4-NPC187022 | -96.10 | -85.42 | -10.68 | 0.00 |
| ABCB4-NPC208890 | -94.01 | -79.52 | -14.49 | 0.00 |
| ABCB4-NPC169387 | -92.86 | -90.86 | -2.00 | 0.00 |
| ABCB4-NPC136860 | -88.28 | -79.85 | -8.43 | 0.00 |
| ABCB4-NPC222524 | -86.16 | -78.11 | -8.05 | 0.00 |
| SLC12A3-NPC321053 | -81.71 | -47.62 | -34.09 | 0.00 |
| ABCB4-NPC188163 | -81.18 | -71.92 | -9.26 | 0.00 |
| ABCB4-NPC128019 | -71.50 | -61.79 | -9.71 | 0.00 |

Table 2.8: Molecular Docking results for Heart Palpitations

| Hub Gene & Natural Component | Energy | VDW | HBond | Elec |
|------------------------------|---------|--------|--------|------|
| GATA4-NPC101636 | -107.59 | -90.62 | -16.97 | 0.00 |
| GATA4-NPC100818 | -103.25 | -67.84 | -35.40 | 0.00 |
| GATA4-NPC100887 | -98.92 | -67.75 | -31.18 | 0.00 |
| GATA4-NPC10097 | -89.52 | -56.87 | -32.65 | 0.00 |
| GATA4-NPC100985 | -84.82 | -62.35 | -22.47 | 0.00 |
| SCN5A-NPC162417 | -83.58 | -73.08 | -10.50 | 0.00 |
| SCN5A-NPC64436 | -82.21 | -74.38 | -7.83 | 0.00 |
| SCN5A-NPC470971 | -81.67 | -71.48 | -10.19 | 0.00 |
| GATA4-NPC101366 | -79.21 | -54.46 | -24.75 | 0.00 |
| SCN5A-NPC322433 | -78.26 | -60.08 | -18.18 | 0.00 |
| SCN5A-NPC265100 | -77.86 | -70.08 | -7.78 | 0.00 |
| SCN5A-NPC136112 | -77.71 | -66.44 | -11.27 | 0.00 |
| GATA4-NPC101294 | -76.70 | -69.70 | -7.00 | 0.00 |
| SCN5A-NPC26285 | -76.27 | -73.59 | -2.68 | 0.00 |
| SCN5A-NPC242933 | -75.49 | -60.87 | -14.62 | 0.00 |
| GATA4-NPC10027 | -74.31 | -65.03 | -9.28 | 0.00 |
| SCN5A-NPC319645 | -72.42 | -64.05 | -8.37 | 0.00 |
| SCN5A-NPC226143 | -72.40 | -68.95 | -3.45 | 0.00 |
| GATA4-NPC100986 | -71.88 | -55.38 | -16.50 | 0.00 |
| SCN5A-NPC248462 | -71.41 | -58.66 | -12.75 | 0.00 |
| SCN5A-NPC70406 | -70.84 | -52.41 | -18.43 | 0.00 |
| SCN5A-NPC141739 | -70.80 | -65.80 | -5.00 | 0.00 |
| SCN5A-NPC26524 | -68.43 | -63.12 | -5.30 | 0.00 |
| SCN5A-NPC173295 | -63.42 | -55.17 | -8.26 | 0.00 |
| SCN5A-NPC57051 | -63.00 | -60.62 | -2.38 | 0.00 |

| | | | | |
|----------------|--------|--------|-------|------|
| SCN5A-NPC42383 | -61.93 | -56.37 | -5.56 | 0.00 |
| SCN5A-NPC65408 | -61.57 | -55.44 | -6.13 | 0.00 |
| SCN5A-NPC97811 | -58.36 | -55.41 | -2.94 | 0.00 |
| SCN5A-NPC24777 | -57.83 | -50.23 | -7.60 | 0.00 |

Table 2.9: Molecular Docking results for Dizziness

| Hub Gene & Natural Component | Energy | VDW | HBond | Elec |
|------------------------------|--------|--------|--------|-------|
| RET-NPC56271 | -94.65 | -85.16 | -9.49 | 0.00 |
| RYR2-NPC122235 | -85.94 | -78.79 | -7.15 | 0.00 |
| NF1-NPC117032 | -83.94 | -80.65 | -2.74 | -0.55 |
| SCN5A-NPC162417 | -83.58 | -73.08 | -10.50 | 0.00 |
| SCN5A-NPC64436 | -82.21 | -74.38 | -7.83 | 0.00 |
| SCN5A-NPC470971 | -81.67 | -71.48 | -10.19 | 0.00 |
| SCN5A-NPC322433 | -78.26 | -60.08 | -18.18 | 0.00 |
| SCN5A-NPC265100 | -77.86 | -70.08 | -7.78 | 0.00 |
| SCN5A-NPC136112 | -77.71 | -66.44 | -11.27 | 0.00 |
| SCN5A-NPC26285 | -76.27 | -73.59 | -2.68 | 0.00 |
| SCN5A-NPC242933 | -75.49 | -60.87 | -14.62 | 0.00 |
| CACNA1G-NPC264400 | -74.95 | -58.77 | -16.18 | 0.00 |
| CACNA1G-NPC55529 | -73.98 | -62.68 | -11.30 | 0.00 |
| RYR2-NPC150323 | -73.75 | -47.76 | -26.00 | 0.00 |
| CACNA1G-NPC470926 | -73.59 | -71.09 | -2.50 | 0.00 |
| SCN5A-NPC319645 | -72.42 | -64.05 | -8.37 | 0.00 |
| SCN5A-NPC226143 | -72.40 | -68.95 | -3.45 | 0.00 |
| CACNA1G-NPC256452 | -71.53 | -61.21 | -10.32 | 0.00 |
| SCN5A-NPC248462 | -71.41 | -58.66 | -12.75 | 0.00 |
| SCN5A-NPC70406 | -70.84 | -52.41 | -18.43 | 0.00 |

| | | | | |
|------------------|--------|--------|--------|------|
| SCN5A-NPC141739 | -70.80 | -65.80 | -5.00 | 0.00 |
| SCN5A-NPC26524 | -68.43 | -63.12 | -5.30 | 0.00 |
| RYR2-NPC319645 | -68.19 | -57.69 | -10.50 | 0.00 |
| CACNA1G-NPC71140 | -66.84 | -59.10 | -7.75 | 0.00 |
| SCN5A-NPC173295 | -63.42 | -55.17 | -8.26 | 0.00 |
| RYR2-NPC226794 | -63.12 | -47.87 | -15.24 | 0.00 |
| SCN5A-NPC57051 | -63.00 | -60.62 | -2.38 | 0.00 |
| SCN5A-NPC42383 | -61.93 | -56.37 | -5.56 | 0.00 |
| SCN1B-NPC264400 | -61.81 | -41.42 | -20.39 | 0.00 |
| SCN5A-NPC65408 | -61.57 | -55.44 | -6.13 | 0.00 |
| SCN5A-NPC97811 | -58.36 | -55.41 | -2.94 | 0.00 |
| SCN5A-NPC24777 | -57.83 | -50.23 | -7.60 | 0.00 |

Table 2.10: Molecular Docking results for Joint Pain

| Hub Gene & Natural Component | Energy | VDW | HBond | Elec |
|------------------------------|---------|--------|--------|------|
| FAS-NPC115624 | -115.52 | -84.27 | -31.25 | 0.00 |
| FAS-NPC116759 | -115.52 | -77.79 | -37.72 | 0.00 |
| FAS-NPC103197 | -110.38 | -97.35 | -13.03 | 0.00 |
| FAS-NPC152424 | -108.06 | -81.95 | -26.11 | 0.00 |
| FAS-NPC100465 | -103.29 | -71.46 | -31.83 | 0.00 |
| FAS-NPC119910 | -103.26 | -89.55 | -13.71 | 0.00 |
| FAS-NPC14294 | -102.40 | -73.18 | -29.22 | 0.00 |
| FAS-NPC150943 | -97.35 | -71.67 | -25.68 | 0.00 |
| FAS-NPC115281 | -95.30 | -76.80 | -18.51 | 0.00 |
| FAS-NPC163527 | -91.19 | -70.10 | -21.08 | 0.00 |
| CR2-NPC139397 | -70.48 | -63.87 | -6.61 | 0.00 |

Table 2.11: Molecular Docking results for Depression and Anxiety

| Hub Gene & Natural Component | Energy | VDW | HBond | Elec |
|------------------------------|--------|--------|--------|------|
| CDH23-NPC67043 | -53.12 | -42.68 | -10.44 | 0.00 |

Table 2.12: Molecular Docking results for Tinnitus

| Hub Gene & Natural Component | Energy | VDW | HBond | Elec |
|------------------------------|---------|--------|--------|-------|
| CACNA1D-NPC36836 | -109.75 | -94.82 | -16.40 | 1.47 |
| TERT-NPC230098 | -97.62 | -85.53 | -12.09 | 0.00 |
| RET-NPC56271 | -94.65 | -85.16 | -9.49 | 0.00 |
| TERT-NPC474324 | -92.79 | -82.29 | -10.50 | 0.00 |
| CACNA1D-NPC63370 | -92.73 | -85.36 | -8.25 | 0.88 |
| TERT-NPC319549 | -91.83 | -71.07 | -20.76 | 0.00 |
| TERT-NPC33256 | -85.58 | -69.20 | -16.38 | 0.00 |
| TERT-NPC237044 | -85.10 | -75.60 | -9.50 | 0.00 |
| NF1-NPC117032 | -83.94 | -80.65 | -2.74 | -0.55 |
| TERT-NPC474325 | -83.26 | -62.39 | -20.87 | 0.00 |
| TERT-NPC298186 | -83.22 | -72.58 | -10.94 | 0.30 |
| TERT-NPC304675 | -81.06 | -65.27 | -15.79 | 0.00 |
| TERT-NPC301189 | -80.12 | -69.50 | -10.61 | 0.00 |
| CACNA1D-NPC190945 | -79.50 | -61.18 | -18.10 | -0.22 |
| TERT-NPC165797 | -78.17 | -69.69 | -8.47 | 0.00 |

Table 2.13: Molecular Docking results for Anorexia (Loss of Appetite)

| Hub Gene & Natural Component | Energy | VDW | HBond | Elec |
|------------------------------|---------|--------|--------|------|
| MEN1-NPC474814 | -114.41 | -96.24 | -18.17 | 0.00 |
| MEN1-NPC199737 | -95.91 | -61.68 | -34.24 | 0.00 |
| MEN1-NPC471778 | -94.89 | -77.62 | -17.27 | 0.00 |
| MEN1-NPC112336 | -90.02 | -69.56 | -20.45 | 0.00 |
| MEN1-NPC301702 | -82.55 | -47.69 | -34.86 | 0.00 |

| | | | | |
|-----------------|--------|--------|--------|-------|
| MEN1-NPC181526 | -74.30 | -46.46 | -27.84 | 0.00 |
| SMAD4-NPC189301 | -73.85 | -53.31 | -21.34 | 0.80 |
| MEN1-NPC323798 | -73.31 | -50.23 | -23.08 | 0.00 |
| SMAD4-NPC176164 | -73.26 | -47.26 | -26.00 | 0.00 |
| SMAD4-NPC226027 | -70.85 | -56.50 | -13.59 | -0.75 |
| SMAD4-NPC174246 | -69.07 | -55.26 | -15.09 | 1.28 |
| SMAD4-NPC183845 | -67.90 | -31.13 | -36.77 | 0.00 |
| MEN1-NPC316574 | -67.29 | -39.65 | -27.64 | 0.00 |
| SMAD4-NPC112890 | -65.48 | -31.35 | -32.29 | -1.84 |
| SMAD4-NPC140872 | -64.27 | -46.55 | -17.72 | 0.00 |
| SMAD4-NPC118459 | -59.74 | -38.78 | -20.96 | 0.00 |
| SMAD4-NPC245027 | -55.96 | -44.00 | -10.50 | -1.46 |
| SMAD4-NPC162620 | -54.80 | -47.63 | -7.17 | 0.00 |

APPENDIX C**MANUSCRIPT****IDENTIFICATION OF NATURAL REMEDIES FOR LONG COVID BASED ON
HUB GENE BIOMARKERS AND REPURPOSED DRUGS**

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ABSTRACT: Long COVID is a phenomenon in which individuals experience persistent symptoms after recovering from COVID-19. The symptoms are discovered to be unique for every individual and can affect multiple organs systems in the body. This study aims to identify effective natural remedies for long COVID by analyzing hub genes associated with the symptoms of the condition and evaluating the repurposed drugs catered and used for treating the symptoms of long COVID. The most common and prevalent symptoms of long COVID were identified; Fatigue, Shortness of Breath, Loss of Smell, Headache, Brain Fog, Chest Pain, Insomnia, Heart Palpitations, Dizziness, Joint Pain, Depression, Anxiety, Tinnitus, Loss of Appetite. Hub genes for each of the symptoms provided an insight on the key biological pathways of the symptom. Repurposed drugs identified, provided the template to identify the natural compounds with similar structure as a potential therapeutic drug. The natural compounds were retrieved using fingerprint search of the repurposed drugs from the NPASS Database. The findings of this study suggest several natural remedies for each symptom based on the molecular docking of the hub gene and natural compound using iGEMDOCK. The identified natural remedies may hold promise in treating long COVID, but further research is required to explore the efficacy and effectiveness of the proposed natural compounds. The results of the study pose important implications for the development of effective treatments for long COVID.

Keywords: Long COVID, Natural Compounds, Hub Genes, Therapeutic Drug

INTRODUCTION

The Coronavirus disease 2019, which made its first appearance in 2019, is still active and spreading in many forms throughout the world. This disease has caused a great impact on the lives of humans as millions of people have fallen to the virus. Contrary to the previous years, the fight against this disease has decreased as COVID cases are being thought of and treated similar to common flu cases. This is plausible due to the improvements and advancement in treatment and prevention of COVID-19. The Severe Acute Respiratory Syndrome Coronavirus 2 (SAR-CoV-2) virus not only causes COVID-19 by infecting individuals but has found another way to instigate problems to people. This new problem goes by many names but is commonly referred to as “Long COVID” (Raveendran et al., 2021).

In the early days (2020), several clinical studies have identified that some symptoms of COVID-19 were found to remain in patients despite their recovery from the disease. This condition was initially referred as long COVID, post-acute sequelae of COVID (PASC), or post-acute COVID-19 syndrome (PACS) as it describes the persistence of the prolonged symptoms that presents after the acute SARS-CoV-2 infection (Deer et al., 2021). PCR tests conducted for the illness results negative, indicating that the syndrome is the delay between the microbial recovery and clinical recovery from the acute infection phase (Garg et al., 2021). The term “Long COVID” was generally accepted to describe the illness where long COVID is defined as the wide range of symptoms that persists for over weeks and months experienced by COVID-19 survivors.

Many reviews have documented the wide spectrum of persistent symptoms experienced by the patients (Akbarialiabad et al., 2021, Davis et al., 2023, Lopez-Leon et al., 2021, Crook et al., 2021). The common symptoms diagnosed under long COVID include fatigue, shortness of breath, heart palpitations, headache, joint pain, insomnia, loss of smell, chest pain and more (Sudre et al., 2021). The manifestation of the symptoms has been identified to be correlated to not a singular organ system but to multiple organ systems, primarily the respiratory, neurological, cardiovascular and musculoskeletal systems.

Classification of the condition poses a great challenge due to the wide range of symptoms and the symptom pattern that varies from individuals. Hence, two distinct categories were established for the symptoms: Post-acute COVID, where symptoms persist from more than three weeks but less than twelve weeks, and Chronic COVID, where the symptoms persist more than twelve weeks (Raveendran et al., 2021).

Treatment for long COVID remains undefined as no singular validated treatment strategy or drugs is available, providing coverage for all symptoms. Different types of symptoms are connected to different types of biological pathways (Davis et al., 2023). Thus, each treatment strategy for each symptom would be unique. A multidisciplinary approach involving assessment, symptomatic treatments, underlying problem treatment, physical therapy, occupational therapy, and psychological support is required to identify a suitable treatment plan for long COVID patients. Various synthetic drugs and repurposed drugs are being tested in clinical settings and are proposed as treatment for long COVID.

Similarly, natural products or compounds as natural remedies for long COVID is a field of research that could provide promising therapeutic drugs. Multiple studies have shown the use of natural remedies for COVID-19 such as herbal remedies and vitamin supplements. But there is limited research on the effectiveness of natural remedies for long COVID symptoms. Therefore, the study aims to explore the potential ability of the natural products and compounds as natural remedies designed for long COVID symptoms.

METHODOLOGY

The Identification of natural remedies for long COVID based on hub gene biomarkers and repurposed drugs involved various bioinformatics tools and databases; PubChem, NCBI, NPASS, Open Babel, AlphaFold database, UniProt, iGEMDOCK. The overall flow of the methodology is simplified and represented in a flowchart in Figure 1.1.

Data Retrieval

The common symptoms of Long COVID were obtained based on publications that reported for long COVID symptoms- systematic reviews, meta-analyses, and case study publications retrieved from public databases such as PubMed, LitCovid database and more. Over 250 symptoms were reported in journals and databases in relation to long COVID. However, only the most common and frequently appearing symptoms were subjected to the study.

A total of 17 symptoms were determined to be the most recurring symptoms experienced by patients in the case studies and review articles. Fatigue, shortness of breath, loss of smell, muscle pain, headache, brain fog, chest pain, difficulty sleeping, heart palpitations, dizziness, joint pain, depression, anxiety, tinnitus, diarrhea, loss of appetite and skin rash are the 17 identified symptoms. The hub gene list and FDA-approved drugs (for each hub gene) was created for each symptom, containing the top hub genes associated with each gene. Hub genes for each symptom serves as a biomarker that is thought to play a critical role in the regulation of the various biological processes of the symptom. Drugs targeting the hub genes affect the biological pathways of the gene, used in treating specific diseases and conditions. The hub genes that are associated with the 17 symptoms and the repurposed drugs for the hub genes were obtained from a study conducted by Sanisha Das on long COVID G Protein-Coupled Receptors (Das & Kumar, 2022).

Identification of Natural Compounds

The presence of natural remedies for long COVID was to be determined by analyzing the FDA approved repurposed drugs. This approach was utilized as over 50% of drugs that have been approved are either derived from natural products or are natural products themselves (Atanasov et al., 2021). Therefore, by using the Natural Product Activity and Species Source Database (NPASS), the natural compounds or their derivatives can be found from the repurposed drugs. For each symptom, there are a total of 10 hub genes and each gene has its respective FDA-approved repurposed drug. The FDA-approved drugs for each gene were taken individually and searched in the PubChem database to identify their canonical SMILES, which were used to identify the natural compounds using

the NPASS database. The natural compounds were retrieved using fingerprint search of the repurposed drugs from the NPASS database. The queries were searched by structure and the fingerprint type was set as PubChem-881 fp and with a threshold of ≥ 0.80 . These settings were used to at least identify one natural compound from the FDA drug. For the results with more than 10 compounds, the top 10 natural compounds that were identified will be taken into consideration. Hub genes and drugs that result in no natural compounds or natural products will be redundant. The remainder of natural compounds will advance to the next stage.

Hub Genes Data Collection (3D Structure)

The 3D structure of the hub gene (protein) provides an insight to their functions in multiple biological pathways. Thus, the UniProt database was used to retrieve and download the 3D structure of the filtered list of hub genes based on the presence of natural compounds. Each hub gene was searched in the database individually and the following filters were applied in the search query; Organism: Homo sapiens and Status: Reviewed (Swiss-Prot). The AlphaFold structure was downloaded to obtain the 3D structure of the hub genes. As not all AlphaFold 3D structures are available, for hub genes that do not have the AlphaFold structure, the SMR structure was obtained.

2D Structure of Natural Compounds

The resulting prioritized natural compounds obtained from the structure of the FDA-approved drugs were recorded from the NPASS database and further studied its structure. The PubChem database, a public database containing information on millions of chemical compounds, was utilized to extract the 2D structures of each natural compound. The secondary structures of each natural compound were downloaded in the SDF (Structure Data File) format from the PubChem database which would allow the analysis of the molecular structure of each compound in detail and to determine its properties.

Conversion of 2D structures to 3D structures (Natural Compounds)

The 3D structure of the natural compounds provides information on the physicochemical properties of the compound, such as solubility and toxicity, and to understand the interaction of the compound with target proteins. The 2D structures of the natural compounds were converted from a 2D SDF file format to 3D PDB, Mol or Mol2 formats. The OpenBabel cheminformatics online conversion tool (<http://www.cheminfo.org/Chemistry/Cheminformatics/FormatConverter/index.html>) was utilized for this process. The conversion process used the SDF file and some utilized the canonical SMILES for the 2D structures of the natural compounds to produce the 3D structures in either one of the applicable 3D structure formats; PDB, Mol and Mol2. The settings used in the conversion tools were set to convert the 2D structure into 3D structure and the rest were set to default.

Molecular Docking of the Hub Genes and Natural Compounds

Molecular docking is an in-silico structure-based method well established in the field of drug discovery (Pinzi & Rastelli, 2019). The molecular docking process utilized the iGEMDOCK docking tool to identify the binding affinity of each of the hub genes with their respective natural compounds. This tool is a Generic Evolutionary Method for molecular DOCKing. Thus, GEMDOCK. It is a software program that is commonly used for computing and analyzing the ligand conformation and orientation relative to the active site of a particular target protein. The tool was downloaded from the official iGEMDOCK webpage (<http://gemdock.life.nctu.edu.tw/dock/igemdock.php>). The hub genes were uploaded into the tool as the binding site and all the natural compounds for a specific gene were uploaded at the same time under compounds. Multiple compounds were docked against a single binding site at the same time. The results produced were saved in a text file format where the binding energy, VDW, Hbond and Elec are recorded. The natural compounds were then analyzed to identify the best natural compound to be used as a therapeutic natural remedy/drug for symptoms of long COVID based on the binding affinity of the complexes.

RESULTS

All the seventeen symptoms with ten hub genes and their corresponding FDA approved drug were analyzed and fourteen among the seventeen symptoms presented with at least one natural compound. The remaining three symptoms, muscle pain, diarrhea and skin rash symptoms resulted in no natural compounds. This was because of either the absence of canonical SMILES of the FDA approved drugs or the lack of identical or similar structured natural compounds for the particular drug.

For each symptom, the repurposed drugs for the different hub genes had provided a series of natural compounds but among the ten hub genes, not all genes resulted with a natural compound. Fatigue presented with four hub genes; NF1, SMAD4, RET and ERBB4 genes. Shortness of breath symptoms resulted in three genes; CHRNE, CHRND and CHRNA1 genes. Loss of smell with two genes, GNRH1 and TAC3. Headaches presented with five genes where some of the genes were of those from fatigue; ESR1, NF1, SMAD4, RET and TERT. Brain fog includes KCNT1, GABRA1 and CACNA1B genes. Chest pain resulted in NF1, SMAD4 and RET genes, which are frequently seen amongst different symptoms. Insomnia was found with four genes; NR1H4, ABCB4, ABCB11 and SLC12A3. Heart palpitations showed two genes called SCN5A and GATA4. Dizziness shares the SCN5A with heart palpitation as well as CACNA1G, RYR2, SCN1A, NF1 and RET genes. Joint pain resulted in CR2 and FAS genes. Depression and anxiety presented with the same gene, CDH23. Tinnitus with NF1, RET genes again and TERT and CACNA1D genes. Finally, loss of appetite (anorexia) resulted with SMAD4, MEN1 and PALB2 genes.

Among all genes, NF1, RET and SMAD4 were found to be associated with more than two symptoms. This shows that they contribute and play a major role in the biological pathway related to multiple symptoms of long COVID

Molecular docking conducted between the hub gene and natural compound provided the binding energy between the compounds. The binding energy of the complexes were tabulated, based on the symptoms, from the complex with the highest negative binding

energy to the lowest (Supplementary Tables 1.2 to 1.13). The natural compound with the highest negative binding energy resulted with greater binding affinity towards the hub gene and as a promising therapeutic agent for the symptom.

DISCUSSION

The study identified a total of 250 hub gene-natural compound complexes from the molecular docking process which shows the potential therapeutic candidates for the seventeen long COVID symptoms. However, only the top binding energy complexes are to be selected as potential candidates due to the difference in the binding affinity and interaction between the complexes. Thus, only a certain number of complexes for each symptom were studied and found to provide promising data.

Fatigue is the feeling of exhaustion and weakness causing a reduction in the ability to perform physical and mental activities. It is found in over 64% of long COVID patients and is associated with the neuro and cardiovascular systems (Joli et al., 2022, Castanares-Zapatero et al., 2022). The mechanism of fatigue caused by long COVID remains unknown but is speculated to be due to the inflammation in the associated organ systems. NF1, SMAD4, RET and ERBB4 were the hub gene biomarkers linked to fatigue. Among the four genes and the identified natural compounds, three complexes with the highest binding affinity showed favorable results (Table 1.1): RET-NPC56271 (-94.65), ERBB4-NPC56271 (-94.29) and NF1-NPC117032 (-83.94). The natural compound Gefitinib (NPC56271), was found to interact with both RET and ERBB4 genes whereas Dehydroevidiamine (NPC117032) is the natural compound for the NF1 gene. The docking results have provided significant evidence that Gefitinib and Dehydroevidiamine can be used as a drug for their respective genes to reduce the significance of the neurologic symptom, fatigue.

Dyspnoea or shortness of breath is a distress symptom that induces breathing discomforts and is usually associated with lung disease, neurodegenerative diseases and chronic heart failure (Hentsch et al., 2021). The damage the SARS-CoV-2 virus causes to

the respiratory system results in inflammation, scarring of lung tissues and damaged blood vessels leading to dyspnoea that is prolonged from six months to a year (Vijayakumar et al., 2022). The genes from the nicotinic acetylcholine receptor (nAChR) gene family, CHRNE, CHRND and CHRNA1, are the hub genes that play roles in the muscular and nerve cell signaling pathways. A total of five hub gene-natural compound complexes were determined for this symptom (Table 1.2). CHRNE-NPC108434 (-106.33), CHRNA1-NPC10908 (-104.70), CHRND-NPC11296 (-103.00), CHRNA1-NPC115284 (-102.98) and CHRND-NPC10871 (-101.86), where Lindoldhamine (NPC108434), Isotetrandrine (NPC10908), Daphnandrine (NPC11296), Fanchinin (NPC115284) and Tetrandrine (NPC10871) are the natural compounds. All five compounds are alkaloids, specifically, bisbenzylisoquinoline alkaloids, which are anti-cancer agents that possess anti-inflammatory, antiparasitic and antiviral properties (Atanasov et al., 2015). The anti-inflammatory property of these compounds makes it an excellent candidate and natural remedy for shortness of breath.

Loss of smell is also known as anosmia and is a symptom that is experienced by 40% of long COVID patients. It is a symptom that is not harmful to the body but is found to negatively affect the quality of life. Anosmia is considered as a neurological symptom where the dysfunction in the olfactory system in the form of inflammation caused by SARS-CoV-2 virus results in prolonged loss of smell (Davis et al., 2023, Castanares-Zapatero et al., 2022, Park et al., 2022). The TAC3 and GRNH1 genes were the hub gene biomarkers for anosmia but have shown no evidence of association with the olfactory system. Regardless, the study had identified the genes as components involved in the biological pathway of the symptom. Hence, molecular docking was conducted and resulted in two main complexes from the GRNH1 gene (Table 1.3); GRNH1-NPC69843 (Tunicyclin D) with -105.95 binding energy and GRNH1-NPC322594 (Deoxyuridine triphosphate) with -100.52 binding energy. Similar to the hub gene, the natural compounds showed no relationship towards the symptom. Thus, our study hypothesized an unknown property in the genes and natural compounds that enables it to be a potential natural remedy for anosmia.

With a prevalence of 13 to 74% and persists for over more than three months, headache is determined as one of the common long COVID symptoms (Membrilla et al., 2021). It is theorized that the damages as a result of the acute infection in different organs systems to be the underlying mechanism of the occurrence of headaches (Tana et al., 2022). The potential natural remedies for headaches revolve around the natural compounds associated with the five hub genes; ESR1, RET, SMAD4, NF1 and TERT. However, the top identified hub gene-natural compound complex involves only the TERT, ESR1 and RET genes. TERT-NPC230098 (unidentified gene based from PubChem and ChEMBL), ESR1-NPC136948 (Norselic Acid D) and RET-NPC56271 (Gefitinib) are the top 3 complexes with -97.62, -96.97 and -94.65 binding energy respectively (Table 1.4). As an unidentified compound, the TERT gene complex's properties remained a mystery whereas Norselic Acid D presented with antimicrobial activity that is not fully understood in relation to the cause of headache (Sheung et al., 2009). Gefitinib, on the other hand, revealed its ability to relieve headaches in erlotinib-induced liver injury (Nakatomi et al., 2011). This mechanism of the Gefitinib compound may provide an insight to combat the headache caused by long COVID.

Brain fog is a cognitive impairment that causes mental confusion, forgetfulness, concentration difficulties, intellectual functions and short-term memory loss. It was identified with a prevalence of 31 to 69% in long COVID patients (Nouraeinejad, 2022). Dysfunction in the brain is proposed to be the mechanism behind the symptom. The hub gene biomarkers (KCNT1, GABRA1 and CACNA1B) for the symptoms are intertwined with the neurological pathways that may provide an understanding of brain fog. But the potential natural remedy and therapeutic drug resulted from the CACNA1B gene (Table 1.5): CACNA1B-NPC198254 (-119.29) and CACNA1B-NPC473404 (-115.68). The NPC198254 is also referred to as Micropeptin B and NPC473404 as Anabaenopeptin F. Both natural compounds were derived from a form of cyanobacteria that is effective in reducing neuroinflammation (Kirk et al., 2021), providing probable cause to conduct

further studies on the association of the natural compounds and brain fog in long COVID patients.

Chest pain, a form of cardiac anomaly, is experienced by 58% of long COVID patients and is related to the respiratory and cardiovascular systems (Roca-Fernandez et al., 2022). The inflammation by the virus in the myocardium and the inflammation by the immune response in the lungs are suspected to be the causes of chest pain. The SMAD4, NF1 and RET gene, also known as the commonly recurring gene in multiple symptoms, are found to be linked to the biological pathways of chest pain. Similar to the results from fatigue, the natural compounds, Gefitinib and Dehydroevidiamine were identified as potential remedies as the NF1 and RET gene complex (RET-NPC56271 (-94.65) and NF1-NPC117032 (-83.94)) posed to have higher binding energy compared to the SMAD4 gene complex (Table 1.6). Gefitinib, with its anti-inflammatory effects, is able to provide relief to chest pain symptoms present in non-small cell lung cancer and is assumed to play a role in improving cardiac conditions and symptoms (Natale, 2004). Dehydroevidiamine is speculated to have anti-inflammatory properties (Amaravathi et al., 2021) as well, providing evidence to study the compound to identify its properties and as a potential natural remedy.

Stress caused by the COVID-19 pandemic is one of the factors impacting the sleep cycle of an individual leading to insomnia, a sleep disorder. Environmental stressors and persistent inflammatory responses have been associated with insomnia in long COVID patients. The dysregulation of neurotransmitters in the brainstem caused by the virus is responsible for the human body sleep cycle (Yong, 2021). This leads to a broad range of neurological disorders including insomnia. Interestingly, the four hub genes for insomnia have been found to be primarily associated with the renal system/pathways. However, the implications of the renal system towards insomnia enables us to hypothesize the hub genes, ABCB4, ABCB11, NR1H4 and SLC12A3 to affect the renal system in a manner which causes insomnia to long COVID patients. A singular natural compound interrelated with two hub genes was identified as the potential natural remedy/therapeutic drug (Table 1.7).

ABCB11-NPC100366 (-107.98) and NR1H4-NPC100366 (-97.79) are the two complexes associated with the same natural compound, Ethyl(4R,20S,24R)-Epoxy-4,25,28-Trihydroxy-3,4-Secodammar-3-Oate. Isolated from the stem bark of the *dysoxylum binectiferum*, the natural compound is assumed to possess cytotoxic and anti-inflammatory activity (Huijiao et al., 2014). The link between the natural compound and the hub gene and the symptoms is lacking. Thus, additional studies are required to understand the implications of the hub genes and the natural remedies proposed for long COVID insomnia.

Heart palpitation is the fluttering and pounding feelings in the chest that is caused by an irregular heartbeat. It not only is associated with the neuro-cardiovascular system and is found in 20% of long COVID patients (DePace & DePace, 2022). Myocardial injuries and inflammations (due to autonomic dysfunctions) result in the persistent cardiac symptoms including an abnormal heartbeat. The SCN5A gene and GATA4 genes were found to be linked to the heart and have a main role in the biological pathway related to heart palpitations. The top two hub gene-natural compound complexes from molecular docking with more than -100 energy were identified to have high binding energies towards the gene (Table 1.8). Both complexes are from the GATA4 gene; GATA4-NPC101636 (Apigenin 7-O-Alpha-L-3-O-Acetyl Rhamnopyranosyl-(1->6)-Beta-D-Glucopyranoside) and GATA4-NPC100818 (Asphodelin A-4'-O-beta-glucoside). No link was identified for the

Apigenin 7-O-Alpha-L-3-O-Acetyl Rhamnopyranosyl-(1->6)-Beta-D-Glucopyranoside compound in relation to the GATA4 gene and heart palpitations. However, asphodelin A-4'-O-beta-glucoside expresses moderate antimicrobial activity against certain species of bacteria and fungi (El-Seedi, 2007). This allows the speculation of the potential of the natural compound to reduce and provide relief for the inflammation of the heart caused by SARS-CoV-2 virus.

Dizziness is one of the common long COVID symptoms that causes disturbed impaired spatial orientation with a distorted sense of motion. It was assumed to be only the

manifestation of neurological elements but factors such as inner ear problems, low blood sugar, low blood pressure, side effects of medications and other nonspecific common neurological symptoms were found to cause dizziness as well (Korres et al., 2022). The RYR2, SCN1B, SCN5A, NF1, RET and CACNA1G genes were found to be associated with the biological pathway related to the symptom. Molecular docking resulted in the RET-NPC56271 complex and RYR2-NPC122235 with the top two highest binding energies (Table 1.9). The RET gene natural compound, Gefitinib, showed evidence of association with dizziness where the use of the compound for non-small cell lung cancer alleviates a number of symptoms including dizziness (Z. Gao et al., 2012). The RYR2 gene's natural compound, Linalyl anthranilate, is commonly found in plants that exhibit antimicrobial activities (S. Yang et al., 2021). This compound also induces oxidative stress that is likely to be related to dizziness (S. Yang et al., 2021). However, the study was conducted on bacterial cells and not animal cells. Thus, the effects of Linalyl anthranilate must be extensively studied to understand the potential association between dizziness and Linalyl anthranilate.

A symptom that is more prevalent in females than males and is known to affect the joints in the body is the joint pain long COVID symptom. The pathophysiology of the symptoms is considered to be either due to the excessive release of cytokines and tumor necrosis or tissue inflammation causing rheumatic/musculoskeletal symptoms. In addition, as a hub gene biomarker for joint pain, the CR2 and FAS genes were also the causative agent of an autoimmune disorder (systemic lupus erythematosus (SLE)) which presents joint pain as a common symptom. The natural compounds identified via molecular docking (Table 1.9) were FAS-NPC115624 (4'-Demethyl Deoxypodophyllotoxin beta-D-glucopyranoside) and FAS-NPC116759 (4-Demethyl-Epipodophyllotoxin-7'-O-Beta-D-Glucopyranoside) where both natural components are identified to be isolated from the plant species, *Podophyllum hexandrum*. The natural compounds extracted from *Podophyllum hexandrum* the plant species showed no relation to the symptom as they exhibit anti-cancer properties (Zilla et al., 2014). Though it remains curious on how a compound associated with the cancer cell pathways can be determined as a potential natural

remedy to joint pain. Thus, further investigation of the natural compounds is required to reveal its hidden properties.

The long COVID psychological symptoms are anxiety and depression that are hypothesized to appear due to the impact of the COVID-19 virus on the central nervous system (CNS). External environmental stressors too, influence the severity and duration of both mental health disorders. The CDH23 gene (cadherin-related 23 gene) belonging to the cadherin superfamily, was identified as the single gene associated with both anxiety and depression. Long-term conditions such as hearing impairment and balance disorders are linked to the gene where they cause psychological issues including anxiety and depression. The natural compound, (2S)-2-(methylazaniumyl)-3-phenylpropanoate (NPC67043), and CD23 gene interaction resulted in the binding affinity of -53.12 (Table 1.10). The lack of another complex to make a comparison makes the CDH23-NPC67043 to be the potential therapeutic candidate and natural remedy targeted for both long COVID symptoms.

The condition where a person is hearing a buzzing or a ringing in the ears, phantom perception, without an external source creating the sound is referred to as tinnitus. Tinnitus is a long COVID symptom where its prevalence among the general population ranges between 16 to 26% (Degen et al., 2022). Research on the pathophysiology of tinnitus resulted in two hypothetical mechanisms; hearing loss and the abnormal neural activity caused by inflammation induced by the virus. The previously discussed long COVID symptoms, depression and anxiety are emotional distress that potentially increases the duration of tinnitus in patients. The hub genes of tinnitus (CACNA1D, NF1, RET and TERT genes) presented with no direct link towards the symptom itself but showed an association to hearing loss, a condition that is a closely related symptom to tinnitus. The CACNA1D-NPC36836 complex and TERT-NPC230098 complex are the top two complexes proposed as prospective therapeutic targets candidates for tinnitus (Table 1.12). NPC36836 is the calcium channel blocker called Nicardipine, usually employed in vascular disorders and as an anti-inflammatory agent as it is found to be more selective for the blood vessels in the nervous and cardiac systems (B. Huang et al., 2014). NPC230098, as

examined in the headache symptom, is an unknown natural compound with unknown properties in relation to its hub gene, TERT. As both natural compounds provide a suitable link to tinnitus, further examinations are needed to investigate the properties and therapeutic benefits of both natural compounds as they are determined viable targets for managing tinnitus.

Anorexia is an eating disorder, also known as, loss of appetite has an overall negative impact in life by causing malnutrition and sudden weight-loss. It is a long COVID symptom where the SARS-CoV-2 virus invades the gastrointestinal system. This causes several complications including the increased production of leptin, the hormone that provides satiety sensations resulting in the loss of appetite (Van Der Voort et al., 2020). Furthermore, pandemic-induced fear and stress also influences the eating habits of an individual. The hub gene biomarkers (SMAD4 and MEN1) for anorexia were found to have no direct relationship with the symptom. A faint and indirect link was identified for the MEN1 gene as this gene is known to cause hyperparathyroidism, which then induces hypoglycemia, leading to a range of symptoms including anorexia. The exact mechanism for both genes however remains unidentified. The MEN1-NPC474814 complex was initially analyzed as a natural remedy with the binding energy of -114.41 (Table 1.13). But the origin and the properties of the natural compound was unidentified. Thus, the second-best complex, MEN1-NPC199737 was investigated. The natural compound is known as Lavendustin B, a weak inhibitor and is majorly used as a negative control analogue among other compounds of the Lavendustin family. The relationship of both compounds to anorexia is not well understood as no evidence is present linking both together. Further research is required to analyze the properties of these natural components as both are found to be associated with the biological pathway of anorexia based on our study.

The limitations of the study include the unavailability of specific literature on the natural compounds as they prevented us from further analyzing the properties of the natural compounds as a natural remedy against long COVID. Furthermore, as a field of research that is not fully understood, the exact mechanism behind the underlying conditions of long

COVID are undetermined. Regardless, as per the objective of the study, the potential natural remedies and therapeutic drugs for the symptoms of long COVID have been identified and studied based on the interaction between the hub gene and natural compounds via molecular docking.

CONCLUSION

Long COVID has been determined as a major worldwide health concern but the research on the syndrome/condition is still lacking and the mechanism of the condition is currently unknown. The identification of natural compounds for long COVID as natural remedies based on hub gene biomarkers and repurposed drugs shows promising potential for improving the symptoms caused by the condition. The identified natural remedies for the fourteen common symptoms, provides an insight and a stable foundation of research on the undiscovered potential of the natural compounds and the unidentified mechanisms of long COVID symptoms. The results from this study also aids in the development of effective therapeutic drugs and interventions catering to each individual symptom based on the hub gene biomarkers and repurposed drugs associated.

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FIGURES

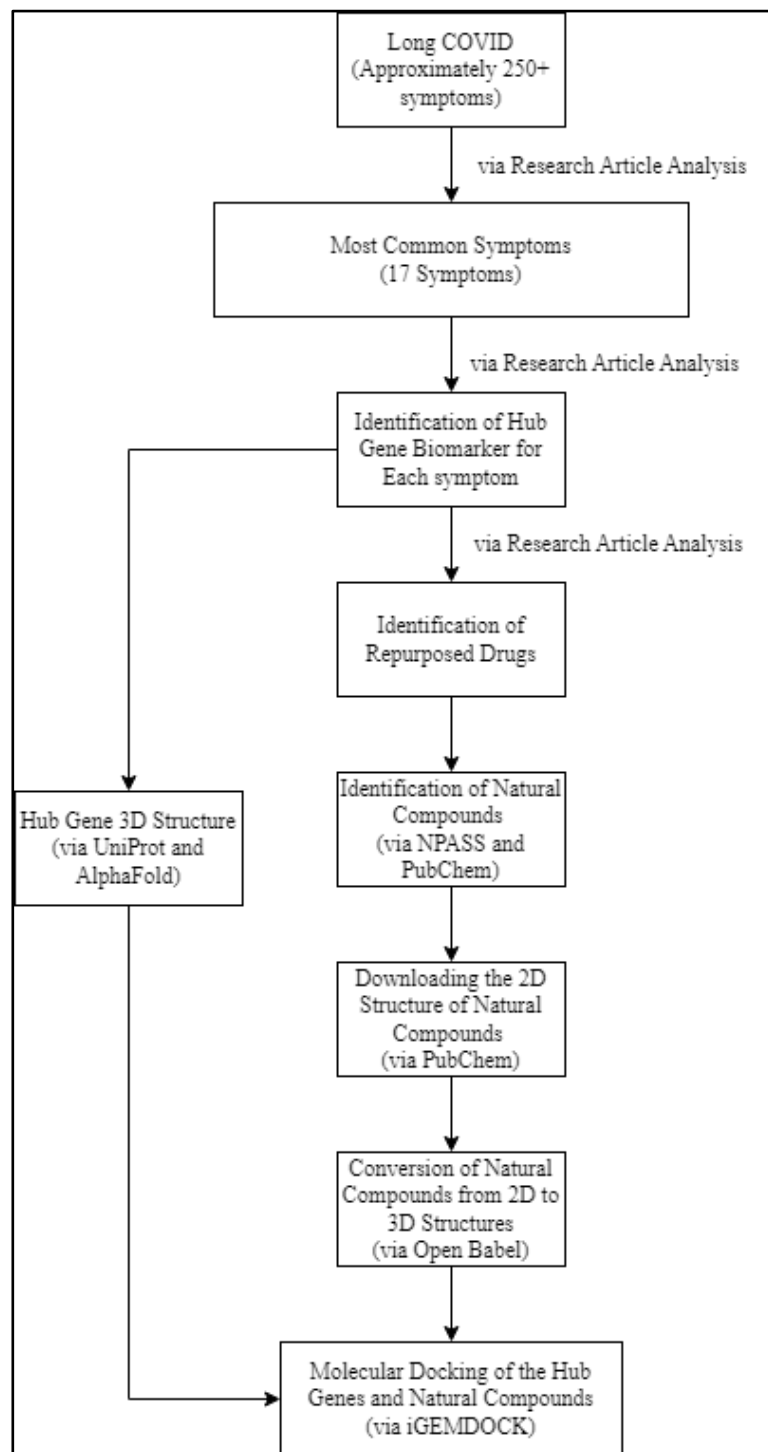


Figure 3.1: The overall workflow of the identification of natural remedies for the 17 common long COVID symptoms using the hub gene biomarkers and repurposed drugs

TABLES

Table 1.1: Molecular Docking results for Fatigue

| Hub Gene & Natural Component | Energy | VDW | HBond | Elec |
|------------------------------|--------|--------|--------|-------|
| RET-NPC56271 | -94.65 | -85.16 | -9.49 | 0.00 |
| ERBB4-NPC56271 | -94.29 | -85.58 | -8.71 | 0.00 |
| NF1-NPC117032 | -83.94 | -80.65 | -2.74 | -0.55 |
| SMAD4-NPC189301 | -73.85 | -53.31 | -21.34 | 0.80 |
| SMAD4-NPC176164 | -73.26 | -47.26 | -26.00 | 0.00 |
| SMAD4-NPC226027 | -70.85 | -56.50 | -13.59 | -0.75 |
| SMAD4-NPC174246 | -69.07 | -55.26 | -15.09 | 1.28 |
| SMAD4-NPC183845 | -67.90 | -31.13 | -36.77 | 0.00 |
| SMAD4-NPC112890 | -65.48 | -31.35 | -32.29 | -1.84 |
| SMAD4-NPC140872 | -64.27 | -46.55 | -17.72 | 0.00 |
| SMAD4-NPC118459 | -59.74 | -38.78 | -20.96 | 0.00 |
| SMAD4-NPC245027 | -55.96 | -44.00 | -10.50 | -1.46 |
| SMAD4-NPC162620 | -54.80 | -47.63 | -7.17 | 0.00 |

Table 1.2: Molecular Docking results for Shortness of Breath

| Hub Gene & Natural Component | Energy | VDW | HBond | Elec |
|------------------------------|---------|--------|--------|------|
| CHRNE-NPC108434 | -106.33 | -93.35 | -12.99 | 0.00 |
| CHRNA1-NPC10908 | -104.70 | -92.98 | -11.72 | 0.00 |
| CHNRD-NPC11296 | -103.00 | -83.34 | -19.66 | 0.00 |
| CHRNA1-NPC115284 | -102.98 | -94.69 | -8.29 | 0.00 |
| CHNRD-NPC10871 | -101.86 | -78.73 | -23.43 | 0.29 |
| CHRNA1-NPC108434 | -98.10 | -89.39 | -8.72 | 0.00 |
| CHNRD-NPC108434 | -97.35 | -78.84 | -18.50 | 0.00 |

| | | | | |
|------------------|--------|--------|--------|------|
| CHRNE-NPC10871 | -96.64 | -84.54 | -12.10 | 0.00 |
| CHRNb1-NPC116465 | -94.42 | -81.36 | -13.06 | 0.00 |
| CHNRD-NPC115284 | -94.09 | -93.60 | -0.49 | 0.00 |
| CHRNE-NPC11296 | -93.10 | -74.49 | -18.61 | 0.00 |
| CHRNb1-NPC10871 | -93.05 | -90.87 | -2.90 | 0.73 |
| CHRNb1-NPC11296 | -93.02 | -88.30 | -4.72 | 0.00 |
| CHNRD-NPC13916 | -92.25 | -74.21 | -18.04 | 0.00 |
| CHRNb1-NPC13916 | -92.22 | -81.32 | -10.89 | 0.00 |
| CHRNb1-NPC104196 | -91.30 | -87.10 | -4.20 | 0.00 |
| CHNRD-NPC104196 | -88.75 | -71.78 | -16.98 | 0.00 |
| CHRNE-NPC106295 | -86.51 | -84.75 | -1.76 | 0.00 |
| CHNRD-NPC106295 | -84.77 | -83.12 | -1.65 | 0.00 |
| CHRNE-NPC10908 | -84.70 | -74.68 | -10.02 | 0.00 |
| CHNRD-NPC116465 | -83.01 | -68.36 | -14.65 | 0.00 |
| CHRNE-NPC115284 | -82.07 | -79.57 | -2.50 | 0.00 |
| CHRNb1-NPC106295 | -81.97 | -81.97 | 0.00 | 0.00 |
| CHRNb1-NPC114124 | -81.78 | -65.48 | -16.30 | 0.00 |
| CHRNb1-NPC103379 | -81.66 | -60.92 | -20.74 | 0.00 |
| CHRNE-NPC13916 | -81.21 | -65.30 | -15.91 | 0.00 |
| CHNRD-NPC10908 | -79.41 | -79.41 | 0.00 | 0.00 |
| CHRNE-NPC116465 | -79.33 | -73.68 | -5.65 | 0.00 |
| CHRNE-NPC114124 | -78.43 | -60.67 | -17.77 | 0.00 |
| CHNRD-NPC103379 | -78.37 | -70.02 | -8.35 | 0.00 |
| CHRNE-NPC104196 | -78.19 | -73.19 | -5.00 | 0.00 |
| CHNRD-NPC114124 | -77.32 | -65.32 | -12.00 | 0.00 |
| CHNRD-NPC123323 | -76.01 | -73.71 | -2.30 | 0.00 |
| CHRNb1-NPC123323 | -74.20 | -66.93 | -7.27 | 0.00 |

| | | | | |
|-----------------|--------|--------|--------|------|
| CHRNE-NPC123323 | -73.07 | -73.07 | 0.00 | 0.00 |
| CHRNE-NPC103379 | -72.46 | -59.26 | -13.20 | 0.00 |

Table 1.3: Molecular Docking results for Loss of Smell

| Hub Gene & Natural Component | Energy | VDW | HBond | Elec |
|------------------------------|---------|--------|--------|-------|
| GNRH1-NPC69843 | -105.95 | -91.90 | -12.60 | -1.46 |
| GNRH1-NPC322594 | -100.52 | -82.39 | -18.12 | 0.00 |
| GNRH1-NPC328779 | -95.88 | -46.03 | -49.85 | 0.00 |
| GNRH1-NPC120887 | -92.41 | -70.14 | -22.27 | 0.00 |
| GNRH1-NPC17892 | -88.11 | -63.65 | -24.47 | 0.00 |
| GNRH1-NPC320249 | -82.83 | -55.55 | -27.28 | 0.00 |
| GNRH1-NPC324390 | -77.60 | -56.91 | -20.69 | 0.00 |
| GNRH1-NPC226769 | -73.68 | -44.94 | -28.74 | 0.00 |
| TAC3-NPC17760 | -70.87 | -64.87 | -6.00 | 0.00 |
| GNRH1-NPC474926 | -70.68 | -48.60 | -22.08 | 0.00 |
| GNRH1-NPC229249 | -70.02 | -70.02 | 0.00 | 0.00 |
| TAC3-NPC241086 | -69.42 | -59.93 | -9.49 | 0.00 |
| TAC3-NPC197239 | -68.37 | -61.77 | -6.61 | 0.00 |
| TAC3-NPC282087 | -67.22 | -51.77 | -15.45 | 0.00 |
| GNRH1-NPC106780 | -66.35 | -66.35 | 0.00 | 0.00 |
| TAC3-NPC259800 | -66.01 | -50.62 | -15.39 | 0.00 |
| GNRH1-4-NPC107135 | -58.84 | -36.30 | -22.54 | 0.00 |
| TAC3-NPC142638 | -58.49 | -38.96 | -19.54 | 0.00 |
| TAC3-NPC106551 | -57.96 | -45.84 | -12.78 | 0.66 |
| TAC3-NPC281686 | -57.96 | -44.77 | -13.83 | 0.64 |
| GNRH1-NPC43655 | -55.76 | -47.40 | -8.36 | 0.00 |
| TAC3-NPC188867 | -54.67 | -33.17 | -21.51 | 0.00 |

| | | | | |
|----------------|--------|--------|--------|------|
| TAC3-NPC239697 | -52.34 | -39.72 | -12.62 | 0.00 |
|----------------|--------|--------|--------|------|

Table 1.4: Molecular Docking results for Headache

| Hub Gene & Natural Component | Energy | VDW | HBond | Elec |
|------------------------------|--------|--------|--------|-------|
| TERT-NPC230098 | -97.62 | -85.53 | -12.09 | 0.00 |
| ESR1-NPC136948 | -96.97 | -78.32 | -18.65 | 0.00 |
| RET-NPC56271 | -94.65 | -85.16 | -9.49 | 0.00 |
| TERT-NPC474324 | -92.79 | -82.29 | -10.50 | 0.00 |
| ESR1-NPC123319 | -92.16 | -77.78 | -14.38 | 0.00 |
| TERT-NPC319549 | -91.83 | -71.07 | -20.76 | 0.00 |
| TERT-NPC33256 | -85.58 | -69.20 | -16.38 | 0.00 |
| ESR1-NPC1015 | -85.42 | -70.77 | -14.64 | 0.00 |
| TERT-NPC237044 | -85.10 | -75.60 | -9.50 | 0.00 |
| ESR1-NPC139397 | -84.00 | -80.50 | -3.50 | 0.00 |
| NF1-NPC117032 | -83.94 | -80.65 | -2.74 | -0.55 |
| TERT-NPC474325 | -83.26 | -62.39 | -20.87 | 0.00 |
| TERT-NPC298186 | -83.22 | -72.58 | -10.94 | 0.30 |
| ESR1-NPC255253 | -82.47 | -71.97 | -10.50 | 0.00 |
| TERT-NPC304675 | -81.06 | -65.27 | -15.79 | 0.00 |
| ESR1-NPC144258 | -80.38 | -74.17 | -6.21 | 0.00 |
| TERT-NPC301189 | -80.12 | -69.50 | -10.61 | 0.00 |
| TERT-NPC165797 | -78.17 | -69.69 | -8.47 | 0.00 |
| ESR1-NPC126993 | -76.15 | -73.83 | -2.33 | 0.00 |
| ESR1-NPC136548 | -74.01 | -61.01 | -13.00 | 0.00 |
| SMAD4-NPC189301 | -73.85 | -53.31 | -21.34 | 0.80 |
| SMAD4-NPC176164 | -73.26 | -47.26 | -26.00 | 0.00 |
| ESR1-NPC129913 | -73.01 | -64.51 | -8.50 | 0.00 |

| | | | | |
|-----------------|--------|--------|--------|-------|
| SMAD4-NPC226027 | -70.85 | -56.50 | -13.59 | -0.75 |
| SMAD4-NPC174246 | -69.07 | -55.26 | -15.09 | 1.28 |
| SMAD4-NPC183845 | -67.90 | -31.13 | -36.77 | 0.00 |
| SMAD4-NPC112890 | -65.48 | -31.35 | -32.29 | -1.84 |
| SMAD4-NPC140872 | -64.27 | -46.55 | -17.72 | 0.00 |
| SMAD4-NPC118459 | -59.74 | -38.78 | -20.96 | 0.00 |
| SMAD4-NPC245027 | -55.96 | -44.00 | -10.50 | -1.46 |
| SMAD4-NPC162620 | -54.80 | -47.63 | -7.17 | 0.00 |

Table 1.5: Molecular Docking results for Brain Fog

| Hub Gene & Natural Component | Energy | VDW | HBond | Elec |
|------------------------------|---------|--------|--------|-------|
| CACNA1B-NPC198254 | -119.29 | -90.52 | -28.77 | 0.00 |
| CACNA1B-NPC473404 | -115.68 | -86.02 | -33.71 | 4.05 |
| CACNA1B-NPC153554 | -109.71 | -85.83 | -23.88 | 0.00 |
| CACNA1B-NPC240130 | -104.61 | -70.44 | -31.19 | -2.99 |
| CACNA1B-NPC274198 | -100.59 | -76.19 | -24.98 | 0.58 |
| KCNT1-NPC329708 | -85.81 | -77.31 | -8.50 | 0.00 |
| KCNT1-NPC193238 | -85.17 | -70.08 | -15.09 | 0.00 |
| KCNT1-NPC274291 | -84.64 | -70.82 | -13.82 | 0.00 |
| GABRA1-NPC317054 | -83.96 | -77.67 | -6.30 | 0.00 |
| KCNT1-NPC47059 | -80.67 | -73.67 | -7.00 | 0.00 |
| KCNT1-NPC203754 | -78.36 | -72.36 | -6.00 | 0.00 |
| KCNT1-NPC165349 | -76.72 | -72.59 | -4.13 | 0.00 |
| KCNT1-NPC264166 | -74.93 | -72.43 | -2.50 | 0.00 |
| KCNT1-NPC118832 | -70.83 | -62.29 | -8.54 | 0.00 |
| KCNT1-NPC150048 | -70.42 | -65.85 | -4.57 | 0.00 |
| KCNT1-NPC231986 | -69.20 | -65.70 | -3.50 | 0.00 |

Table 1.6: Molecular Docking results for Chest Pain

| Hub Gene & Natural Component | Energy | VDW | HBond | Elec |
|------------------------------|--------|--------|--------|-------|
| RET-NPC56271 | -94.65 | -85.16 | -9.49 | 0.00 |
| NF1-NPC117032 | -83.94 | -80.65 | -2.74 | -0.55 |
| SMAD4-NPC189301 | -73.85 | -53.31 | -21.34 | 0.80 |
| SMAD4-NPC176164 | -73.26 | -47.26 | -26.00 | 0.00 |
| SMAD4-NPC226027 | -70.85 | -56.50 | -13.59 | -0.75 |
| SMAD4-NPC174246 | -69.07 | -55.26 | -15.09 | 1.28 |
| SMAD4-NPC183845 | -67.90 | -31.13 | -36.77 | 0.00 |
| SMAD4-NPC112890 | -65.48 | -31.35 | -32.29 | -1.84 |
| SMAD4-NPC140872 | -64.27 | -46.55 | -17.72 | 0.00 |
| SMAD4-NPC118459 | -59.74 | -38.78 | -20.96 | 0.00 |
| SMAD4-NPC245027 | -55.96 | -44.00 | -10.50 | -1.46 |
| SMAD4-NPC162620 | -54.80 | -47.63 | -7.17 | 0.00 |

Table 1.7: Molecular Docking results for Insomnia

| Hub Gene & Natural Component | Energy | VDW | HBond | Elec |
|------------------------------|---------|--------|--------|------|
| ABCB11-NPC100366 | -107.98 | -85.27 | -22.72 | 0.00 |
| NR1H4-NPC100366 | -97.79 | -87.37 | -10.42 | 0.00 |
| ABCB4-NPC213206 | -96.30 | -79.94 | -16.35 | 0.00 |
| ABCB4-NPC187022 | -96.10 | -85.42 | -10.68 | 0.00 |
| ABCB4-NPC208890 | -94.01 | -79.52 | -14.49 | 0.00 |
| ABCB4-NPC169387 | -92.86 | -90.86 | -2.00 | 0.00 |
| ABCB4-NPC136860 | -88.28 | -79.85 | -8.43 | 0.00 |
| ABCB4-NPC222524 | -86.16 | -78.11 | -8.05 | 0.00 |
| SLC12A3-NPC321053 | -81.71 | -47.62 | -34.09 | 0.00 |

| | | | | |
|-----------------|--------|--------|-------|------|
| ABCB4-NPC188163 | -81.18 | -71.92 | -9.26 | 0.00 |
| ABCB4-NPC128019 | -71.50 | -61.79 | -9.71 | 0.00 |

Table 1.8: Molecular Docking results for Heart Palpitations

| Hub Gene & Natural Component | Energy | VDW | HBond | Elec |
|------------------------------|---------|--------|--------|------|
| GATA4-NPC101636 | -107.59 | -90.62 | -16.97 | 0.00 |
| GATA4-NPC100818 | -103.25 | -67.84 | -35.40 | 0.00 |
| GATA4-NPC100887 | -98.92 | -67.75 | -31.18 | 0.00 |
| GATA4-NPC10097 | -89.52 | -56.87 | -32.65 | 0.00 |
| GATA4-NPC100985 | -84.82 | -62.35 | -22.47 | 0.00 |
| SCN5A-NPC162417 | -83.58 | -73.08 | -10.50 | 0.00 |
| SCN5A-NPC64436 | -82.21 | -74.38 | -7.83 | 0.00 |
| SCN5A-NPC470971 | -81.67 | -71.48 | -10.19 | 0.00 |
| GATA4-NPC101366 | -79.21 | -54.46 | -24.75 | 0.00 |
| SCN5A-NPC322433 | -78.26 | -60.08 | -18.18 | 0.00 |
| SCN5A-NPC265100 | -77.86 | -70.08 | -7.78 | 0.00 |
| SCN5A-NPC136112 | -77.71 | -66.44 | -11.27 | 0.00 |
| GATA4-NPC101294 | -76.70 | -69.70 | -7.00 | 0.00 |
| SCN5A-NPC26285 | -76.27 | -73.59 | -2.68 | 0.00 |
| SCN5A-NPC242933 | -75.49 | -60.87 | -14.62 | 0.00 |
| GATA4-NPC10027 | -74.31 | -65.03 | -9.28 | 0.00 |
| SCN5A-NPC319645 | -72.42 | -64.05 | -8.37 | 0.00 |
| SCN5A-NPC226143 | -72.40 | -68.95 | -3.45 | 0.00 |
| GATA4-NPC100986 | -71.88 | -55.38 | -16.50 | 0.00 |
| SCN5A-NPC248462 | -71.41 | -58.66 | -12.75 | 0.00 |
| SCN5A-NPC70406 | -70.84 | -52.41 | -18.43 | 0.00 |
| SCN5A-NPC141739 | -70.80 | -65.80 | -5.00 | 0.00 |

| | | | | |
|-----------------|--------|--------|-------|------|
| SCN5A-NPC26524 | -68.43 | -63.12 | -5.30 | 0.00 |
| SCN5A-NPC173295 | -63.42 | -55.17 | -8.26 | 0.00 |
| SCN5A-NPC57051 | -63.00 | -60.62 | -2.38 | 0.00 |
| SCN5A-NPC42383 | -61.93 | -56.37 | -5.56 | 0.00 |
| SCN5A-NPC65408 | -61.57 | -55.44 | -6.13 | 0.00 |
| SCN5A-NPC97811 | -58.36 | -55.41 | -2.94 | 0.00 |
| SCN5A-NPC24777 | -57.83 | -50.23 | -7.60 | 0.00 |

Table 1.9: Molecular Docking results for Dizziness

| Hub Gene & Natural Component | Energy | VDW | HBond | Elec |
|------------------------------|--------|--------|--------|-------|
| RET-NPC56271 | -94.65 | -85.16 | -9.49 | 0.00 |
| RYR2-NPC122235 | -85.94 | -78.79 | -7.15 | 0.00 |
| NF1-NPC117032 | -83.94 | -80.65 | -2.74 | -0.55 |
| SCN5A-NPC162417 | -83.58 | -73.08 | -10.50 | 0.00 |
| SCN5A-NPC64436 | -82.21 | -74.38 | -7.83 | 0.00 |
| SCN5A-NPC470971 | -81.67 | -71.48 | -10.19 | 0.00 |
| SCN5A-NPC322433 | -78.26 | -60.08 | -18.18 | 0.00 |
| SCN5A-NPC265100 | -77.86 | -70.08 | -7.78 | 0.00 |
| SCN5A-NPC136112 | -77.71 | -66.44 | -11.27 | 0.00 |
| SCN5A-NPC26285 | -76.27 | -73.59 | -2.68 | 0.00 |
| SCN5A-NPC242933 | -75.49 | -60.87 | -14.62 | 0.00 |
| CACNA1G-NPC264400 | -74.95 | -58.77 | -16.18 | 0.00 |
| CACNA1G-NPC55529 | -73.98 | -62.68 | -11.30 | 0.00 |
| RYR2-NPC150323 | -73.75 | -47.76 | -26.00 | 0.00 |
| CACNA1G-NPC470926 | -73.59 | -71.09 | -2.50 | 0.00 |
| SCN5A-NPC319645 | -72.42 | -64.05 | -8.37 | 0.00 |
| SCN5A-NPC226143 | -72.40 | -68.95 | -3.45 | 0.00 |

| | | | | |
|-------------------|--------|--------|--------|------|
| CACNA1G-NPC256452 | -71.53 | -61.21 | -10.32 | 0.00 |
| SCN5A-NPC248462 | -71.41 | -58.66 | -12.75 | 0.00 |
| SCN5A-NPC70406 | -70.84 | -52.41 | -18.43 | 0.00 |
| SCN5A-NPC141739 | -70.80 | -65.80 | -5.00 | 0.00 |
| SCN5A-NPC26524 | -68.43 | -63.12 | -5.30 | 0.00 |
| RYR2-NPC319645 | -68.19 | -57.69 | -10.50 | 0.00 |
| CACNA1G-NPC71140 | -66.84 | -59.10 | -7.75 | 0.00 |
| SCN5A-NPC173295 | -63.42 | -55.17 | -8.26 | 0.00 |
| RYR2-NPC226794 | -63.12 | -47.87 | -15.24 | 0.00 |
| SCN5A-NPC57051 | -63.00 | -60.62 | -2.38 | 0.00 |
| SCN5A-NPC42383 | -61.93 | -56.37 | -5.56 | 0.00 |
| SCN1B-NPC264400 | -61.81 | -41.42 | -20.39 | 0.00 |
| SCN5A-NPC65408 | -61.57 | -55.44 | -6.13 | 0.00 |
| SCN5A-NPC97811 | -58.36 | -55.41 | -2.94 | 0.00 |
| SCN5A-NPC24777 | -57.83 | -50.23 | -7.60 | 0.00 |

Table 1.10: Molecular Docking results for Joint Pain

| Hub Gene & Natural Component | Energy | VDW | HBond | Elec |
|------------------------------|---------|--------|--------|------|
| FAS-NPC115624 | -115.52 | -84.27 | -31.25 | 0.00 |
| FAS-NPC116759 | -115.52 | -77.79 | -37.72 | 0.00 |
| FAS-NPC103197 | -110.38 | -97.35 | -13.03 | 0.00 |
| FAS-NPC152424 | -108.06 | -81.95 | -26.11 | 0.00 |
| FAS-NPC100465 | -103.29 | -71.46 | -31.83 | 0.00 |
| FAS-NPC119910 | -103.26 | -89.55 | -13.71 | 0.00 |
| FAS-NPC14294 | -102.40 | -73.18 | -29.22 | 0.00 |
| FAS-NPC150943 | -97.35 | -71.67 | -25.68 | 0.00 |
| FAS-NPC115281 | -95.30 | -76.80 | -18.51 | 0.00 |

| | | | | |
|---------------|--------|--------|--------|------|
| FAS-NPC163527 | -91.19 | -70.10 | -21.08 | 0.00 |
| CR2-NPC139397 | -70.48 | -63.87 | -6.61 | 0.00 |

Table 1.11: Molecular Docking results for Depression and Anxiety

| Hub Gene & Natural Component | Energy | VDW | HBond | Elec |
|------------------------------|--------|--------|--------|------|
| CDH23-NPC67043 | -53.12 | -42.68 | -10.44 | 0.00 |

Table 1.12: Molecular Docking results for Tinnitus

| Hub Gene & Natural Component | Energy | VDW | HBond | Elec |
|------------------------------|---------|--------|--------|-------|
| CACNA1D-NPC36836 | -109.75 | -94.82 | -16.40 | 1.47 |
| TERT-NPC230098 | -97.62 | -85.53 | -12.09 | 0.00 |
| RET-NPC56271 | -94.65 | -85.16 | -9.49 | 0.00 |
| TERT-NPC474324 | -92.79 | -82.29 | -10.50 | 0.00 |
| CACNA1D-NPC63370 | -92.73 | -85.36 | -8.25 | 0.88 |
| TERT-NPC319549 | -91.83 | -71.07 | -20.76 | 0.00 |
| TERT-NPC33256 | -85.58 | -69.20 | -16.38 | 0.00 |
| TERT-NPC237044 | -85.10 | -75.60 | -9.50 | 0.00 |
| NF1-NPC117032 | -83.94 | -80.65 | -2.74 | -0.55 |
| TERT-NPC474325 | -83.26 | -62.39 | -20.87 | 0.00 |
| TERT-NPC298186 | -83.22 | -72.58 | -10.94 | 0.30 |
| TERT-NPC304675 | -81.06 | -65.27 | -15.79 | 0.00 |
| TERT-NPC301189 | -80.12 | -69.50 | -10.61 | 0.00 |
| CACNA1D-NPC190945 | -79.50 | -61.18 | -18.10 | -0.22 |
| TERT-NPC165797 | -78.17 | -69.69 | -8.47 | 0.00 |

Table 1.13: Molecular Docking results for Anorexia (Loss of Appetite)

| Hub Gene & Natural Component | Energy | VDW | HBond | Elec |
|------------------------------|---------|--------|--------|------|
| MEN1-NPC474814 | -114.41 | -96.24 | -18.17 | 0.00 |

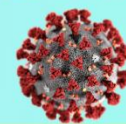
| | | | | |
|-----------------|--------|--------|--------|-------|
| MEN1-NPC199737 | -95.91 | -61.68 | -34.24 | 0.00 |
| MEN1-NPC471778 | -94.89 | -77.62 | -17.27 | 0.00 |
| MEN1-NPC112336 | -90.02 | -69.56 | -20.45 | 0.00 |
| MEN1-NPC301702 | -82.55 | -47.69 | -34.86 | 0.00 |
| MEN1-NPC181526 | -74.30 | -46.46 | -27.84 | 0.00 |
| SMAD4-NPC189301 | -73.85 | -53.31 | -21.34 | 0.80 |
| MEN1-NPC323798 | -73.31 | -50.23 | -23.08 | 0.00 |
| SMAD4-NPC176164 | -73.26 | -47.26 | -26.00 | 0.00 |
| SMAD4-NPC226027 | -70.85 | -56.50 | -13.59 | -0.75 |
| SMAD4-NPC174246 | -69.07 | -55.26 | -15.09 | 1.28 |
| SMAD4-NPC183845 | -67.90 | -31.13 | -36.77 | 0.00 |
| MEN1-NPC316574 | -67.29 | -39.65 | -27.64 | 0.00 |
| SMAD4-NPC112890 | -65.48 | -31.35 | -32.29 | -1.84 |
| SMAD4-NPC140872 | -64.27 | -46.55 | -17.72 | 0.00 |
| SMAD4-NPC118459 | -59.74 | -38.78 | -20.96 | 0.00 |
| SMAD4-NPC245027 | -55.96 | -44.00 | -10.50 | -1.46 |
| SMAD4-NPC162620 | -54.80 | -47.63 | -7.17 | 0.00 |

POSTER

IDENTIFICATION OF NATURAL REMEDIES FOR LONG COVID BASED ON HUB GENE BIOMARKERS AND REPURPOSED DRUGS

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ABSTRACT

Long COVID is a phenomenon in which individuals experience persistent symptoms after recovering from COVID-19. The symptoms are discovered to be unique for every individual and can affect multiple organs systems in the body. This study aims to identify effective natural remedies for long COVID by analyzing hub genes associated with the symptoms of the condition and evaluating the repurposed drugs catered and used for treating the symptoms of long COVID. The most common and prevalent symptoms of long COVID were identified; Fatigue, Shortness of Breath, Loss of Smell, Headache, Brain Fog, Chest Pain, Insomnia, Heart Palpitations, Dizziness, Joint Pain, Depression, Anxiety, Tinnitus, Loss of Appetite. Hub genes for each of the symptoms provided an insight on the key biological pathways of the symptom. Repurposed drugs identified, provided the template to identify the natural compounds with similar structure as a potential therapeutic drug. The natural compounds were retrieved using fingerprint search of the repurposed drugs from the NPASS Database. The findings of this study suggest several natural remedies for each symptom based on the molecular docking of the hub gene and natural compound using iGEMDOCK. The identified natural remedies may hold promise in treating long COVID, but further research is required to explore the efficacy and effectiveness of the proposed natural compounds. The results of the study pose important implications for the development of effective treatments for long COVID.

Keywords: Long COVID, Natural Compounds, Hub Genes, Therapeutic Drug

INTRODUCTION

- Long COVID results in an individual experiencing persistent symptoms after recovering from the acute COVID-19 (Raveendran et al., 2021).
- The symptoms are discovered to be unique for every individual and can affect multiple organs systems in the body (Castanares-Zapatero et al., 2022).
- Treatment for long COVID is a complex process; No treatment or drug is effectively able to cover the wide range of symptoms.
- Natural compounds that can be used remedies by studying the currently available repurposed drugs for long COVID

OBJECTIVES

General Objective

- To identify the natural compounds that can be used as therapeutic drug for long COVID based on the hub genes and repurposed drugs using bioinformatics tools.

Specific Objectives

- To study the hub gene biomarkers for the most common long COVID symptoms
- To identify the natural compounds associated with hub genes of each long COVID symptoms
- To examine the Molecular Docking between the hub gene biomarkers and natural compounds

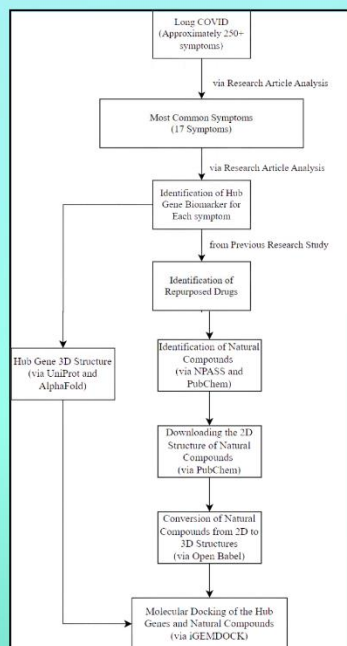
DISCUSSION

Natural Products Identified

Gefitinib
Dehydroevodiamine
Tuniclylin D
Norselic Acid D
Lindolthamine
Isotetradine
Micropeptin B
Anabaenoceptin F
Linalyl anthranilate
Asphodelin A-4'-O-
beta-glucoside
Nicardipine
Lavendustin B
25)-(2-methylazaniumyl)-
3-phenylpropanoate
4)-Demethyl Deoxypodophyllotoxin
beta-D-glucopyranoside

Most of the identified natural products presents with anti-microbial and anti-inflammatory properties which can be used as natural remedies for the treatment of symptoms of long COVID

METHODOLOGY



RESULTS

Common Long COVID Symptoms & Hub Gene Biomarkers

- Fatigue — NFI, SMAD4, RET and ERB84
- Shortness of breath — CHME, CHRD and CHRB1
- Loss of smell — GNRH1 and TAC3
- Headache — ESRI, NFI, SMAD4, RET and TERT
- Brain fog — KCNT1, GABRA1 and CANCA1B
- Chest pain — NFI, SMAD4 and RET
- Insomnia — NR1H4, ABCB4, ABCB1 and SLC12A5
- Heart palpitations — SCN5A and GATA4
- Dizziness — CANCA1G, RYR2, SCN1A, SCN5A, NFI and RET
- Joint pain — C92 and FAS
- Anxiety — CDH25
- Depression — CDH25
- Tinnitus — NFI, RET, TERT and CANCA1D
- Loss of appetite — SMAD4, MEN1 and PALB2

Top Molecular Docking

Results

- | | |
|---------------------|-----------------------------------------------------|
| Fatigue | REF-NPC56274 (-1.46), EMBB-NPC52714 (-1.09) |
| Shortness of breath | CHNE-NPC58841 (1.66, 35), CHRE-NPC14926 |
| Loss of smell | ONBN-NPC5843 (-105.95), ONBN-NPC52294 (-105.92) |
| Headache | TEST-NPC253099 (-97.62), ESH-NPC69448 (-96.97) |
| Bran fog | CACAN-NPC82524 (-129.72), CACAN-NPC47434 (-150.68) |
| Chest pain | REF-NPC56271 (-1.65), NF1-NPC107052 (1.85, 94) |
| Insomnia | ABCI-NPC100566 (-107.58), NR94-NPC100566 (-97.79) |
| Heart palpitations | GATA4-NPC10686 (-107.59), GATA4-NPC106818 (-108.25) |
| Dizziness | REF-NPC56271 (-1.65), RYR2-NPC122355 (-1.55, 92) |
| Joint pain | FAS-NPC56264 (-1.92, 63), TEST-NPC22379 (-185.94) |
| Anxiety | REF-NPC56271 (-1.65), NF1-NPC107052 (1.85, 94) |
| Depression | CD245-NPC5745 (133.12) |
| Tinnitus | CACAN-NPC56855 (-109.75), TEST-NPC220098 (-97.92) |
| Loss of appetite | REF-NPC47484 (-114.41), MEN1-NPC99757 (-95.91) |

CONCLUSION

The study shows promising potential for improving the symptoms caused by Long COVID. It provides a stable foundation of research on the undiscovered potential of the natural compounds and mechanisms of long COVID symptoms. It also aids in the development of effective therapeutic drugs catering to each symptom based on the hub gene biomarkers and repurposed drugs.

REFERENCES

- [illegible]