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Short Communication

Critical pediatric neurological illness associated with COVID-19 (Omicron BA.2.3.7 variant) infection in Taiwan: immunological assessment and viral genome analysis in tertiary medical center



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ABSTRACT

Objectives: Since April 2022, another wave of the Omicron epidemic has struck Taiwanese society, and children with severe neurological complications have been reported frequently. A few cases even developed acute fulminant encephalitis. To investigate the possible causes of the increased incidence of such complications in Taiwan, we reviewed several cases of pediatric patients with severe neurological symptoms.

Methods: We collected the medical records of pediatric patients with COVID-19 infection who presented with severe neurological symptoms. The COVID-19 infection was diagnosed by nasal swab reverse transcriptase-polymerase chain reaction. The remaining samples were sent for whole genome sequencing and spike (S) protein amino acid variation mapping.

Results: The increase of several inflammatory markers was observed in all patients included in this study. However, none of the cerebrospinal fluid samples tested positive for SARS-CoV-2. The result of whole genome sequencing showed that all the sequences belonged to the lineage BA.2.3.7. However, the sequences had a K97E mutation in the S protein that differed from other BA.2.3.7 lineage strains, which was located at the S protein N-terminal domain.

Conclusion: The new mutation in the S protein, which had not previously been observed but was discovered in this study, potentially explains the sudden increase in incidence of extremely adverse neurological symptoms in pediatric patients.

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Abbreviation: NTD, N-terminal domain.

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1. Introduction

Since the COVID-19 pandemic outbreak, people all around the world have continued to fight it (Jian *et al.*, 2022). However, as the virus evolves, more variants of concern have been reported (Chung *et al.*, 2022). Patients with COVID-19 infection have also experienced neurological symptoms during the course of the infection (Nordvig *et al.*, 2021). However, severe neurological complications

Table 1
Patient characteristics and laboratory findings.

Patient N	Age	Sex	Clinical symptoms	SARS-CoV-2 PCR		Serum					CSF			
				Swab	CSF	LDH (U/l) 140-271	Ferritin (ng/ml) M: 30-400 F: 13-150	PCT (ng/ml) -0.05	D-dimer (mg/l) -0.5	IL-6 (pg/ml) <7.0	Opening pressure cmH2O	Cell count (<5/ μ l)	Glucose (mg/dl)	Total protein 15-45 (mg/dl)
#1	5y	F	Fever, drowsiness and nonsensical babbling, GTCS, shock complicated with multiple organ failure	pos.	N.A.	>12000	>20000	6.95	>20	1111	N.A.	N.A.	N.A.	N.A.
#2	4y	F	Fever, GTCS, drowsiness	pos.	neg.	451	164	0.5	<0.27	31.4	26.5	1251	87	112
#3	1y	M	Fever, vomit, dizziness, GTCS	pos.	neg.	216	188	0.85	<0.27	6.5	17.5	4	72	21
#4	3y	F	Fever, abdominal pain, vomit, GTCS	pos.	N.A.	300	86.3	1.51	N.A.	6.8	N.A.	N.A.	N.A.	N.A.
#5	2y5m	M	Fever, bilateral upward gaze with loss of consciousness and cyanotic change of lip, GTCS	pos.	neg.	258	75.2	1.08	0.62	103.3	15	5	68	26

CSF, cerebrospinal fluid; GTCS, generalized tonic-clonic seizure; IL-6, interleukin-6; LDH, lactate dehydrogenase; N.A., not available; PCR, polymerase chain reaction; PCT, Procalcitonin; pos., positive.

have tended to be more common in children recently (Dilber *et al.*, 2021; Valderas *et al.*, 2022).

In Taiwan, another wave of the pandemic began in April 2022. Among the reported pediatric cases, several patients presented with severe symptoms, including seizures, meningeal symptoms, and encephalopathy. These complications were also reported in Hong Kong (Tso *et al.*, 2022) and Japan over the previous Omicron epidemic. However, studies in the reports lacked viral genome sequencing data. Therefore, we investigated the pediatric patients with similar complications admitted to our hospital during the outbreak, and we attempted to elucidate commonalities between them.

2. Materials and methods

2.1. Study design and clinical specimens

We included the medical records and clinical samples of five patients with COVID-19 who were admitted to Tri-Service General Hospital, Taipei City, Taiwan, for suspected meningoencephalitis in May 2022. Participants were confirmed as positive for COVID-19 infection by reverse transcriptase-polymerase chain reaction, using nasopharyngeal swabs.

2.2. Whole genome sequencing of SARS-CoV-2

Ovation RNA-Seq System V2 (Nugen Technologies, San Carlos, CA, USA) was used to synthesize complementary DNA, which was then processed into a library. Whole genome sequences of the SARS-CoV-2 isolates (TSGH-70, TSGH-71, TSGH-72, TSGH-73, TSGH-74, TSGH-75, TSGH-76, and TSGH-77) were obtained from RNA using the Ion Torrent™ NGS Reverse Transcription Kit (ThermoFisher Scientific). Library construction was performed according to the Ion AmpliSeq™ SARS-CoV-2 Insight Research Assay (ThermoFisher Scientific) protocol. Sequencing was performed

on the Ion GeneStudio S5 Prime (ThermoFisher Scientific), according to Ion 510 & 520 & 530-Chef Kit specifications. Assembled sequences were uploaded to the Global initiative on sharing all influenza data (GISAID) database (<http://www.gisaid.org/>).

2.3. Mapping amino acid variation in spike (S) protein and phylogenetic relationship analysis

A total of 12 highly similar Taiwanese COVID-19 Omicron sequences identified by GISAID (including the three uploaded TSGH sequences from this study) were collected. CoV-GLUE (<http://cov-glue.cvr.gla.ac.uk>) was used to investigate amino acid variations in the three strains from our study and to identify S protein mutations. To investigate the amino acid variations in the S region of these Taiwanese strains and classify pathogen evolution relationships, further sequence analyses were executed using Nextclade (<http://clades.nextstrain.org/>) with Augur developed by Nextstrain (Hadfield *et al.*, 2018).

3. Results

3.1. Patient characteristics

The age of the participants ranged from 1 to 5 years (median = 3 years). All patients presented with neurological symptoms (Table 1) within 1-2 days after the onset of respiratory symptoms and febrile episodes.

Patient #1 ultimately passed away due to acute cerebral edema with brain stem compression and multiple organ failure. All other patients recovered fully and were discharged after a median of 6 days (4-9 days) stay in the hospital.

3.2. Laboratory findings

The biochemistry assays, which reflect inflammation status, showed prominent elevation, such as procalcitonin, lactate de-

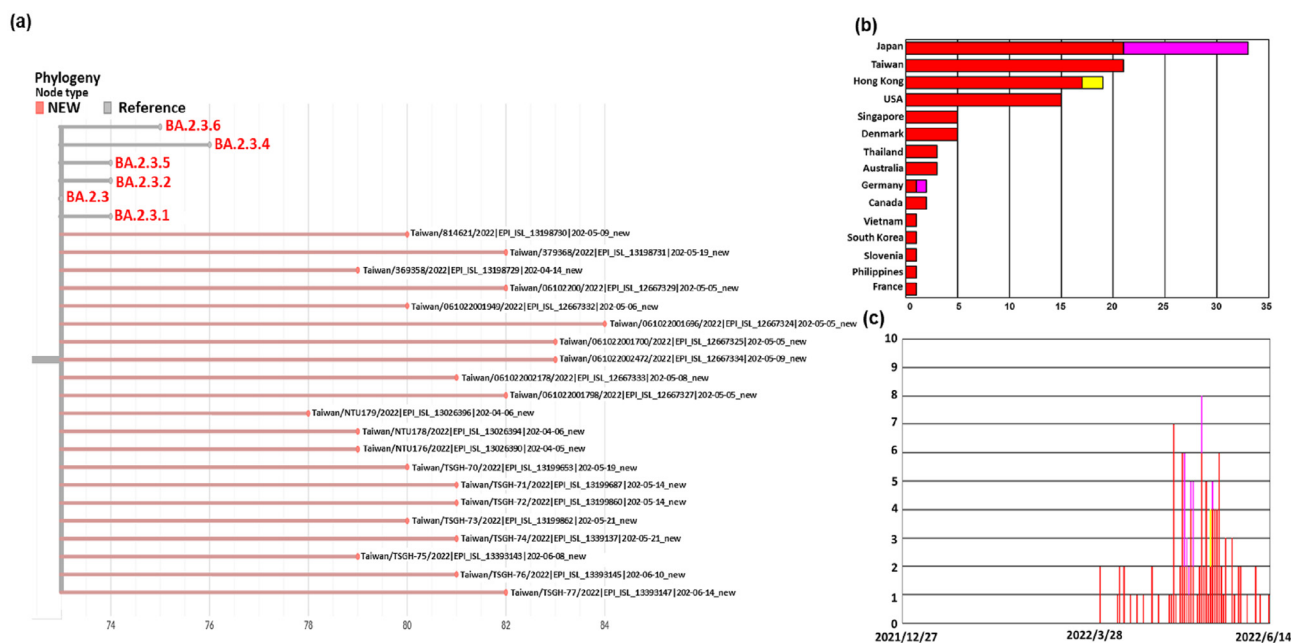


Figure 1. (a) Phylogenetic relationship of 21 Taiwanese Omicron sequences based on Nextclade. Major clade presents that these sequences is 21L when aligned to the reference sequences (Wu-Han SARS-CoV2). Branch point BA.2.3 shows only Taiwanese sequences (red line) were belonged to the new node as a potential subspecies of BA.2.3 lineage. (b) and (c): Using sequences with only K97E mutation point in spike protein shows Japan, Hong Kong, and USA are major high relationship and detection time is fit to the time of Omicron epidemic occurs in Taiwan. Red: Lineage BA.2.3. Yellow: BA.2. Pink: Unclassified lineage.

hydrogenase, and ferritin. Elevation of interleukin-6 was seen in three patients, which appeared to correlate with disease severity and intensive care unit length of the stay. Three patients had cerebrospinal fluid samples, and all were negative for SARS-CoV-2 polymerase chain reaction.

3.3. Whole genome sequencing and S protein amino acid variation mapping

Nextclade showed that the phylogenetic relationships of all the sequences were clustered as a new node different from other BA.2.3.7 lineage strains (Figure 1a). Surprisingly, all sequences in this subgroup displayed the S protein K97E mutation. Mutation diversity events in this group also indicated the same results in K97E, and this missense mutation point was in the N-terminal domain (NTD) region of the S protein, which was identified in host immune issues.

It is also interesting that when the S protein K97E mutation information was used in GISAID mapping, only Japan, Hong Kong, and the United States showed high correction (Figure 1b). Moreover, these K97E mutation strains appeared at the end of March 2022, corresponding with the beginning of the Taiwan COVID-19 Omicron epidemic (Figure 1c).

4. Discussion

Based on our patients' findings, the increased cytokine and inflammation markers, although having negative polymerase chain reaction results in cerebrospinal fluid, can lead to the conclusion that the causes of these severe neurological symptoms might be related to hyperimmune states rather than a direct viral invasion of the central nervous system.

Our analysis of the eight TSGH sequences indicated that a new Omicron BA.2.3.7 subspecies with special S protein K97E might have been produced in Taiwan, and these sequences are similar to Japan, the United States, and Hong Kong.

The location of K97 is between two beta sheets and the edge of the druggable cavity region of S protein NTD domain (Di Gaetano et al., 2021) and some extensive ubiquitination events have been observed in them (Stukalov et al., 2021), which might have an effect on immune regulation. Furthermore, mutations of the NTD region in S protein that allow for immune evasion have also been reported (Harvey et al., 2021).

Therefore, the K97E mutation, which has not been observed in Taiwan previously, potentially explains the sudden increase in the incidence of severe neurological symptoms in pediatric patients due to its possible effect on immune regulation.

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Ethical approval statement

This study was approved by the institutional review board of Tri-Service General Hospital (TSGHIRB number: C202005041) and registered on April 14, 2022.

Declaration of competing interests

The authors have no competing interests to declare.

CRediT authorship contribution statement

Chi-Sheng Chen: Methodology, Investigation, Data curation, Writing – original draft, Visualization. **Chia-Ning Chang:** Resources. **Chih-Fen Hu:** Resources. **Ming-Jr Jian:** Software. **Hsing-Yi Chung:** Software. **Chih-Kai Chang:** Software. **Cherng-Lih Perng:** Software, Validation. **Kuo-Sheng Hung:** Software, Validation.

Feng-Yee Chang: Supervision. **Chih-Hung Wang:** Supervision. **Shyi-Jou Chen:** Conceptualization, Data curation, Writing – review & editing. **Hung-Sheng Shang:** Conceptualization, Methodology, Writing – review & editing, Visualization.

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