Biological factors associated with long COVID and comparative analysis of SARS-CoV-2 spike protein variants: a retrospective study in Thailand

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ABSTRACT

Background. Post-acute COVID-19 syndrome (long COVID) refers to the persistence of COVID-19 symptoms or exceptional symptoms following recovery. Even without conferring fatality, it represents a significant global public health burden. Despite many reports on long COVID, the prevalence and data on associated biological factors remain unclear and limited. This research aimed to determine the prevalence of long COVID during the two distinct epidemic periods in Thailand, due to the Delta and Omicron variants of SARS-CoV-2, and to investigate the biological factors associated with long COVID. In addition, the spike protein amino acid sequences of the Delta and Omicron variants were compared to determine the frequency of mutations and their potential biological implications.

Methods. A retrospective cross-sectional study was established to recruit confirmed COVID-19 participants at Maharat Nakhon Ratchasima Hospital who had recovered for at least three months and were infected between June 2021 and August 2022. The demographic data and long COVID experience were collected via telephone interview. The biological factors were analyzed through binary logistic regression. The datasets of the SARS-CoV-2 spike protein amino acid sequence of the Delta and Omicron variants in Thailand were retrieved from GIDSAID to determine mutation frequencies and to identify possible roles of the mutations based on published data.

Results. Data was collected from a total of 247 participants comprising 106 and 141 participants of the Delta and Omicron epidemic periods, respectively. Apart from the COVID-19 severity and health status, the baseline participant data of the two time periods were remarkably similar. The prevalence of long COVID observed in the Omicron period was higher than in the Delta period (74.5% *vs.* 66.0%). The biological factors associated with long COVID were epidemic variant, age, treatment with symptomatic medicines, and vaccination status. When the spike protein sequence data of the two variants were compared, it was observed that the Omicron variant exhibited a greater quantity of amino acid changes in its receptor-binding domain (RBD) and receptor-binding motif (RBM). The critical changes of the Omicron variant

within these regions had a significant function in enhancing virus transmissibility and host immune response resistance.

Conclusion. This study revealed informative data associated with long COVID in Thailand. More attention should be given to long COVID caused by unique virus variants and other biological factors to prepare a healthcare management strategy for COVID-19 patients after recovery.

INTRODUCTION

Since March 11, 2020, COVID-19 caused by SARS-CoV-2 has been classified as a newly emerging disease by the World Health Organization (*WHO, 2020*), a designation it has held for the last four years. Variants of SARS-CoV-2 have emerged continuously on a global scale. Certain variants have demonstrated effective adaptation in order to infect and spread among humans more easily. Since the beginning of COVID-19 era until the present, Thailand has been encountered with three unique epidemic SARS-CoV-2 variants: alpha (April to early June 2021), Delta (June to November 2021), and Omicron (December 2021 until now). The Department of Disease Control of the Ministry of Public Health in Thailand has documented a cumulative count of 4,758,116 confirmed cases and 34,547 fatalities attributed to COVID-19 as of January 28, 2024 resulting in an accumulated case fatality rate of 0.73% (*DDC, 2024*). Although recovery and a low mortality rate of 3–5% have been observed in the majority of individuals infected with COVID-19, a considerable proportion of patients have experienced long-term complications referred to as long COVID (*Batiha et al., 2022*). The prevalence of long COVID in Thailand is limited. However, few studies have suggested a prevalence range of 30–65% for long COVID (*Wangchalabovorn, Weerametachai & Leesri, 2022*; *Wongsermsin, Chinoraso & Yeekian, 2022*; *Tancharoensukjit, 2023*).

Long COVID or post-acute COVID conditions (PCC) are defined by the presence of diverse clinical symptoms that persist for several months shortly after an acute infection with the emerging respiratory virus named severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2). This long-term impact cannot be diagnosed using a specific biomarker or guideline. The disparity in time-frame for genetic variants of concern (VoC) of SARS-CoV-2 investigations contributed to significant differences in the prevalence data of long COVID. (*Montagne et al., 2020*; *Dennis et al., 2021*; *Moreno-Pérez et al., 2021*; *Sudre et al., 2021*). The ongoing COVID-19 pandemic results in the virus adapting through mutations in its genome, particularly in the gene of the spike glycoprotein. Since this protein plays a critical role in facilitating virus entry and is the primary target of neutralizing antibodies, it was hypothesized that this viral structure is responsible for the occurrence of long COVID (*Theoharides, 2022*). Amino acid changes in the spike glycoprotein of the virus allow it to escape the host immune system and to enhance its transmission and pathogenesis. Consequently, infection with distinct genetic variants of SARS-CoV-2 at different times

may have different effects on the occurrence of long COVID symptoms. Our understanding is that there is no published data that indicated a correlation between viral genetic markers and long COVID outcomes. This is because the majority of researchers believe that long COVID is associated with specific host genetic variants (*Batiha et al., 2022*).

Besides the genetic variants of SARS-CoV-2, long COVID might be connected to several biological host variables. The development of long COVID has been associated with several characteristics, including the severity of the illness, aging, gender, and rising levels of certain inflammatory markers (*Batiha et al., 2022*). Nevertheless, the data pertaining to the Asian population, particularly Thai, is extremely limited. This study aimed to identify biological risk factors associated with the development of long COVID in Thai, specifically focusing on the SARS-CoV-2 variants and host factors. Furthermore, the amino acid variations found in the spike protein of the SARS-CoV-2 Delta and Omicron variants were compared for their role in pathogenesis and transmission.

MATERIALS AND METHODS

Study design and participants

This cross-sectional study collected retrospective data from confirmed COVID-19 cases who acquired the SARS-CoV-2 infection during the Delta and Omicron epidemic periods, as reported by the Department of Medical Science of the Ministry of Public Health in Thailand (*DMSc, 2023*). Data were analyzed to determine the difference in prevalence and characteristics of long COVID symptoms between the two periods and to identify which biological factors were associated with long COVID. A minimum sample size of 206 positive COVD-19 cases was required, which was calculated using the n4Studies application (https://www.facebook.com/n4Studies/) based on an estimation infinite population proportion method by setting a prevalence difference proportion of 16% (*ONS, 2022*), and an alpha error of 0.05. Based on the medical records of the confirmed SARS-CoV-2 RT-qPCR positive cases at Maharat Nakhon Ratchasima Hospital from June to November 2021 (Delta period) and from December 2021 to the first week of August 2022 (Omicron period), all confirmed COVID-19 cases who were ≥ 18 years old, infected by SARS-CoV-2 for the first time within these periods, and had recovered from the infection for a minimum of three months were included as eligible participants. They were approached at random *via* telephone between March and April 2023 and requested to provide verbal consent for a single remote interview *via* telephone. To reduce the recall bias that influenced the validity assessment, we conducted a primary evaluation of the memorization participants by asking the time period of their infections. Fortunately, all participants were able to recall the disease period. Individuals who were pregnant during the COVID-19 infection, missed contact, had no recollection of long COVID experiences, passed away prior to interview, or lacked the ability to communicate in Thai were excluded from the study.

A peer-evaluated questionnaire was employed to record and analyze retrospective self-report data from each participant during the interview process. The self-reported questionnaire asked about basic demographic data and the experience of COVID-19

disease, including COVID-19 severity, the presence of long COVID symptoms, drug(s) for treatment, and vaccine status. According to the World Health Organization (*WHO, 2022a*), the definition of long COVID in this study is the persistence of existing infection symptoms or the emergence of new symptoms within three months of the initial SARS-CoV-2 infection. These symptoms must persist for a minimum of two months without any other explanation. In this investigation, the spectrum of long COVID disorders is categorized into seven systems, including cardiovascular: chest pain, fast-beating or heart palpitations; neurology: headaches, lightheadedness, pins-and-needles feelings, change in smell or taste, difficulty thinking or concentrating; dermatology: hair loss, rash; psychology: depression or anxiety, sleep problems; respiratory: difficulty breathing or shortness of breath, coughing; generalized symptoms of fatigue and myalgia; and others: diarrhea, stomach pain, constipation, *etc.*

The questionnaire for COVID-19 severity was evaluated by employing the specific criteria classification explanation, which classified the condition as mild, moderate, or severe according to the NIH categories (*NIH, 2022*). Participants were asked regarding the drug(s) they used for treatment in the five most used categories in Thailand, which include Andrographolide herbal medicine, Flavipiravir, Molnupiravir, symptomatic medicines (any medical therapy of a disease that only affects its symptoms, not the underlying cause, such as antipyretic medicine, cold medicine, cough medicine, *etc.*), and steroids and others.

This study was approved by the Ethical Committee of the Maharat Nakhon Ratchasima Hospital Institutional Review Board (MNRH IRB), Ministry of Public Health, Thailand (Certificate No. 139/2022). Participant identifiers were removed from the data and replaced with a participant code. The collected data will be destroyed immediately after publication.

Comparing genetic variations in the spike gene between the Delta and Omicron variants

The Global Initiative for Sharing Avian Influenza Data (GISAID) database (https://gisaid.org) is a free, open-access database that is available online. Its purpose is to facilitate the rapid exchange of data from key pathogens, such as influenza, SARS-CoV-2, respiratory syncytial virus (RSV), Mpox virus, and arboviruses such as chikungunya, dengue, and Zika. The database also offers genetic sequence and related clinical and epidemiological data associated with human viruses, as well as geographical and species-specific data associated with avian and other animal viruses, to further the informative data on the evolution and transmission of viruses during epidemics and pandemics.

In this study, GISAID database was accessed for datasets of Delta and Omicron SARS-CoV-2 variants collected in Thailand between July 1, 2021 and August 31, 2022. We collected only complete sequence datasets, which included 206 Delta variant sequences and 292 Omicron sequences. These genetic sequences are derived from the study objectives, who have different backgrounds in terms of gender, clinical status, and living areas in Thailand. Mutations causing amino acid changes within the spike protein of the two variants were examined to compare the percentage of accumulated mutation frequency. Subsequently, key mutations within each variant defined as having a difference in the percentage of accumulated mutation frequency over 50% were reviewed to determine

whether their roles associated with pathogenesis, transmission, and immune evasion of the SARS-CoV-2 variant might help to explain a long COVID outcome.

Statistical analysis

All data were analyzed using the Statistical Package for the Social Sciences (Version 25.0; SPSS Inc., Chicago, IL, USA). Calculation of frequency, percentage, mean and standard deviation was used to characterize the baseline demographic data, to compare the data of long COVID between the Delta and Omicron epidemic periods, and to compare the genetic variations in the spike protein of the SARS-CoV-2 variants. A binary logistic regression test with enter method was used to determine the association between biological factors and long COVID outcome. The strength of association was determined using an odds ratio at 95% confidence interval and a P-value < 0.05 was considered as significant association. Furthermore, the model summary or goodness of fit derived from a binary logistic regression analysis was elucidated by the values of -2Log Likelihood (-2ll) and Nagelkerke R^2 (Pseudo R^2).

RESULTS

Demographic characteristics

Between June 2021 and August 2022, during the Delta and Omicron epidemic periods in Thailand, a total of 490 confirmed COVID-19 cases at Maharat Nakhon Ratchasima Hospital were randomly asked for informed consent to participate in a telephone interview. A total of 251 subjects enrolled voluntarily; however, 247 were selected for analysis based on the defined inclusion and exclusion criteria. The number of participants who were infected by SARS-CoV-2 during the Delta and Omicron epidemic periods was 106 and 141, respectively. The flowchart of the subject enrollment process is shown in Fig. 1.

The baseline demographics and clinical characteristics of confirmed COVID-19 cases of the Delta and Omicron epidemic periods were compared are shown in Table 1. The mean age of the participants was 40.75 ± 14.20 years during Delta and 35.61 ± 11.70 years during Omicron. Female gender, age range of 18 to 29 years, obesity, and blood group O were the most prevalent characteristics of the participants in both epidemic periods. Nevertheless, during the Delta period, more than half of the cases ($n = 54$, or 50.9%) involved immunocompromised hosts who had at least one underlying disease. Hypertension, diabetes, hypercholesterolemia, asthma, and allergies were common underlying conditions. In contrast, infection with SARS-CoV-2 during the Omicron period primarily affected healthy hosts ($n = 125$, 88.7%). Almost one-third of the Delta cases ($n = 38$, 35.8%) exhibited symptoms classified as moderate to severe, whereas the Omicron cases predominantly presented mild symptoms. The antiviral drug Favipiravir and symptomatic drug usage was the key treatment utilized by the participants during both variant periods. The majority of the participants affected during the Delta period had not yet been immunized against COVID-19 ($n = 63$, 59.4%). Conversely, a significant numbers of participants affected during the Omicron period had received at least three vaccinations ($n = 80$, 56.7%). In respect to the vaccine type, the inactivated vaccine was predominant during the Delta period ($n = 24$, 55.8%), whereas the mRNA vaccine was
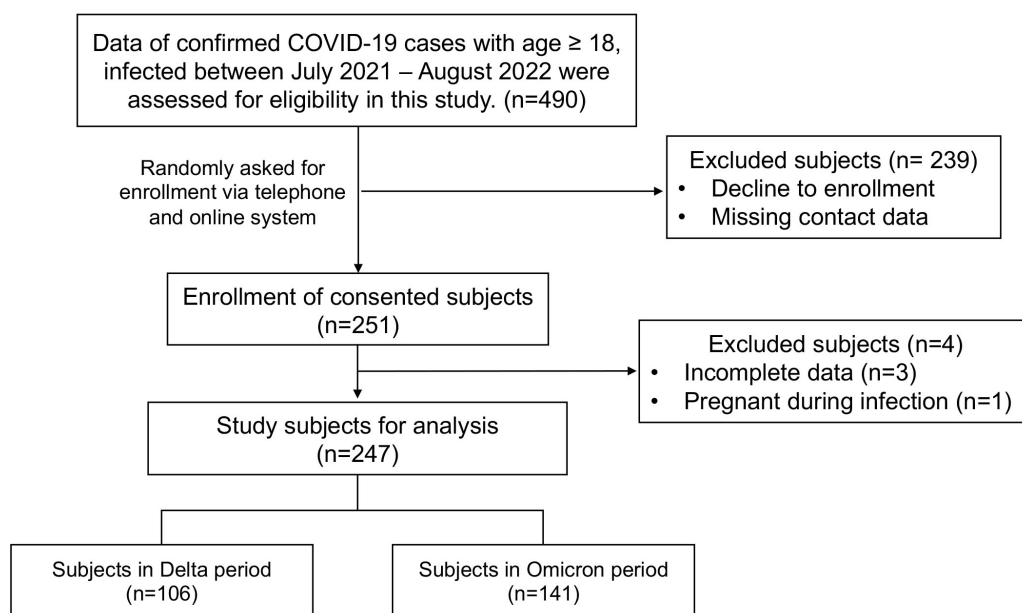
Figure 1 Flowchart of subject enrollment process and study subject groups.

Full-size ⬜ DOI: 10.7717/peerj.17898/fig-1

predominant during the Omicron period ($n = 75$, 56.4%). Significantly, throughout both epidemic periods, the majority of participants reported having sustained long COVID, with a number of symptoms having comparable prevalence rates between the Delta and Omicron periods.

The prevalence of self-reported long COVID symptoms between the Delta and Omicron epidemic periods was compared (Fig. 2). The seven most frequently reported symptoms in long-term COVID patients were as follows: cardiovascular, neurology, dermatology, psychology, respiratory, generalized symptoms of fatigue and myalgia, and others. Both participant groups reported all symptoms but the rate of observation for each symptom varied between the groups. Participants who were infected during the Delta period showed a predominant psychological disorder, including insomnia, anxiety, and depression. Conversely, abnormalities in the respiratory and neuron systems were prevalent during the Omicron period.

A comparison of the baseline demographics of the participants between non-long COVID cases and long COVID cases is presented in Table 2. Non-long COVID cases had a mean age of 40.86 ± 13.74 years and long COVID cases were 36.56 ± 12.59 years old. Comparative frequencies were observed for almost all analyzed variables in the two groups. Significantly, the prevalence of long COVID experience was greater during the Omicron period (60%) compared to the Delta period (40%); additionally, a greater proportion of participants with moderate to severe COVID-19 exhibited a higher rate of long COVID.

A binary logistic regression analysis was used to determine biological factors associated with long COVID. Variant epidemic period, age, treatment with symptomatic medicines, and vaccination status were found to be significant predictors of long COVID outcomes.

Table 1 Baseline demographics and clinical details of confirmed COVID-19 cases compared between the Delta and Omicron epidemic periods in Thailand ($n = 247$).

Variable	Delta ($n = 106$)	Omicron ($n = 141$)
Age, years	40.75 ± 14.20	35.61 ± 11.70
18–29	29 (27.4%)	49 (34.8%)
30–39	28 (26.4%)	47 (33.3%)
40–49	23 (21.7%)	24 (17.0%)
more than 50	26 (24.5%)	21 (14.9%)
Sex		
Male	36 (34.0%)	67 (47.5%)
Female	70 (66.0%)	74 (52.5%)
BMI (kg/m^2)	25.21 ± 4.60	24.08 ± 5.09
BMI weight status[a]		
Underweight (<18.5)	3 (2.8%)	15 (10.6%)
Normal (18.5–22.9)	30 (28.3%)	47 (33.3%)
Overweight (23–24.9)	27 (25.5%)	29 (20.6%)
Obese ($\geq$25)	46 (43.4%)	50 (35.5%)
Blood group		
A	12 (11.3%)	20 (14.2%)
B	27 (25.5%)	29 (20.6%)
AB	10 (9.4%)	13 (9.2%)
O	35 (33.0%)	47 (33.3%)
Unknown	22 (20.8%)	32 (22.7%)
Having underlying diseases		
Yes	54 (50.9%)	16 (11.3%)
No	52 (49.1%)	125 (88.7%)
No. of underlying diseases		
1	35 (64.8%)	16 (100.0%)
$\geq$2	19 (35.2%)	0 (0.0%)
Key underlying diseases		
Hypertension	29 (27.4%)	1 (0.7%)
Diabetes	29 (27.4%)	1 (0.7%)
Heart disease	2 (1.9%)	1 (0.7%)
Cerebrovascular disease	1 (0.9%)	0 (0.0%)
Thyroid disease	2 (1.9%)	3 (2.1%)
Asthma and Allery	9 (8.5%)	4 (2.8%)
Cancer	2 (1.9%)	0 (0.0%)
Hypercholesterolemia	12 (11.3%)	0 (0.0%)
Disease severity[b]		
Mild	68 (64.2%)	132 (93.6%)
Moderate	28 (26.4%)	9 (6.4%)
Severe-ICU	10 (9.4%)	0 (0.0%)

Table 1 (*continued*)

Variable	Delta ($n = 106$)	Omicron ($n = 141$)
Key drugs for treatment		
Andrographolide	15 (14.2%)	27 (19.1%)
Flavipiravir	61 (57.5%)	58 (41.1%)
Molnupiravir	1 (0.9%)	9 (6.4%)
Symptomatic medicines[c]	46 (43.4%)	72 (51.1%)
Steroid and others	7 (6.6%)	2 (1.4%)
Vaccination status		
Unvaccinated	63 (59.4%)	8 (5.7%)
1–2 doses	39 (36.8%)	53 (37.6%)
≥3 doses	4 (3.8%)	80 (56.7%)
Type of the latest vaccine		
Inactivated	24 (55.8%)	15 (11.3%)
Viral vector	15 (34.9%)	43 (32.3%)
mRNA	4 (9.3%)	75 (56.4%)
Long COVID status		
Yes	70 (66.0%)	105 (74.5%)
No	36 (34.0%)	36 (25.5%)
No. of long COVID symptoms		
1	29 (27.4%)	41 (29.1%)
2	21 (19.8%)	28 (19.9%)
3	9 (8.5%)	18 (12.8%)
≥4	11 (10.4%)	18 (12.8%)

Notes.

Data reported as n (%) for categorical variables, and mean ± SD for continuous variables.

[a] Based on *Weir & Jan (2023)*.

[b] Based on *WHO (2022a)*.

[c] Any form of medical drug that only targets the symptoms of a disease, such as antipyretic medicine, cold medicine, cough medicine, *etc.*, rather than the underlying cause.

Abbreviations: BMI, Body Mass Index; No, Number.

The odds of developing long COVID were significantly higher for SARS-CoV-2 infection during the Omicron period [OR = 2.976 (95% CI [1.202–7.365]); $p = 0.018$] than for infection during the Delta period. A 1-year increase in age was correlated with a decreased likelihood of developing long COVID [OR = 0.969 (95% CI [0.942–0.996]); $p = 0.025$]. In comparison to other antiviral and immunosuppressive drugs, treatment with symptomatic medications for COVID-19 was associated with a higher risk of developing long COVID [OR = 3.804 (95% CI [1.149–12.590]); $p = 0.029$]. It is worth mentioning that in comparison to vaccination with at least one dose, non-vaccination was a significant risk factor for long COVID [OR = 6.434 (95% CI [1.253–33.033]); $p = 0.026$]. All ten independent variables, as shown in Table 2, possessed the potential to account for 19.3% of the long COVID outcome explanation. The remaining 80.7% of the variance could be accounted for by variables that were not included in the binary logistic regression analysis. The dataset for analysis in this part was available in File S1.
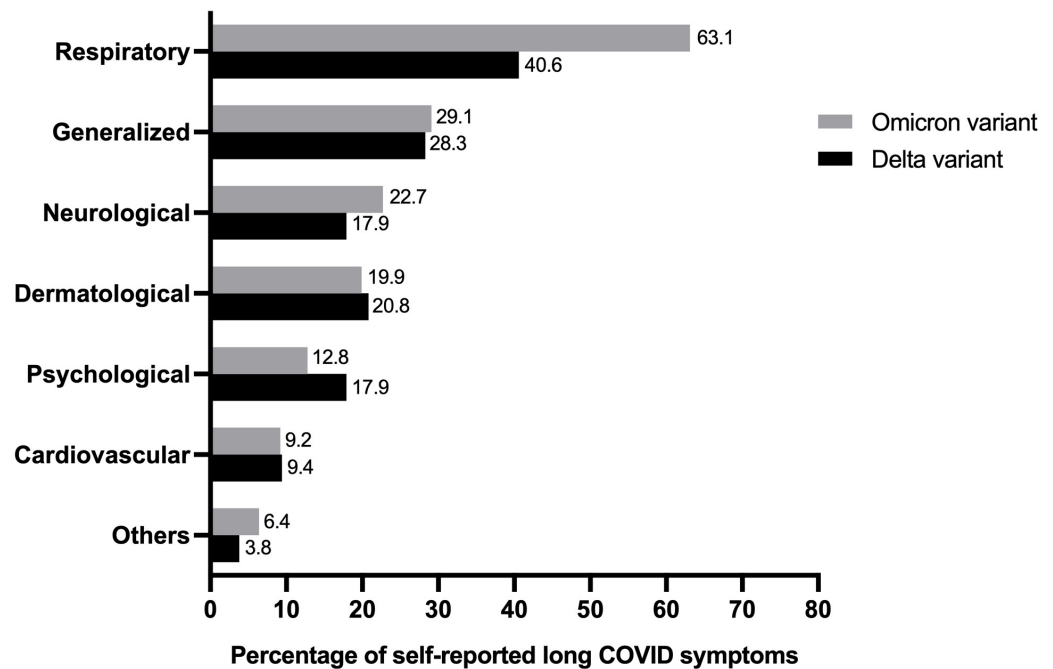
Figure 2 Comparing percentage of individual's experience of self-reported long COVID symptoms from study population in Delta and Omicron variant periods (total $n = 175$).

Genetic variations in the spike gene between the Delta and Omicron variants isolated in Thailand

As it has been hypothesized that the spike protein of SARS-CoV-2 is accountable for long COVID, particularly neuropsychiatric symptoms (*Theoharides, 2022*), the prevalence and characteristics of long COVID outcomes may be influenced differently by the mutations in this protein found in the Delta and Omicron variants. This analysis compared the mutation frequencies observed in each region of the spike protein across the two variants as shown in Fig. 3. Most amino acid changes based on the wild-type spike protein sequence were observed in the S1 subunit compared to the S2 subunit. A higher number of amino acid changes were found in nearly all regions of the S1 subunit of the Omicron variant in comparison to the Delta variant, except for the signal peptide (SP) region. The amino acid substitutions observed in the fusion peptide (FP) and heptad repeat 1 (HR1) region of the S2 subunit were similar between the two variants. No amino acid changes were observed in the cytoplasmic domain (CD) and protease cleavage site of the S2 subunit (S2′) for the Omicron variant.

The frequency of each mutation in the S1 and S2 spike protein subunits was compared between the two variants and is presented as heatmap data in Figs. 4 and 5. The predominant high-frequency mutations were found in the S1 subunit of both variants, especially in the regions of the N-terminal domain (NTD), receptor-binding domain (RBD), receptor-binding motif (RBM), and protease cleavage site between the S1 and S2 subunits (S1S2′).

Table 2 Baseline demographics of study populations comparing between non-long COVID and long COVID cases and variables associated with the risk of long COVID status ($n = 247$).

Variable	Frequency n (%) or mean ± SD		Binary logistic regression		
	Non-long COVID ($n = 72$)	Long COVID ($n = 175$)	B	Odds ratio (95% CI)	P-value
Variant period					
Delta	36 (50.0%)	70 (40.0%)	–	1	–
Omicron	36 (50.0%)	105 (60.0%)	1.090	2.976 (1.202–7.365)	0.018[*]
Age, years	40.86 ± 13.74	36.56 ± 12.59	−0.032	0.969 (0.942–0.996)	0.025[*]
Sex					
Female	42 (58.3%)	102 (58.3%)	–	1	–
Male	30 (41.7%)	73 (41.7%)	−0.449	0.638 (0.304–1.341)	0.236
BMI	25.03 ± 5.05	24.37 ± 4.87	0.003	1.003 (0.905–1.111)	0.960
Blood group					
O	22 (41.5%)	60 (42.9%)	–	1	–
A	9 (16.9%)	23 (16.4%)	−0.209	0.812 (0.306–2.153)	0.675
B	16 (30.2%)	40 (28.6%)	−0.132	0.876 (0.397–1.936)	0.744
AB	6 (11.3%)	17 (12.1%)	0.510	1.666 (0.553–5.018)	0.364
Underlying diseases					
No	48 (66.7%)	129 (73.7%)	–	1	–
Yes	24 (33.3%)	46 (26.3%)	−0.365	0.694 (0.213–2.267)	0.546
Hypertension	14 (19.4%)	16 (9.1%)	−1.161	0.313 (0.084–1.164)	0.083
Diabetes	7 (9.7%)	9 (5.1%)	−0.803	0.448 (0.114–1.757)	0.249
Disease severity					
Mild	64 (88.9%)	136 (77.7%)	–	1	–
Moderate	7 (9.7%)	30 (17.1%)	0.994	2.701 (0.948–7.697)	0.063
Severe-ICU	1 (1.4%)	9 (5.1%)	2.070	7.925 (0.768–81.786)	0.082
Key drugs for treatment[a]					
Andrographolide	11 (15.3%)	31 (17.7%)	0.340	1.405 (0.510–3.867)	0.511
Flavipiravir	35 (48.6%)	84 (48.0%)	0.901	2.463 (0.670–9.057)	0.175
Molnupiravir	5 (6.9%)	5 (2.9%)	−0.183	0.832 (0.128–5.402)	0.848
Symptomatic medicines[b]	30 (41.7%)	88 (50.3%)	1.336	3.804 (1.149–12.590)	0.029[*]
Steroid and others	1 (1.4%)	8 (4.6%)	0.609	1.839 (0.186–18.166)	0.602
Vaccination status					
Vaccinated	51 (70.8%)	125 (71.4%)	–	1	–
Unvaccinated	21 (29.2%)	50 (28.6%)	1.862	6.434 (1.253–33.033)	0.026[*]
Type of the latest vaccine					
Inactivated	11 (21.6%)	28 (22.4%)	–	1	–
Viral vector	19 (37.3%)	39 (31.2%)	−0.083	0.921 (0.331–2.563)	0.874
mRNA	21 (41.2%)	58 (46.4%)	0.250	1.284 (0.395–4.173)	0.677
[c]-2Log Likelihood (-2ll) = 262.136 $R^2 = 0.193$					

Notes.

[a]Reference group was non-selection in each drug category to treat.

[b]Any form of medical drug that only targets the symptoms of a disease, such as antipyretic medicine, cold medicine, cough medicine, *etc.*, rather than the underlying cause.

[c]To elucidate the model summary or goodness of fit following binary logistic regression analysis, the -2Log Likelihood (-2ll) and Nagelkerke R^2 (Pseudo R^2) statistic values were reported.
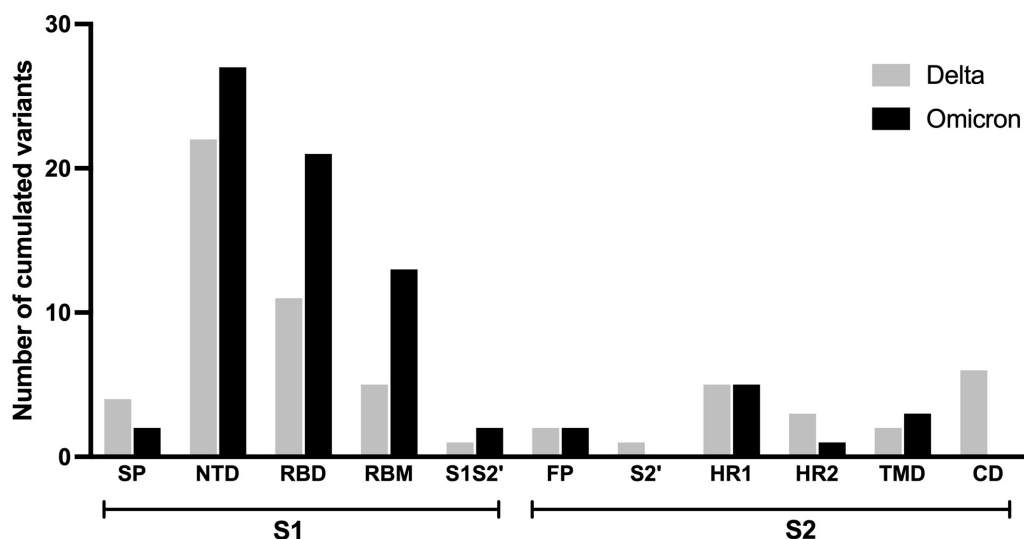
[*]Significant level at P-value < 0.05.

Figure 3 Comparing number of accumulated mutations between Delta and Omicron variant isolates in S1 and S2 subunits of spike SARS-CoV-2. SP, signal peptide; NTD, N-terminal domain; RBD, receptor-binding domain; RBM, receptor-binding motif; S1S2′, protease cleavage site between S1 and S2 subunits; FP, fusion peptide; S2′, protease cleavage site in S2 subunit; HR1, heptad repeat 1; HR2, heptad repeat 2; TMD, transmembrane domain; CD, cytoplasmic domain.

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For the S2 subunit, high-frequency mutations were observed in the FP region of the Omicron variant and the HR1 region of both variants.

Finally, the literature review of the pathogenesis evidence or suspected evidence regarding the role of the key mutations of each variant, defined here as percentage frequency differences between variants greater than 50%, is shown in Table 3. The Omicron variant sequences obtained from Thailand had a larger number of key mutations compared to the Delta variant sequences. Most of the significant mutations identified in the Delta variant sequences were associated with ACE2 affinity binding enhancement, viral infectivity, and immune evasion. The sole mutation, D950B, located in the HR1 region of the S2 subunit, lacked any evidence regarding its function. The greater part of the key mutations in the Omicron variant exhibited comparable functions to those observed in the Delta variant. However, a few mutations played addition roles in facilitating spike cleavage, virus fusion, and virus transmissibility; spike function impairment had also been published. Additionally, the function of five mutations in the NTD and RBD regions of the S1 subunit remained unknown: L24del, P25del, P26del, A27S, and R408S. The dataset for analysis in this part was available in tFile S2.

DISCUSSION

The potential of a high public health burden due to long COVID must be considered amid the ongoing global transmission of SARS-CoV-2 and its rapidly emerging variants. Research comparing the variations in long COVID prevalence and characteristics caused by distinct virus variants is scarce. Thus, this study collected informative data on long
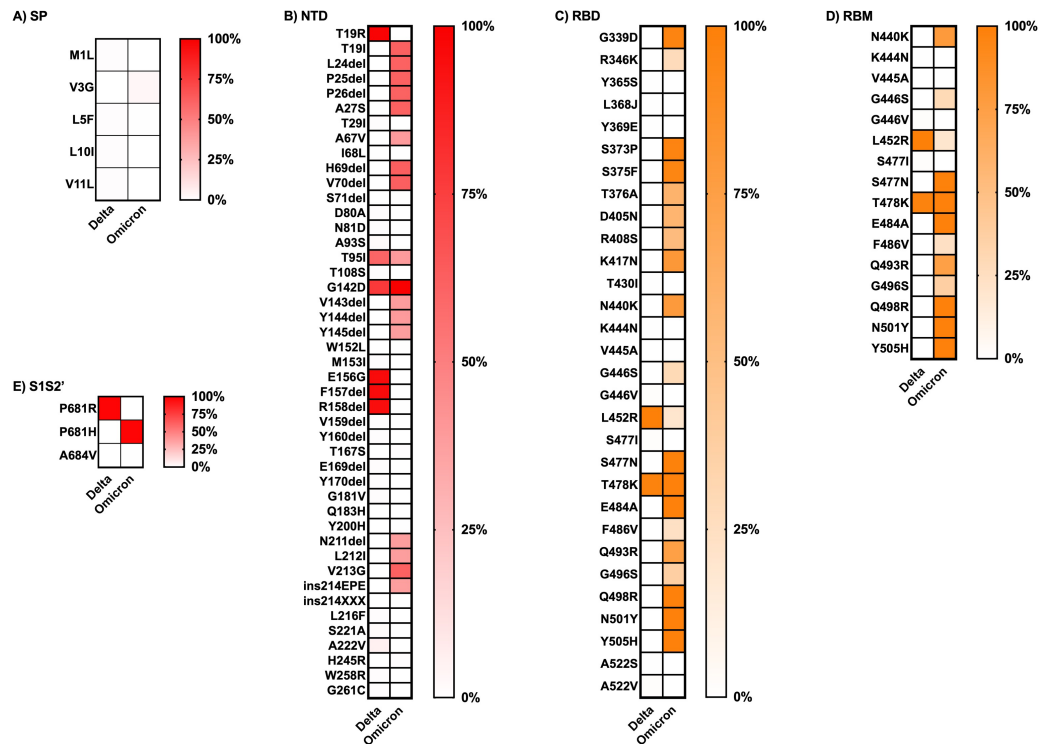
Kiatratdasakul et al. (2024), *PeerJ*, DOI 10.7717/peerj.17898

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Figure 4 Comparing the percentage of frequency mutations between Delta and Omicron variant isolates in the S1 subunit of spike SARS-CoV-2. (A) SP: signal peptide; (B) NTD: N-terminal domain; (C) RBD: receptor-binding domain; (D) RBM: receptor-binding motif; and (E) S1S2′: protease cleavage site between S1 and S2 subunits.

Full-size 🖼 DOI: 10.7717/peerj.17898/fig-4

COVID prevalence and clinical symptoms by comparing the Delta and Omicron epidemic periods in Thailand.

Our demographic analysis was consistent with previous studies indicating that most participants in both epidemic periods were female (*Antonelli et al., 2022*; *WHO, 2022b*). The Omicron variant had a higher potential for infection among healthy people compared to the preceding Delta variant. This may be correlated with the biological characteristic of an increased transmissibility of Omicron. It must be emphasized that all strains of SARS-CoV-2 are capable of inducing long COVID. This is despite the fact that the prevalence of long COVID varied considerably between studies due to dissimilarities in the COVID-19 participant selection process, study sites, ethnicity, self-report bias, and time frame analysis (*Antonelli et al., 2022*; *Du et al., 2022*). Over sixty percent of our two participant groups had long COVID. Long COVID prevalence in the Delta period found in this study was comparable with a previous report from Thailand (*Wangchalabovorn, Weerametachai & Leesri, 2022*). However, the data collected from participants' self-reports may be influenced by confounding factors, especially recall bias in the memorization of events that occurred a long time ago. Although this study decreased this bias by employing a question to assess the memory of participants, it may not entirely prevent it.
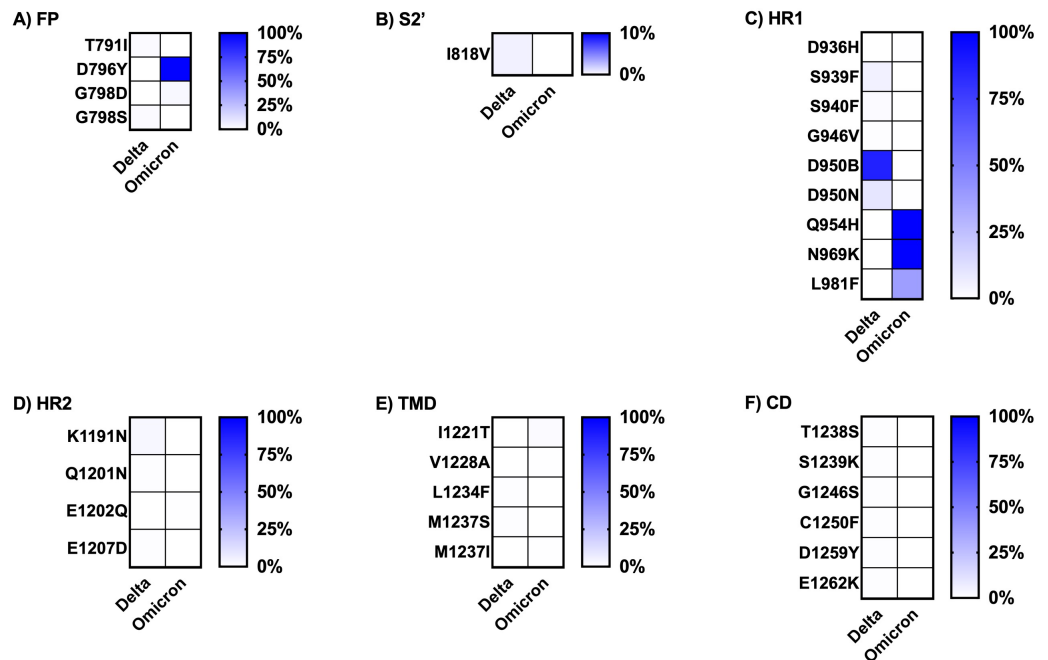
Figure 5 Comparing the percentage of frequency mutations between Delta and Omicron variant isolates in the S2 subunit of spike SARS-CoV-2. (A) FP: fusion peptide, (B) S2′: protease cleavage site in S2 subunit, (C) HR1: heptad repeat 1, (D) HR2: heptad repeat 2, (E) TMD: transmembrane domain, and (F) CD: cytoplasmic domain.

Full-size 🖼 DOI: 10.7717/peerj.17898/fig-5

Post-acute COVID-19 syndrome or casually long COVID comprises a wide spectrum of clinical symptoms as described in this study. Even though the Delta and Omicron groups presented long COVID characteristics at varying rates, all abnormality classifications were discernible in both groups. As reported in studies from Europe and Asia (*Menges et al., 2021*; *Xiong et al., 2021*), fatigue that was categorized as a generalized symptom and respiratory symptoms remained prevalent. Although the precise mechanisms underlying the development of long COVID remain unknown, several hypotheses have been proposed, including immune dysregulation, persistent inflammatory reactions, antibody-dependent enhancement (ADE) from non-neutralizing antibody response, autoimmune mimicry, viral persistence, reactivation of latent pathogens, and alterations in the host microbiome (*Batiha et al., 2022*; *Chen et al., 2023*). With respect to viral persistence and shedding, the upper respiratory tract, lower respiratory tract, gastrointestinal tract, and blood were observed to have the longest durations of viral RNA shedding at 83, 59, 126, and 60 days, respectively (*Chen et al., 2023*). The sustained presence of SARS-CoV-2 RNA or antigen and immune responses directed against it may determine the development of long COVID (*Files et al., 2021*; *Chen et al., 2023*).

In this study, the biological factors that were found significantly associated with the prognosis of long COVID were variant epidemic period, age, treatment with symptomatic medicines, and vaccination status as determined by binary logistic regression analysis. Infection during the Omicron period was associated with an almost three times increased

Table 3 The frequency difference of mutations in spike SARS-CoV-2 protein between Delta and Omicron variant isolates has a value greater than 50%.

Region	Mutation	Accumulated frequency (%)		% Δ	Pathogenesis or suspected evidence (x)								Reference
		Delta (N = 206)	Omicron (N = 292)		Not found	Immune escape	Involve in infectivity	Enhance binding affinity to ACE2 receptor	Enhance viral fusion	Enhance spike clavage	Enhance transmissibility	Impair spike function	
A). S1 subunit													
NTD	T19R	203 (98.5)	0 (0.0)	98.5		x							*Singh et al. (2022)*
	T19I	0 (0.0)	180 (61.6)	61.6			x						*Pastorio et al. (2022)*
	L24del	0 (0.0)	179 (61.3)	61.3	x								
	P25del	0 (0.0)	179 (61.3)	61.3	x								
	P26del	0 (0.0)	178 (60.9)	60.9	x								
	A27S	0 (0.0)	179 (61.3)	61.3	x								
	H69del	0 (0.0)	181 (61.9)	61.9			x						*Fatihi et al. (2021)*
	V70del	0 (0.0)	182 (62.3)	62.3			x						*Fatihi et al. (2021)*
	E156G	197 (95.6)	0 (0.0)	95.6		x	x	x					*Dang, Ren & Wang (2022)*
	F157del	197 (95.6)	1 (0.3)	95.2		x	x	x					*Dang, Ren & Wang (2022)*
	R158del	195 (94.7)	1 (0.3)	94.3		x	x	x					*Dang, Ren & Wang (2022)*
	V213G	0 (0.0)	179 (61.3)	61.3		x							*Wang et al. (2023)*
RBD	G339D	0 (0.0)	282 (96.6)	96.6		x	x				x		*Cao et al. (2022)*
	S373P	0 (0.0)	281 (96.2)	96.2		x	x				x		*Cao et al. (2022)*
	S375F	0 (0.0)	280 (95.9)	95.9		x	x				x		*Cao et al. (2022)*
	T376A	0 (0.0)	173 (59.2)	59.2			x					x	*Pastorio et al. (2022)*
	D405N	0 (0.0)	170 (58.2)	58.2			x					x	*Bugatti et al. (2023)*
	R408S	0 (0.0)	153 (52.4)	52.4	x								
	K417N	0 (0.0)	235 (80.5)	80.5		x	x	x					*Greaney et al. (2021)*
RBD/ RBM	N440K	0 (0.0)	230 (78.8)	78.8	x								*Cao et al. (2022)*
	L452R	205 (99.5)	59 (20.2)	79.3		x	x	x					*Dang, Ren & Wang (2022), Wilhelm et al. (2021)*
	S477N	0 (0.0)	287 (98.3)	98.3			x	x					*Dang, Ren & Wang (2022), Zahradník et al. (2021)*
	E484A	0 (0.0)	288 (98.6)	98.6		x							*Greaney et al. (2021)*
	Q493R	0 (0.0)	218 (74.7)	74.7		x	x	x			x		*Focosi et al. (2021)*
	Q498R	0 (0.0)	288 (98.6)	98.6			x	x					*Dang, Ren & Wang (2022), Zahradník et al. (2021)*
	N501Y	0 (0.0)	288 (98.6)	98.6			x	x			x		*Niu et al. (2021)*
	Y505H	0 (0.0)	288 (98.6)	98.6			x	x					*Khan et al. (2022)*
S1S2′	P681R	202 (98.1)	0 (0.0)	98.1			x		x				*Saito et al. (2022)*
	P681H	0 (0.0)	287 (98.3)	98.3		x	x			x			*Lista et al. (2022)*
B). S2 subunit													
FP	D796Y	0 (0.0)	289 (98.9)	98.9		x							*Elko et al. (2024)*
HR1	D950B	179 (86.9)	0 (0.0)	86.9	x								
	Q954H	0 (0.0)	290 (99.3)	99.3			x		x				*Park et al. (2023)*
	N969K	0 (0.0)	290 (99.3)	99.3			x		x				*Park et al. (2023)*

Notes.

Symbol and abbreviations: △, different value; x, presence; NTD, N-terminal domain; RBD, receptor-binding domain; RBM, receptor-binding motif; S1S2′, protease cleavage site between S1 and S2 subunits; FP, fusion peptide; HR1, heptad repeat 1.

risk of long COVID sequelae in comparison to the Delta period. This finding contradicts the results of previous studies (*Antonelli et al., 2022*; *Du et al., 2022*). Although the prevalence of long COVID caused by different strains did not differ significantly, *Du et al. (2022)* identified a significant distinction in specific symptoms observed in each strain through a systematic review and meta-analysis. As we utilized a lengthy period during the Omicron epidemic covering the variant epidemics of BA.1, BA.2, BA.4, and BA.5, which contain many critical mutations that increased the fitness for infection (*Tian et al., 2022*), the discrepancy in the period of analysis may account for the outcome of our study. In agreement with the findings of previous studies (*Peghin et al., 2021*; *Maglietta et al., 2022*; *Notarte et al., 2022*; *Subramanian et al., 2022*; *Yoo et al., 2022*), advanced age did not support long COVID induction in this analysis. However, it differed from some studies (*Sudre et al., 2021*; *Thompson et al., 2022*). Based on our current understanding, this study represents the first report that establishes treatment with symptomatic medications and vaccination status as substantial risk factors for the development of long COVID. As long COVID may be caused by the persistence of viral antigen, which results in an ongoing activation of the host immune response, long COVID can be induced by the use of symptomatic medications lacking antiviral activity. Compared to those who had received at least one dose of vaccination, non-immunized individuals exhibited a six-fold increased risk of developing long COVID. This crucial information was substantiated by a recent cohort research (*Catala et al., 2024*). Moreover, our analysis data, along with those of other prior studies, emphasized the absence of a correlation between long COVID development and female gender or initial severity of COVID-19 (*Townsend et al., 2020*; *Simani et al., 2021*; *Al-Kuraishy et al., 2022*; *Al-Thomali et al., 2022*).

The accumulation of mutations in the spike protein gene of SARS-CoV-2 is a significant factor in the emergence of novel variants of concern (VOCs). The Omicron variant exhibited the highest number of mutations compared to earlier VOCs (Alpha, Beta, Gamma, and Delta), with more than 60% of the mutations accumulated in the spike protein gene as opposed to other regions of the genome (*Tian et al., 2022*). Viral infection and transmissibility are determined by amino acid changes in the receptor-binding domain (RBD) and receptor-binding motif (RBM) of the SARS-CoV-2 spike protein. Furthermore, the resistance of the host antibody (Ab) immune response is attributed to the amino acid changes occurring in the spike protein, which serves as the primary target for neutralization (*Tian et al., 2022*). Recently, there has been speculation regarding the potential contribution of the spike protein, either alone or in conjunction with other inflammatory mediators, in the induction of long COVID (*Theoharides, 2022*).

Unfortunately, the amino acid sequence of the spike protein obtained directly from participants with long COVID and those without long COVID in this study could not be accessible for the purpose of explicitly comparing and discussing the viral mutations associated with the development of long COVID. We instead performed a comprehensive analysis by retrieving the available spike protein sequence datasets from GIDSAID. In light of this constraint, a direct prediction of a long COVID induced by a viral mutation is not achievable. The analysis of the amino acid sequence of the spike protein from Delta and Omicron variants in Thailand resulted in insightful findings regarding the frequency of

mutations and the function of critical mutations that occur in these two variants. Consistent with a previous study (*Harvey et al., 2021*), the Omicron variant dataset contained two or more times the number of mutations located in the RBD and RBM compared to the Delta variant dataset. The observed variation in the critical region of the spike protein might support an association between spike protein function and progression of long COVID. The enhanced binding affinity to the angiotensin-converting enzyme-2 (ACE2) receptor, which is specific for SARS-CoV-2 infection, on mast cells may be linked to the development of mast cell activation syndrome (MCAS), one of the underlying mechanisms of long COVID, as hypothesized to result from the amino acid changes in the RBD and RBM regions. Extraordinary symptoms may be induced in long COVID patients due to dysregulation in the release of inflammatory mediators in MCAS (*Batiha et al., 2022*).

CONCLUSION

The SARS-CoV-2 variant, age, treatment with symptomatic medications, and non-immunization status were identified as biological factors associated with long COVID progression in our retrospective cross-sectional study. The identification of these factors should help to facilitate the development of a suitable health management strategy. Furthermore, the analysis of the frequency and biological implications of mutations observed in the spike protein gene of the Delta and Omicron variants could support the explanation for long COVID development and provide a scientific reference for monitoring, prevention, and vaccine development.

ACKNOWLEDGEMENTS

ADDITIONAL INFORMATION AND DECLARATIONS

Competing Interests
The authors declare there are no competing interests.

Author Contributions
- Supanchita Kiatratdasakul performed the experiments, analyzed the data, prepared figures and/or tables, and approved the final draft.
- Pirom Noisumdaeng conceived and designed the experiments, authored or reviewed drafts of the article, and approved the final draft.
- Nattamon Niyomdecha conceived and designed the experiments, performed the experiments, analyzed the data, prepared figures and/or tables, authored or reviewed drafts of the article, and approved the final draft.

Ethics

The following information was supplied relating to ethical approvals (i.e., approving body and any reference numbers):

This study was approved by the Ethical Committee of the Maharat Nakhon Ratchasima Hospital IRB, Ministry of Public Health, Thailand (Certificate No. 139/2022).

Data Availability

The following information was supplied regarding data availability:

The raw data are available in the Supplemental Files.

Supplemental Information

Supplemental information for this article can be found online at http://dx.doi.org/10.7717/peerj.17898#supplemental-information.

REFERENCES

Al-Kuraishy HM, Al-Gareeb AI, Alexiou A, Batiha GE. 2022. Central effects of ivermectin in alleviation of COVID-19-induced Dysauto-nomia. *Current Drug Targets* **23(13)**:1277–1287 DOI 10.2174/1389450123666220810102406.

Al-Thomali AW, Al-Kuraishy HM, Al-Gareeb AI, Al-Buhadiliy A, De Waard M, Sabatier JM, Khan Khalil AA, Saad HM, Batiha GE. 2022. Role of neuropilin 1 in COVID-19 patients with acute ischemic stroke. *Biomedicines* **10(8)**:2032 DOI 10.3390/biomedicines10082032.

Antonelli M, Pujol JC, Spector TD, Ourselin S, Steves CJ. 2022. Risk of long COVID associated with Delta versus Omicron variants of SARS-CoV-2. *Lancet* **399(10343)**:2263–2264 DOI 10.1016/S0140-6736(22)00941-2.

Batiha GE, Al-Kuraishy HM, Al-Gareeb AI, Welson NN. 2022. Pathophysiology of post-COVID syndromes: a new perspective. *Virology Journal* **19(1)**:158 DOI 10.1186/s12985-022-01891-2.

Bugatti A, Filippini F, Messali S, Giovanetti M, Ravelli C, Zani A, Ciccozzi M, Caruso A, Caccuri F. 2023. The D405N mutation in the spike protein of SARS-CoV-2 Omicron BA.5 inhibits spike/integrins interaction and viral infection of human lung microvascular endothelial cells. *Viruses* **15(2)**:332 DOI 10.3390/v15020332.

Cao Y, Wang J, Jian F, Xiao T, Song W, Yisimayi A, Huang W, Li Q, Wang P, An R, Wang J, Wang Y, Niu X, Yang S, Liang H, Sun H, Li T, Yu Y, Cui Q, Liu S, Yang X, Du S, Zhang Z, Hao X, Shao F, Jin R, Wang X, Xiao J, Wang Y, Xie XS. 2022. Omicron escapes the majority of existing SARS-CoV-2 neutralizing antibodies. *Nature* **602(7898)**:657–663 DOI 10.1038/s41586-021-04385-3.

Catala M, Mercade-Besora N, Kolde R, Trinh NTH, Roel E, Burn E, Rathod-Mistry T, Kostka K, Man WY, Delmestri A, Nordeng HME, Uuskula A, Duarte-Salles T, Prieto-Alhambra D, Jodicke AM. 2024. The effectiveness of COVID-19 vaccines to prevent long COVID symptoms: staggered cohort study of data from the UK, Spain, and Estonia. *The Lancet Respiratory Medicine* **S2213–S2600(23)**:00414–00419 DOI 10.1016/S2213-2600(23)00414-9.

Kiatratdasakul et al. (2024), *PeerJ*, DOI 10.7717/peerj.17898

17/22

Chen B, Julg , Mohandas S, Bradfute SB, Recover Mechanistic Pathways Task Force. 2023. Viral persistence, reactivation, and mechanisms of long COVID. *Elife* **12**:e86015 DOI 10.7554/eLife.86015.

Dang S, Ren L, Wang J. 2022. Functional mutations of SARS-CoV-2: implications to viral transmission, pathogenicity and immune escape. *Chinese Medical Journal* **135(10)**:1213–1222 DOI 10.1097/CM9.0000000000002158.

Dennis A, Wamil M, Alberts J, Oben J, Cuthbertson DJ, Wootton D, Crooks M, Gabbay M, Brady M, Hishmeh L, Attree E, Heightman M, Banerjee R, Banerjee A. 2021. Multiorgan impairment in low-risk individuals with post-COVID-19 syndrome: a prospective, community-based study. *BMJ Open* **11(3)**:e048391 DOI 10.1136/bmjopen-2020-048391.

Department of Disease Control of Thailand (DDC). 2024. COVID-19-dashborad. *Available at* https://ddc.moph.go.th/covid19-dashboard/ (accessed on 28 January 2024).

Department of Medical Science of Thailand (DMSc). 2023. Government data catalog-dataset/sars-cov-2-variants. *Available at* https://ddc.moph.go.th/covid19-dashboard/ (accessed on 18 January 2023).

Du M, Ma Y, Deng J, Liu M, Liu J. 2022. Comparison of long COVID-19 caused by different SARS-CoV-2 strains: a systematic review and meta-analysis. *International Journal of Environmental Research and Public Health* **19(23)**:16010 DOI 10.3390/ijerph192316010.

Elko EA, Mead HL, Nelson GA, Zaia JA, Ladner JT, Altin JA. 2024. Recurrent SARS-CoV-2 mutations at spike D796 evade antibodies from pre-omicron convalescent and vaccinated subjects. *Microbiology Spectrum* **12(2)**:e0329123 DOI 10.1128/spectrum.03291-23.

Fatihi S, Rathore S, Pathak AK, Gahlot D, Mukerji M, Jatana N, Thukral L. 2021. A rigorous framework for detecting SARS-CoV-2 spike protein mutational ensemble from genomic and structural features. *Current Research in Structural Biology* **3**:290–300 DOI 10.1016/j.crstbi.2021.11.002.

Files JK, Sarkar S, Fram TR, Boppana S, Sterrett S, Qin K, Bansal A, Long DM, Sabbaj S, Kobie JJ, Goepfert PA, Erdmann N. 2021. Duration of post-COVID-19 symptoms is associated with sustained SARS-CoV-2-specific immune responses. *JCI Insight* **6(15)**:e151544 DOI 10.1172/jci.insight.151544.

Focosi D, Novazzi F, Genoni A, Dentali F, Gasperina DD, Baj A, Maggi F. 2021. Emergence of SARS-COV-2 spike protein escape mutation Q493R after treatment for COVID-19. *Emerging Infectious Diseases* **27(10)**:2728–2731 DOI 10.3201/eid2710.211538.

Greaney AJ, Loes AN, Crawford KHD, Starr TN, Malone KD, Chu HY, Bloom JD. 2021. Comprehensive mapping of mutations in the SARS-CoV-2 receptor-binding domain that affect recognition by polyclonal human plasma antibodies. *Cell Host & Microbe* **29(3)**:463–476 DOI 10.1016/j.chom.2021.02.003e6.

Harvey WT, Carabelli AM, Jackson B, Gupta RK, Thomson EC, Harrison EM, Ludden C, Reeve R, Rambaut A, COVID-19 Genomics UK (COG-UK) Consortium, Peacock SJ, Robertson DL. 2021. SARS-CoV-2 variants, spike mutations and immune escape. *Nature Reviews. Microbiology* **19(7)**:409–424 DOI 10.1038/s41579-021-00573-0.

Khan A, Khan SA, Zia K, Altowyan MS, Barakat A, Ul-Haq Z. 2022. Deciphering the impact of mutations on the binding efficacy of SARS-CoV-2 omicron and delta variants with human ACE2 receptor. *Frontiers in Chemistry* **10**:892093 DOI 10.3389/fchem.2022.892093.

Lista MJ, Winstone H, Wilson HD, Dyer A, Pickering S, Galao RP, De Lorenzo G, Cowton VM, Furnon W, Suarez N, Orton R. 2022. The P681H mutation in the spike glycoprotein of the alpha variant of SARS-CoV-2 escapes IFITM restriction and is necessary for type I interferon resistance. *Journal of Virology* **96(23)**:e0125022 DOI 10.1128/jvi.01250-22.

Maglietta G, Diodati F, Puntoni M, Lazzarelli S, Marcomini B, Patrizi L, Caminiti C. 2022. Prognostic factors for post-COVID-19 syndrome: a systematic review and meta-analysis. *Journal of Clinical Medicine* **11(6)**:1541 DOI 10.3390/jcm11061541.

Menges D, Ballouz T, Anagnostopoulos A, Aschmann HE, Domenghino A, Fehr JS, Puhan MA. 2021. Burden of post-COVID-19 syndrome and implications for healthcare service planning: a population-based cohort study. *PLOS ONE* **16(7)**:e0254523 DOI 10.1371/journal.pone.0254523.

Montagne A, Nation DA, Sagare AP, Barisano G, Sweeney MD, Chakhoyan A, Pachicano M, Joe E, Nelson AR, D'Orazio LM, Buennagel DP, Harrington MG, Benzinger TLS, Fagan AM, Ringman JM, Schneider LS, Morris JC, Reiman EM, Caselli RJ, Chui HC, Tcw J, Chen Y, Pa J, Conti PS, Law M, Toga AW, Zlokovic BV. 2020. APOE4 leads to blood–brain barrier dysfunction predicting cognitive decline. *Nature* **581(7806)**:71–76 DOI 10.1038/s41586-020-2247-3.

Moreno-Pérez O, Merino E, Leon-Ramirez JM, Andres M, Ramos JM, Arenas-Jiménez J, Asensio S, Sanchez R, Ruiz-Torregrosa P, Galan I, Scholz A, Amo A, González-delaAleja P, Boix V, Gil J, COVID19-ALC research group. 2021. Post-acute COVID-19 syndrome. Incidence and risk factors: A Mediterranean cohort study. *Journal of Infection* **82(3)**:378–383 DOI 10.1016/j.jinf.2021.01.004.

National Institutes of Health (NIH). 2022. Clinical spectrum of SARS-CoV-2 infection. *Available at* https://www.covid19treatmentguidelines.nih.gov/overview/clinical-spectrum/ (accessed on 15 June 2022).

Niu Z, Zhang Z, Gao X, Du P, Lu J, Yan B, Wang C, Zheng Y, Huang H, Sun Q. 2021. N501Y mutation imparts cross-species transmission of SARS-CoV-2 to mice by enhancing receptor binding. *Signal Transduction and Targeted Therapy* **6(1)**:284 DOI 10.1038/s41392-021-00704-2.

Notarte KI, De Oliveira MHS, Peligro PJ, Velasco JV, Macaranas I, Ver AT, Pangilinan FC, Pastrana A, Goldrich N, Kavteladze D, Gellaco MML, Liu J, Lippi G, Henry BM, Fernández-de Las-Peñas C. 2022. Age, sex and previous comorbidities as risk factors not associated with SARS-CoV-2 infection for long COVID-19: a

systematic review and meta-analysis. *Journal of Clinical Medicine* **11(24)**:7314 DOI 10.3390/jcm11247314.

Office for National Statistics of the United Kingdom (ONS). 2022. Prevalence of ongoing symptoms following coronavirus (COVID-19) infection in the UK: 3 November 2022. *Available at https://www.ons.gov.uk/peoplepopulationandcommunity/healthandsocialcare/conditionsanddiseases/bulletins/prevalenceofongoingsymptoms followingcoronaviruscovid19 infectionintheuk/3november2022/*.

Park SB, Khan M, Chiliveri SC, Hu X, Irvin P, Leek M, Grieshaber A, Hu Z, Jang ES, Bax A, Liang TJ. 2023. SARS-CoV-2 omicron variants harbor spike protein mutations responsible for their attenuated fusogenic phenotype. *Communications Biology* **6(1)**:556 DOI 10.1038/s42003-023-04923-x.

Pastorio C, Zech F, Noettger S, Jung C, Jacob T, Sanderson T, Sparrer KMJ, kirchhoff F. 2022. determinants of spike infectivity, processing, and neutralization in SARS-CoV-2 Omicron subvariants BA.1 and BA.2. *Cell Host & Microbe* **30(9)**:1255–1268 DOI 10.1016/j.chom.2022.07.006.

Peghin M, Palese A, Venturini M, De Martino M, Gerussi V, Graziano E, Bontempo G, Marrella F, Tommasini A, Fabris M, Curcio F, Isola M, Tascini C. 2021. Post-COVID-19 symptoms 6 months after acute infection among hospitalized and non-hospitalized patients. *Clinical Microbiology and Infection* **27(10)**:1507–1513 DOI 10.1016/j.cmi.2021.05.033.

Saito A, Irie T, Suzuki R, Maemura T, Nasser H, Uriu K, Kosugi Y, Shirakawa K, Sadamasu K, Kimura I, Ito J, Wu J, Iwatsuki-Horimoto K, Ito M, Yamayoshi S, Loeber S, Tsuda M, Wang L, Ozono S, Butlertanaka EP, Tanaka YL, Shimizu R, Shimizu K, Yoshimatsu K, Kawabata R, Sakaguchi T, Tokunaga K, Yoshida I, Asakura H, Nagashima M, Kazuma Y, Nomura R, Horisawa Y, Yoshimura K, Takaori-Kondo A, Imai M, Genotype to Phenotype Japan (G2P-Japan) Consortium, Tanaka S, Nakagawa S, Ikeda T, Fukuhara T, Kawaoka Y, Sato K. 2022. Enhanced fusogenicity and pathogenicity of SARS-CoV-2 Delta P681R mutation. *Nature* **602(7896)**:300–306 DOI 10.1038/s41586-021-04266-9.

Simani L, Ramezani M, Darazam IA, Sagharichi M, Aalipour MA, Ghorbani F, Pakdaman H. 2021. Prevalence and correlates of chronic fatigue syndrome and post-traumatic stress disorder after the outbreak of the COVID-19. *Journal of Neurovirology* **27(1)**:154–159 DOI 10.1007/s13365-021-00949-1.

Singh P, Sharma K, Singh P, Bhargava A, Negi SS, Sharma P, Bhise M, Tripathi MK, Jindal A, Nagarkar NM. 2022. Genomic characterization unravelling the causative role of SARS-CoV-2 Delta variant of lineage B.1.617.2 in 2nd wave of COVID-19 pandemic in Chhattisgarh, India. *Microbial Pathogenesis* **164**:105404 DOI 10.1016/j.micpath.2022.105404.

Subramanian A, Nirantharakumar K, Hughes S, Myles P, Williams T, Gokhale KM, Taverner T, Chandan JS, Brown K, Simms-Williams N, Shah AD, Singh M, Kidy F, Okoth K, Hotham R, Bashir N, Cockburn N, Lee SI, Turner GM, Gkoutos GV, Aiyegbusi OL, McMullan C, Denniston AK, Sapey E, Lord JM, Wraith DC, Leggett E, Iles C, Marshall T, Price MJ, Marwaha S, Davies EH, Jackson LJ,

Matthews KL, Camaradou J, Calvert M, Haroon S. 2022. Symptoms and risk factors for long COVID in non-hospitalized adults. *Nature Medicine* **28**(8):1706–1714 DOI 10.1038/s41591-022-01909-w.

Sudre CH, Murray B, Varsavsky T, Graham MS, Penfold RS, Bowyer RC, Pujol JC, Klaser K, Antonelli M, Canas LS, Molteni E, Modat M, Cardoso MJorge, May A, Ganesh S, Davies R, Nguyen LH, Drew DA, Astley CM, Joshi AD, Merino J, Tsereteli N, Fall T, Gomez MF, Duncan EL, Menni C, Williams FMK, Franks PW, Chan AT, Wolf J, Ourselin S, Spector T, Steves CJ. 2021. Attributes and predictors of long COVID. *Nature Medicine* **27**(4):626–631 DOI 10.1038/s41591-021-01292-y.

Tancharoensukjit T. 2023. Prevalence and factors associated with post acute COVID syndrome (Long COVID) in patients infected coronavirus disease 2019 who received inpatient treatment Lan Krabue hospital. *Journal of the Department of Medical Services* **48**(1):51–59 (in Thai).

Theoharides TC. 2022. Could SARS-CoV-2 Spike Protein Be Responsible for Long-COVID Syndrome? *Molecular Neurobiology* **59**(3):1850–1861 DOI 10.1007/s12035-021-02696-0.

Thompson EJ, Williams DM, Walker AJ, Mitchell RE, Niedzwiedz CL, Yang TC, Huggins CF, Kwong ASF, Silverwood RJ, Di Gessa G, Bowyer RCE, Northstone K, Hou B, Green MJ, Dodgeon B, Doores KJ, Duncan EL, Williams FMK, Open-SAFELY Collaborative, Steptoe A, Porteous DJ, McEachan RRC, Tomlinson L, Goldacre B, Patalay P, Ploubidis GB, Katikireddi SV, Tilling K, Rentsch CT, Timpson NJ, Chaturvedi N, Steves CJ. 2022. Long COVID burden and risk factors in 10 UK longitudinal studies and electronic health records. *Nature Communications* **13**(1)::3528 DOI 10.1038/s41467-022-30836-0.

Tian D, Sun Y, Xu H, Ye Q. 2022. The emergence and epidemic characteristics of the highly mutated SARS-CoV-2 Omicron variant. *Journal of Medical Virology* **94**(6):2376–2383 DOI 10.1002/jmv.27643.

Townsend L, Dyer AH, Jones K, Dunne J, Mooney A, Gaffney F, O'Connor L, Leavy D, O'Brien K, Dowds J, Sugrue JA, Hopkins D, Martin-Loeches I, Cheallaigh CNi, Nadarajan P, McLaughlin AM, Bourke NM, Bergin C, O'Farrelly C, Bannan C, Conlon N. 2020. Persistent fatigue following SARS-CoV-2 infection is common and independent of severity of initial infection. *PLOS ONE* **15**(11):e0240784 DOI 10.1371/journal.pone.0240784.

Wang X, Hu M, Liu B, Xu H, Jin Y, Wang B, Zhao Y, Wu J, Yue J, Ren H. 2023. Evaluating the effect of SARS-CoV-2 spike mutations with a linear doubly robust learner. *Frontiers in Cellular and Infection Microbiology* **13**:1161445 DOI 10.3389/fcimb.2023.1161445.

Wangchalabovorn M, Weerametachai S, Leesri T. 2022. Prevalence of post COVID-19 conditions in SARS-CoV-2 infected patients at 3-month telephone follow-up. *Regional Health Promotion Center* **9**:265–284 (in Thai).

Weir CB, Jan A. 2023. BMI classification percentile and cut off points. In: *StatPearls*. Treasure Island: StatPearls Publishing. *Available at* https://www.ncbi.nlm.nih.gov/books/NBK541070/ (accessed on 15 June 2022).

Wilhelm A, Toptan T, Pallas C, Wolf T, Goetsch U, Gottschalk R, Vehreschild MJGT, Ciesek S, Widera M. 2021. Antibody-mediated neutralization of authentic SARS-CoV-2 B.1.617 variants harboring L452R and T478K/E484Q. *Viruses* **13(9)**:1693 DOI 10.3390/v13091693.

Wongsermsin S, Chinoraso J, Yeekian C. 2022. Symptom and factors effect on severity of long COVID. *Chonburi Hospital Journal* **47(3)**:233–240 (in Thai).

World Health Organization (WHO). 2020. *Clinical management of COVID-19 (Interim Guidance).* Geneva: World Health Organization. *Available at* https://iris.who.int/handle/10665/332196.

World Health Organization (WHO). 2022a. *Post COVID-19 condition (Long COVID).* Geneva: World Health Organization. *Available at* https://www.who.int/europe/news-room/fact-sheets/item/post-covid-19-condition.

World Health Organization (WHO). 2022b. *Severity of disease associated with Omicron variant as compared with Delta variant in hospitalized patients with suspected or confirmed SARS-CoV-2 infection.* Geneva: World Health Organization*Available at* https://www.who.int/publications/i/item/9789240051829.

Xiong Q, Xu M, Li J, Liu Y, Zhang J, Xu Y, Dong W. 2021. Clinical sequelae of COVID-19 survivors in Wuhan, China: a single-centre longitudinal study. *Clinical Microbiology and Infection* **27(1)**:89–95 DOI 10.1016/j.cmi.2020.09.023.

Yoo SM, Liu TC, Motwani Y, Sim MS, Viswanathan N, Samras N, Hsu F, Wenger NS. 2022. Factors associated with post-acute sequelae of SARS-CoV-2 (PASC) after diagnosis of symptomatic COVID-19 in the inpatient and outpatient setting in a diverse cohort. *Journal of General Internal Medicine* **37(8)**:1988–1995 DOI 10.1007/s11606-022-07523-3.

Zahradník J, Marciano S, Shemesh M, Zoler E, Harari D, Chiaravalli J, Meyer B, Rudich Y, Li C, Marton I, Dym O, Elad N, Lewis MG, Andersen H, Gagne M, Seder RA, Douek DC, Schreiber G. 2021. SARS-CoV-2 variant prediction and antiviral drug design are enabled by RBD in vitro evolution. *Nature Microbiology* **6(9)**:1188–1198 DOI 10.1038/s41564-021-00954-4.