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The identification and phylogenetic analysis of SARS-CoV-2 delta variants in Taiwan

Li-Teh Liu[1] | Jih-Jin Tsai[2,3,4] | Justin Jang Hann Chu[5,6,7,8] | Chun-Hong Chen[9,10] | Liang-Jen Chen[11] | Ping-Chang Lin[2] | Ching-Yi Tsai[2] | Miao-Chen Hsu[2] | Wan-Long Chuang[3,12] | Shang-Jyh Hwang[3,13] | Inn-Wen Chong[14,15]

[1]Department of Medical Laboratory Science and Biotechnology, College of Medical Technology, Chung-Hwa University of Medical Technology, Tainan City, Taiwan

[2]Tropical Medicine Center, Kaohsiung Medical University Hospital, Kaohsiung City, Taiwan

[3]School of Medicine, College of Medicine, Kaohsiung Medical University, Kaohsiung City, Taiwan

[4]Division of Infectious Diseases, Department of Internal Medicine, Kaohsiung Medical University Hospital, Kaohsiung City, Taiwan

[5]Department of Microbiology and Immunology, Yong Loo Lin School of Medicine, National University of Singapore, Singapore, Singapore

[6]Infectious Diseases Translational Research Programme, Yong Loo Lin School of Medicine, National University of Singapore, Singapore, Singapore

[7]NUS Medicine BSL3 Core Facility, Yong Loo Lin School of Medicine, National University of Singapore, Singapore, Singapore

[8]Institute of Molecular and Cell Biology (IMCB), A*STAR, Singapore, Singapore

[9]National Mosquito-Borne Diseases Control Research Center, National Health Research Institutes, Miaoli County, Taiwan

[10]National Institute of Infectious Diseases and Vaccinology, National Health Research Institutes, Miaoli County, Taiwan

[11]Department of Family Medicine, Pingtung Christian Hospital, Pingtung City, Taiwan

[12]Hepatobiliary Division, Department of Internal Medicine, Kaohsiung Medical University Hospital, Kaohsiung City, Taiwan

[13]Division of Nephrology, Department of Internal Medicine, Kaohsiung Medical University Hospital, Kaohsiung City, Taiwan

[14]Department of Internal Medicine and Graduate Institute of Medicine, Kaohsiung Medical University, Kaohsiung City, Taiwan

[15]Department of Pulmonary Medicine, Kaohsiung Medical University Hospital, Kaohsiung City, Taiwan

Correspondence
Jih-Jin Tsai, Division of Infectious Diseases, Department of Internal Medicine, Kaohsiung Medical University Hospital, No. 100, Tzyou 1st Road, Kaohsiung 80756, Taiwan.
Email: jijits@cc.kmu.edu.tw

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Abstract

In Taiwan, coronavirus disease 2019 (COVID-19) involving the delta variant occurred after that involving the alpha variant in 2021. In this study, we aimed to analyze the Delta variant. A total of 318 patients in Taiwan infected with delta variants were identified. The case fatality rate (CFR) of patients infected with delta variants was 0.94% in Taiwan compared with that of those infected with alpha variants (5.95%). The possible reasons for the low CFR might be hybrid immunity due to infection and rapid promotion of the COVID-19 vaccination program during the alpha variant outbreak. We identified three 21J delta variants. Two long gene deletions were detected in these severe acute respiratory syndrome coronavirus-2 (SARS-CoV-2) isolates: *ORF7a*Δ91 in KMUH-8 and *Spike*Δ30 in KMUH-9. Protein structure prediction indicates that *ORF7a*Δ91 results in malfunction of NS7a as an interferon antagonist and that *Spike*Δ30 results in a truncated spike protein (N679–A688del), resulting in a lower infection rate compared with the delta

variant without these deletions. The impact of these two deletions on SARS-CoV-2-associated pathogenesis deserves further investigation. Delta variants still exist in many regions in the omicron era, and the backbone of the delta variant genome possibly spread worldwide in the form of delta-omicron hybrids (deltacron; e.g., XBC.1 and XAY.2), which casts a potential threat to public health. Our study further highlighted the importance of more understanding of the delta variants.

KEYWORDS
COVID-19, delta variant, phylogenetic analysis, SARS-CoV-2, whole-genome sequencing

1 | INTRODUCTION

Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) was isolated from patients with coronavirus disease 2019 (COVID-19) in China in December 2019.[1] Inhalation of droplets containing SARS-CoV-2 from expiration of carriers or contact with virus-containing nasal or oral secretions can result in asymptomatic infection or mild to severe COVID-19.[2] Genetic mutations have accumulated during circulation of SARS-CoV-2 worldwide, leading to the emergence of variants with increased disease severity, mortality, and transmissibility as well as development of resistance to antivirals, vaccination, and immune responses. These situations have prompted the isolation and/or characterization of variants of interest and variants of concern (VOCs).[3] Many SARS-CoV-2 variants have been imported into Taiwan, and the most concerning variants before the omicron era were the alpha variant and the delta variant.[4,5] During the COVID-19 outbreak caused by the alpha variant in Taiwan,[4] the COVID-19 pandemic entered the pre-delta (April–May 2021) and delta emergence (June 2021) periods. By November 2021, the delta variant had spread to 179 countries and accounted for 90%–99% of all SARS-CoV-2 sequences submitted to GISAID,[6] with clinical samples being collected during the delta predominance period between July and November 2021. The case fatality rate (CFR) worldwide was 1.4%–1.7% in this period.[7] The epidemic of the delta variant in Taiwan began on June 14, 2021.[5]

In this study, we detected and isolated delta variants from COVID-19 patients infected with SARS-CoV-2 between July 2021 and January 2022. Whole-genome sequencing (WGS) was performed, and sequences were analyzed. Phylogenetic trees were constructed to demonstrate the possible origin of the isolates with other delta variants in Taiwan and worldwide. The CFR of delta variant-infected individuals was explored in comparison to the CFRs of alpha and omicron variant-infected individuals in Taiwan and worldwide.

2 | MATERIALS AND METHODS

2.1 | Ethics and sample collection

Kaohsiung Medical University Hospital (KMUH) is authorized by the Central Epidemic Command Center (CECC) in Taiwan to diagnose and treat suspected COVID-19 patients. Nasopharyngeal swabs from patients suspected to have COVID-19 were collected in Universal Transport Medium (UTM; Viral Transport Medium w/Special Swab, Creative Life Science, Taiwan). This study was reviewed and approved by the Institutional Review Board of Kaohsiung Medical University Hospital (KMUHIRB-E-I-20200013). Informed consent was obtained from all subjects involved in the study.

2.2 | RNA extraction from nasopharyngeal swabs and detection of SARS-CoV-2 genomic RNA

Total RNA in swab-UTM was extracted, and SARS-CoV-2 genomic RNA was evaluated by using quantitative reverse transcription-polymerase chain reaction (qRT-PCR) as described in our previous studies.[4,8] The primers, probes, mixtures, machine, and thermal cycling conditions used are listed in Table S1A–C. Swab-UTM samples with a positive PCR result were sent for SARS-CoV-2 culture.

2.3 | Isolation of SARS-CoV-2 in Vero E6 cells

One hundred microliters of a swab-UTM sample with a positive qRT-PCR result was inoculated into Vero E6 cells to isolate SARS-CoV-2 as described in our previous studies.[4,8] The cytopathic effect (CPE) was examined daily under a phase-contrast microscope. For samples that did not show a CPE after 3 days of incubation, a blind passage was performed until Day 21 to increase the chance for virus propagation and isolation, with medium replacement every 2–3 days.

2.4 | RNA library construction, WGS, and sequence analysis

Viral RNA extraction, RNA library construction, WGS, and sequence analysis were performed using swab-UTM samples, as described in our previous studies.[4,8]

2.5 | PCR amplification and Sanger sequencing

Spike, *n*, *orf7a*, and *orf8* gene fragments were amplified for Sanger sequencing to confirm single nucleotide variant (SNV) and InDel by

the methods described in our previous studies.[4,8] The variants checked and primer pairs used are listed in Table S1D.

2.6 | SARS-CoV-2 genome and phylogenetic analysis

Genomic sequences of SARS-CoV-2 were retrieved and downloaded from the GISAID EpiCoV and NCBI GenBank databases. Theoretical phylogenetic trees were reconstructed with the methods described in our previous studies.[4,8]

3 | RESULTS

3.1 | Delta SARS-CoV-2 in Taiwan

The first COVID-19 case caused by delta recorded in Taiwan was imported from abroad in March 2021 (EPI_ISL_11147495, sublineage AY.103). The first autochthonous COVID-19 case infected with the delta variant was case number 14,407, which was recorded in Taiwan in mid-June 2021. This patient was infected by a traveler who returned from Peru in early June, which was case number 13,332 (EPI_ISL_3279365, sublineage AY.46). The first autochthonous delta variant cluster infection event began with case number 14,407 and spread to their family (case numbers 14,408 and 14,409) in Pingtung County, southern Taiwan. The virus also spread to another patient (case 14,816) when case 14,407 was hospitalized for treatment. The virus spread to case 14,409 (EPI_ISL_3000157, sublineage AY.46), who transmitted it to case 14,298 (EPI_ISL_11362239, sublineage AY.46), a taxi driver who transported the patient. This cluster infection event resulted in 13 sequencing-confirmed COVID-19 patients infected with the delta variant. The six autochthonous cluster delta variant infections recorded in Taiwan are listed in Table 1.[9] A total of 318 delta variant COVID-19 cases were recorded until December 2022: 266 imported cases and 52 autochthonous cases. The CFR for all delta variant cases was 0.94% (3/318).

TABLE 1 Delta variant cluster infections that occurred in Taiwan between June and December 2021.

Cluster infections	Year: 2021	Number of cases[a]
Cluster 1	June 14–July 11	13
Cluster 2	August 28–September 1	3
Cluster 3	August 25–September 14	28
Cluster 4	December 3–December 15	6
Cluster 5	December 13	1
Cluster 6	December 12–December 24	1

Abbreviations: COVID-19, coronavirus disease 2019; SARS-CoV-2, severe acute respiratory syndrome coronavirus-2.
[a]The SARS-CoV-2 sequences in these COVID-19 patients were confirmed by sequencing to be delta variants.

3.2 | Detection, isolation, WGS, and data analysis of SARS-CoV-2 isolates KMUH-8, KMUH-9, and KMUH-10

During the COVID-19 epidemic in Taiwan between July 2021 and January 2022, genomic RNA of SARS-CoV-2 was detected in 10 nasopharyngeal swabs (Table 2). CPEs were observed in Vero E6 cells inoculated with three of these specimens (Figure S1). We isolated three strains (KMUH-8, 9, and 10), which were confirmed by qRT-PCR and the median tissue culture infectious dose ($TCID_{50}$; Table 2).

The genomic sequences of KMUH-8, KMUH-9, and KMUH-10 were deposited in the GenBank (OM278784, OM373110, and ON247405) and GISAID EpiCoV (EPI_ISL_8636666, EPI_ISL_8636668, and EPI_ISL_12005416.1) databases. These sequences were also analyzed by using Nextclade to confirm the SNV and InDel results analyzed in our genome workstation.[10] The KMUH-8, KMUH-9, and KMUH-10 sequences belong to the 21J (delta)/GK (NextStrain_clade/GISAID_clade) and pangolin lineages B.1.617.2 and sublineages AY.126, AY.4.5, and AY.121, respectively. These results are consistent with GISAID and the World Health Organization (WHO) data indicating that delta was the most common VOC during that period, except for KMUH-10 isolated from a clinical sample collected in January 2022, when omicron variants predominated worldwide. The consensus results of SNV and InDel analyses are shown in Table S2. Amino acid deletion events and nonsynonymous codon variations are shown in Table 3. These isolates share many delta variant-specific spike protein mutations, such as T19R, G142D, R158G, L452R, T478K, D614G, and D950N. Additional variations, such as spike protein N74K, V289 L, T678I, I850 L, D1259Y, E156del, and F157del, were detected (Table 3). We noted two long nucleotide deletions in KMUH-8 and KMUH-9. A 91-nt deletion of the *ORF7a* gene (*ORF7a*Δ91) in the KMUH-8 sequence disrupts the triplet reading frame and results in a predicted frameshift mutation with NS7a L49P and a premature termination codon at L77 (L77*; underlined in red in Figure 1A). WGS results also revealed a 30-nt deletion of the *spike* gene (*Spike*Δ30) in the KMUH-9 sequence that results in a truncated spike protein (N679–A688del; Figure 1B). The results of WGS coverage analysis, depth distribution, and some additional and rare SNVs and deletions in KMUH-8 to KMUH-10, as confirmed by Sanger sequencing, are shown in Figure 1. We did not detect any insertion event in these three delta variant sequences. The SNVs and deletions in the delta variant sequences in Table S2 display coverages of 99.69%–99.99% and depths between 10,000 and 200,000 compared with the reference sequence.

We performed nucleotide sequence comparison to determine whether any other SARS-CoV-2 genome contains the same *ORF7a*Δ91 or a similar deletion by using the blastn package of the basic local alignment sequence tool (BLAST). The sequence comparison results returned 100 SARS-CoV-2 genomic sequences with the most significant alignments with the full-length genomic sequence of KMUH-8: 99 other delta 21J AY.126 variants and KMUH-8 itself. There are at least 77 sequences that contain the same *ORF7a*Δ91 or a

TABLE 2 Detection of the presence of SARS-CoV-2 genomic RNA using qRT-PCR and the cytopathic effect in Vero E6 cell culture.

Sample number	Swab-UTM[a] (Ct)			CPE observed DPI[b]		Culture fluid[c] (Ct)					
	E gene	*RdRP* gene	*N* gene	Original swab	Blind passage[d]	*E* gene	*RdRP* gene	*N* gene	Strain	$TCID_{50}$[e]	Sample collection[f]
1	33.0	31.0	>40	Negative	Negative	ND	ND	ND	—	ND	16
2	22.53	22.85	21	7	6	13.35	17.66	22.80	KMUH-8	10^7	1
3	18.1	18.08	16.3	7	6	13.94	18.63	23.36	KMUH-9	$10^{6.7}$	6
4	34.0	32.0	>40	Negative	Negative	ND	ND	ND	—	ND	N
5	>40	26.0	>40	Negative	Negative	ND	ND	ND	—	ND	12
6	>40	29.0	>40	Negative	Negative	ND	ND	ND	—	ND	11
7	38.06	35.5	38	Negative	Negative	ND	ND	ND	—	ND	X
8	36.65	32.1	34.7	Negative	Negative	ND	ND	ND	—	ND	X
9	21.52	21.52	21.3	6	8	12.94	18.47	22.01	KMUH-10	$10^{4.7}$	X
10	33.0	32.0	>40	Negative	Negative	ND	ND	ND	—	ND	N

Abbreviations: CPE, cytopathic effect; SARS-CoV-2, severe acute respiratory syndrome coronavirus-2; UTM, Universal Transport Medium.
[a]RNA extracted from nasopharyngeal swab-UTM.
[b]DPI, days postinoculation.
[c]RNA extracted from culture supernatant from Vero E6 cells with CPE. ND, not determined.
[d]Culture medium from Vero E6 cells without CPE at 3 days postinoculation was transferred to a well with fresh confluent Vero E6 cells, and CPE was examined daily until Day 21.
[e]ND, not determined; $TCID_{50}$, median tissue culture infectious dose.
[f]The sample was collected days after symptom onset. N, The patient had an asymptomatic infection; X, Postsymptom-onset information was missing.

similar deletion as that observed in KMUH-8 (Figure S2A). Next, we performed protein sequence comparison to assess whether any other SARS-CoV-2 NS7a protein contains the same or similar amino acid deletions and premature termination. The sequence comparison returned 100 SARS-CoV-2 NS7a sequences. The results revealed no NS7a protein deposited in GenBank containing the same frameshift mutation and premature termination at L77 as KMUH-8. However, we did find similar truncations and premature termination at L77 in some truncated ORF7a proteins (Figure S2B). Furthermore, no similar *ORF7a*Δ91 among other delta variants have been isolated in Taiwan (Figure S2C).

The genomic sequence of KMUH-9 was aligned with other delta variants isolated in Taiwan to determine whether *Spike*Δ30 is present. Unexpectedly, the results revealed no other delta variant containing *Spike*Δ30, similar to KMUH-9 (Figure S3). To understand the prevalence of the *Spike*Δ30 variant identified in this study, we performed BLAST analysis against the coronavirus genomes in the NCBI datasets (6,592,930 nucleotide records on December 31, 2022) using the full-length genome of KMUH-9 as a query. The BLAST results returned 100 hits. Next, we searched for SARS-CoV-2 variants containing spike N679del and A688del in the GISAID EpiCoV database (14,421,640 viruses on December 31, 2022). The results included 314 spike N679del and 159 spike A688del SARS-CoV-2 sequences; 51 SARS-CoV-2 sequences contain the spike N679–A688 deletion, including wild-type SARS-CoV-2, alpha, and delta variants. Prior to KMUH-9, at most 18 sequences containing *Spike*Δ30 had been identified. These results indicate that only one delta variant (Sweden/DeltaCoV_Isolate_1/2021) has the same *Spike*Δ30 as KMUH-9 (Table 4). Therefore, the frequency of the delta variant with *Spike*Δ30 was two of 2.1 million in GISAID and GenBank (December 31, 2022).

3.3 | Phylogenetic analysis of delta variants isolated in Taiwan

We downloaded the genomes of delta variants from GISAID to understand the phylogenetic correlations of the delta variants isolated in Taiwan. The sequence set contained 39 delta variants, with submission dates between March 2021 and December 2022. These 39 delta variant sequences and two reference sequences were analyzed to find the most appropriate evolutionary model before reconstruction of the phylogenetic tree.[4,8] The results demonstrated that delta lineages 21A, 21I, and 21J clustered, respectively (Figure 2). The results suggest that KMUH-9/August 21, 2021 is most similar to 16,028/August 20, 2021 (EPI_ISL_3987576). Although KMUH-8 and KMUH-10 are phylogenetically similar to some delta variants in the phylogenetic tree, the results indicate that these were not isolated from the same cluster of infected patients. The 13,332/June 14, 2021 variant was isolated from a traveler who returned from Peru in early June, as mentioned above. The 14,409/June 22, 2021 and 14,298/June 22, 2021 variants were isolated from COVID-19 patients in Cluster 1 (Table 1), in which the virus spread from the patient (Case 13,332 in Taiwan) infected with 13,332/June 14, 2021. As we did not have information on NTU92 and NTU112, we could not confirm whether these two delta variants were isolated from cluster infections in Taiwan.

3.4 | Investigation of the possible origin of the three delta variants isolated in this study by using ultrafast sample placement on existing tRee

We performed real-time phylogenetic analysis using ultrafast sample placement on existing tRee (UShER)[13] to find the most similar

TABLE 3 Sequence variation of KMUH-8 to KMUH-10 compared with the reference hCoV-19/Wuhan/WIV04/2019.[a]

Viral gene	Nucleotide position	Genomic variation	Variant type	Protein	AA position	KMUH-8	KMUH-9	KMUH-10
Spike	22,029	6-nt del	Amino acid deletion	Spike	156–157del	**X**	**X**	**X**
	23,595	30-nt del			**679-688del**	O	**X**	O
ORF7a	27,539	91-nt del		NS7a	**L49P, L77***[b]	**X**	O	O
ORF8	28,248	6-nt del		NS8	119–120del	**X**	**X**	**X**
ORF1a	4181	G→U	Codon change[c]	NSP3	A488S	X	X	X
	5861	G→A			V1048I	X	O	O
	6402	C→U			P1228L	X	X	X
	7124	C→U			P1469S	X	X	X
	7851	C→U			A1711V	O	X	X
	9053	G→U		NSP4	V167L	X	X	X
	10,029	C→U			T492I	X	X	X
	11,201	A→G		NSP6	T77A	X	X	X
ORF1b	14,408	C→U		NSP12	P323L	X	X	X
	14,882	A→C			D481A	O	X	O
	15,451	G→A			G671S	X	X	X
	16,466	C→U		NSP13	P77L	X	X	X
	16,726	C→U			H164Y	O	O	X
	17,746	C→U			P504S	O	O	X
	19,220	C→U		NSP14	A394V	X	X	X
	19,859	C→U		NSP15	A80V	O	O	X
	19,984	U→C			F122L	X	O	O
	21,137	A→G		NSP16	K160R	X	O	O
Spike	21,618	C→G		Spike	T19R	X	X	X
	21,784	T→A			N74K	**X**	O	O
	21,846	C→U			T95I	X	X	X
	21,987	G→A			G142D	X	X	X
	22,029	6-nt del			R158G	X	X	X
	22,427	G→U			V289L	O	**X**	O
	22,917	U→G			L452R	X	X	X
	22,995	C→A			T478K	X	X	X
	23,403	A→G			D614G	X	X	X
	23,595	C→U			T678I	**X**	O	O
	23,604	C→G			P681R	X	O	X
	24,110	A→C			I850L	**X**	O	O
	24,410	G→A			D950N	X	X	X
	25,337	G→U			D1259Y	O	**X**	O
ORF3a	25,439	A→C		NS3	K16T	X	O	O
	25,469	C→U			S26L	X	X	X
	25,513	C→U			L41F	X	O	O
	25,996	G→U			V202L	O	O	X
	26,062	G→U			G224C	X	O	O
M	26,767	U→C		M	I82T	X	X	X
ORF7a	27,638	U→C		NS7a	V82A	X	X	X
	27,752	U→T			T120I	X	X	X
ORF7b	27,874	U→T		NS7b	T40I	X	X	X

(Continues)

TABLE 3 (Continued)

Viral gene	Nucleotide position	Genomic variation	Variant type	Protein	AA position	KMUH-8	KMUH-9	KMUH-10
N	28,347	G→U		N	G25V	O	**X**	O
	28,461	A→G			D63G	X	X	X
	28,881	G→U			R203M	X	X	X
	28,916	G→U			G215C	X	X	X
	29,402	G→U			D377Y	X	X	X

[a]X, with this variation; O, without this variation. Please refer to Figure 1 for the Sanger sequencing results of the sequence variations labeled in bold type on the right.
[b]This 91-nt deletion of the *ORF7a* gene disrupts the triplet reading frame and results in a predicted frameshift mutation with NS7a L49P and a premature termination codon at L77 (L77*).
[c]We display only nonsynonymous codon variations in this table; please refer to Table S2 for full information..

complete and high-coverage SARS-CoV-2 sequences from publicly available SARS-CoV-2 databases (e.g., GISAID, GenBank COG-UK, and CNCB; the analysis was performed on December 31, 2022) and reveal the possible origin of the three delta variants isolated in this study. According to the results, KMUH-8 is similar to SARS-CoV-2 sequences isolated in Germany, Spain, England, Turkey, Canada, Sweden, and Switzerland (Figure 3A). When considering the sample collection date, KMUH-8 and Canada/QC-L00379049001/2021|EPI_ISL_6424146|August 19, 2021 likely originated from the same ancestors. The results for KMUH-9 suggest that it is most similar to Taiwan/16028/2021| EPI_ISL_3987576|August 20, 2021 (Figure 3B), which was isolated from COVID-19 case number 16,028 in Taiwan. In addition, KMUH-9 is similar to SARS-CoV-2 sequences isolated in Denmark, Lithuania, and Germany. KMUH-9 was isolated from a patient who returned to Taiwan from Germany, and the patient involved in case 16,028 returned to Taiwan from Japan and had received two doses of SARS-CoV-2 vaccine. The results for KMUH-10 suggest that it is most similar to OV528101.1|December 9, 2021, Germany/BW-RKI-I-349949/2021|EPI ISL 7107280|November 24, 2021, OV469849.1|December 8, 2021, and Germany/BY-RKI-I-387809/2021|EPI ISL 8221217|December 8, 2021, which were isolated in Germany (Figure 3C).

4 | DISCUSSION

The results of WGS and in silico bioinformatics analyses suggested that our three delta variants, KMUH-8, KMUH-9, and KMUH-10, belong to the Pango lineages AY.126, AY.4.5, and AY.121, respectively. These viruses share similar amino acid deletions (e.g., spike 156–157del and NS8 119–120del), nonsynonymous amino acid variations in the spike protein (e.g., spike T19R, T95N, G142D, R158G, L452R, T478K, D614G, and D950N), and a few SNVs across the *ORF1a*, *ORF1b*, *ORF3a*, *M*, *ORF7a*, *ORF7b*, *N*, and *ORF9b* genes. Phylogenetic analysis of the 39 delta variants, which were isolated in Taiwan and deposited in GSAID, with the aid of Pango lineage annotation revealed that the KMUH-8 and KMUH-10 isolates are not from the same cluster of infections as other isolates. Furthermore, the travel history demonstrated that KMUH-9 and the 16,028/August 20, 2021 isolate did not originate from the same cluster infection. In addition to the common sequence variations in delta variants,[14] our data show additional SNVs and deletions in the genomic sequences of KMUH-8 and KMUH-9 that result in predicted amino acid replacement and truncation of viral proteins. The frequencies of spike N74K, V289 L, T678I, I850 L, D1259Y, and N679–A688del in the delta variants deposited in GISAID, with clinical samples collected between January 2020 and December 2022, are shown in Table S3. The virologic and pathological functions of these rare genetic variations have not been systematically investigated and reported in the peer-reviewed literature. In contrast to the additional SNVs and deletions, spike E156G/Δ157-158 or Δ156-157/R158G was a frequent event among delta variants (Table S3). It is worth noting that the spike N74 glycosylation site is predicted to be absent from the naturally occurring K74 variant, and the N74K-D614G variant exhibits stronger binding energy and higher infectivity than wild-type SARS-CoV-2.[15] Among these rare spike variations, only one delta variant isolate in addition to KMUH-9, namely, Sweden/DeltaCoV_Isolate_1/2021, contain spike N679–A688del resulting from *Spike*Δ30. Only 51 SARS-CoV-2 sequences containing the spike N679–A688 deletion were found in the GISAID and GenBank databases; spike N679–A688del is an extremely rare genetic variation event among delta variants. The first documented virus containing the *Spike*Δ30, resulting in spike N679–A688 deletion, was identified in Hong Kong in the first season of 2020. It appears that spike N679–A688del was not retained as the virus evolved. Indeed, it seems more likely that the delta variant acquired spike 679–688del through an independent genetic event during replication, resulting in a variant similar to KMUH-9 (Figure 4). The rarity of the spike N679–A688 deletion in GISAID and GenBank suggests that SARS-CoV-2 with this deletion has never caused superspreading events within the past 3 years. It is also possible that people infected with SARS-CoV-2 harboring the spike N679–A688 deletion had asymptomatic infections or experienced self-limited mild symptoms. Thus, they did not seek medical treatment, and surveillance of this deletion variant was limited. A previous study revealed that a furin cleavage site (FCS) around spike Q675–R685 is essential for

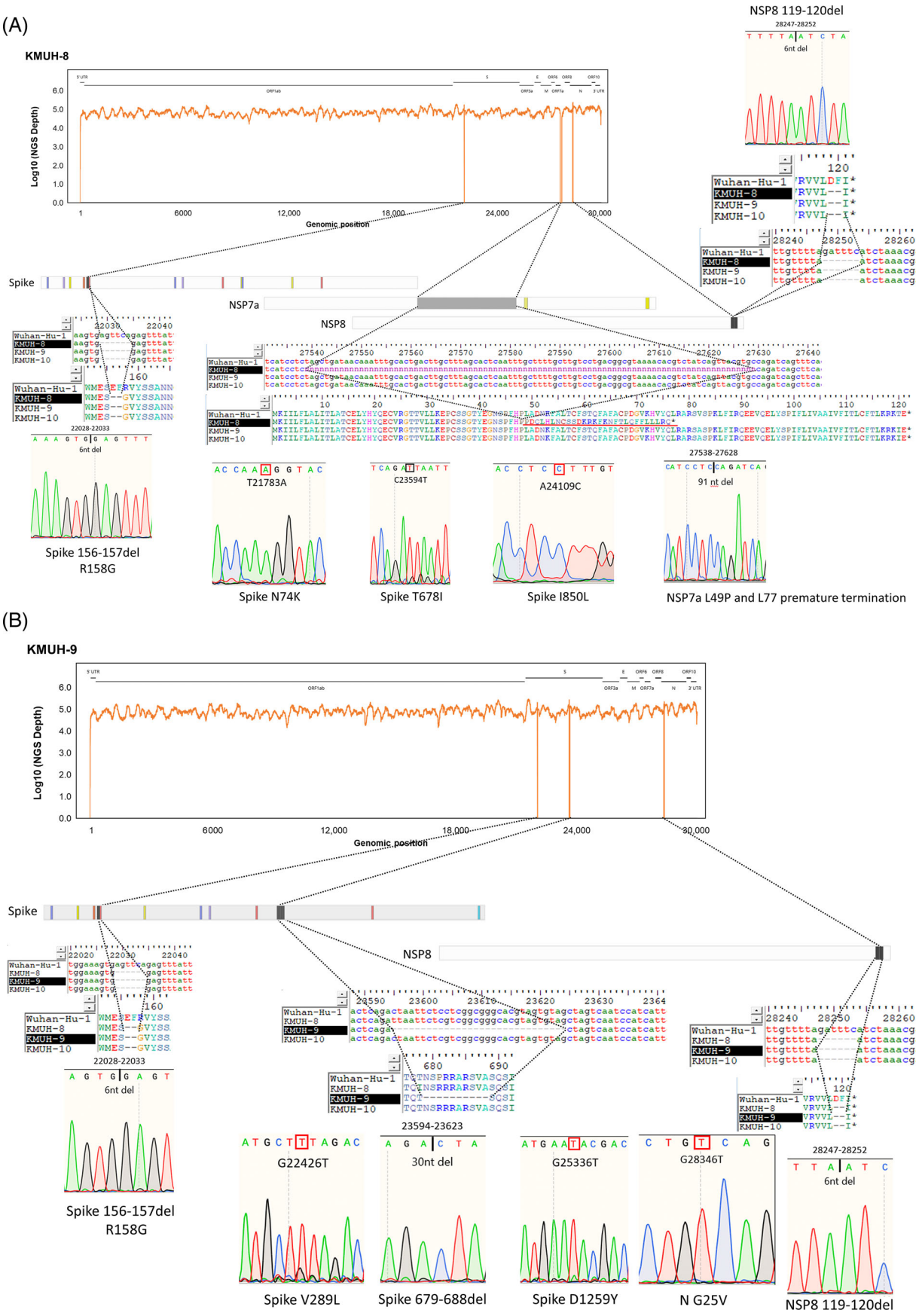

FIGURE 1 Presentation of whole-genome sequencing coverage, depth, and Sanger sequencing results of selected single nucleotide variants and deletions of severe acute respiratory syndrome coronavirus-2 isolated in this study. (A) KMUH-8. (B) KMUH-9. (C) KMUH-10. Genomic RNA sequences and amino acid sequences were aligned by using BioEdit 7.2.[11]

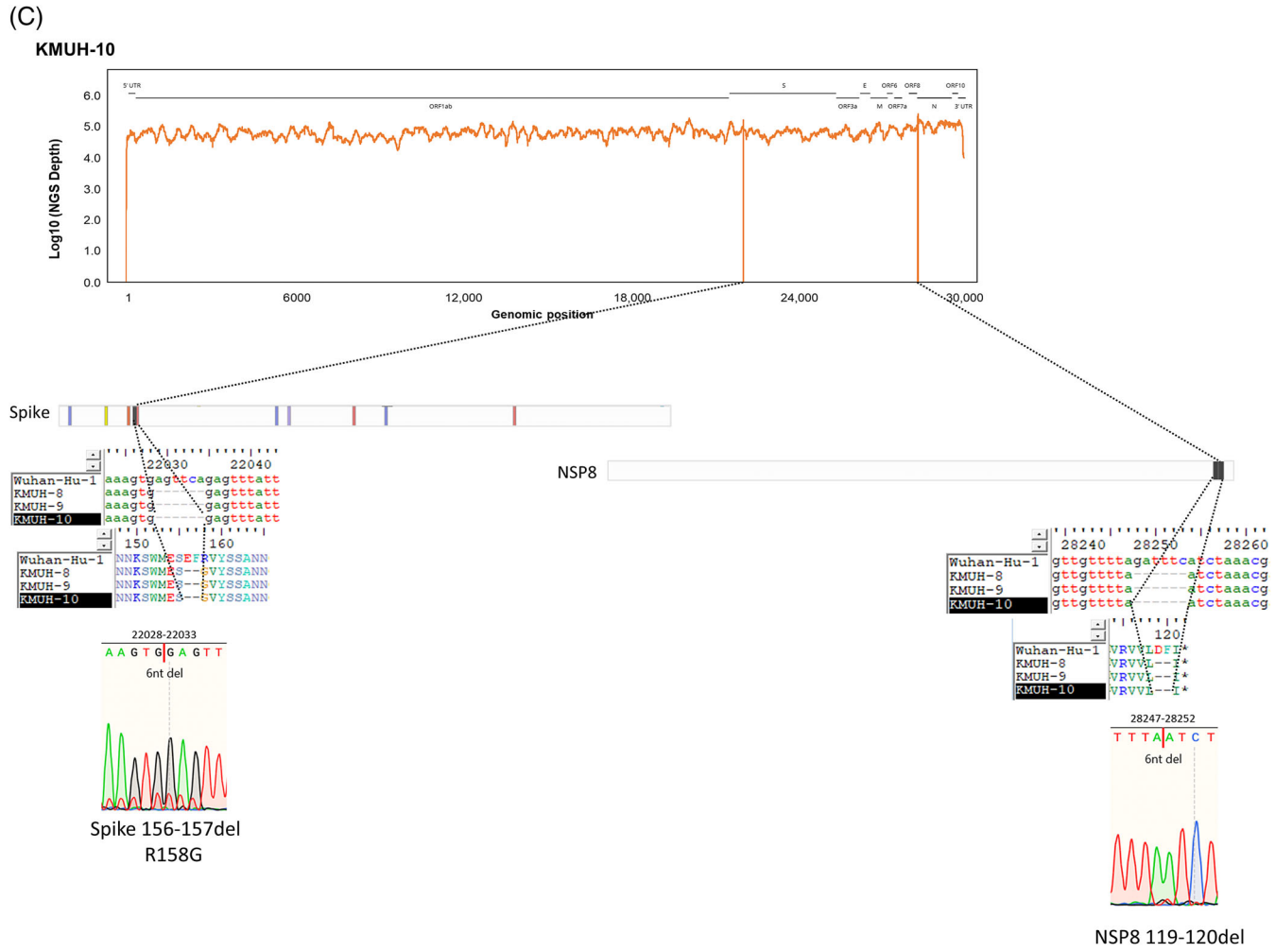

FIGURE 1 (Continued)

viral entry and infection of human lung cells.[16] Moreover, Cantuti-Castelvetri et al.[17] suggested that a variant with spike 675–679del (QTQTN) loses its FCS, impairing NRP-1-potentiating ACE-2 infection of host cells. Spike N679–A688del might also result in loss of the FCS around spike Q675–R685 or affect the 3D structure around the S1/S2 site (Q675–R685), resulting in a lower infection rate compared with delta variants without this deletion. However, more investigations are needed to address these matters.

NS7a, a transmembrane protein with an immunoglobulin-like domain and ~ 85% sequence identity to SARS-CoV-1 NS7a,[18,19] is not essential for viral replication. Nevertheless, it is reported to induce apoptosis via a caspase-dependent pathway.[20] Recent studies have revealed that the SARS-CoV-2 NS7a protein is an immunomodulator of human CD14+ monocytes, triggering an aberrant inflammatory response,[21] and it is described as an effective interferon antagonist.[19] It should be noted that *ORF7a*Δ91 results in amino acid substitutions in the βD of the ectodomain[18] of the NS7a protein (L49P) and a premature termination codon at L77. Crystal structure analysis of NS7a by X-ray crystallography has revealed that it contains seven β-strands and is divided into two tightly packed β-sheets.[18] Additionally, protein structure prediction by sequence alignments for homology modeling[22] suggests alteration of the 3D structure of NS7a of KMUH-8 compared with that of wild-type (Figure S4).[23] Wild-type NS7a contains seven β-strands (Figure S4A), which is similar to the results obtained by x-ray crystallography.[18] The predicted KMUH-8 NS7a includes only two β-strands (Figure S4B), with possible loss of function as an interferon antagonist.[19] Although SARS-CoV-2 defective in antagonizing the IFN-I response might be developed as a live-attenuated vaccine candidate,[24] the impact of *ORF7a*Δ91 on the function of the NS7a protein should be examined for IFN-I antagonism in cell culture and animal models.

The CFR of COVID-19 patients infected with delta variants in Taiwan was 0.94% (3/318) between June 2021 and December 2022. The estimated CFR for delta variants, which accounted for 90%–99% of COVID-19 cases worldwide between July 2021 and November 2021, was 1.4%–1.7% in the delta predominance period.[7] The CFR of COVID-19 patients infected with alpha variants was 5.95% (820/13,795) during the fourth wave of the outbreak in Taiwan between May and June 2021.[4] Previous studies have suggested that the delta variant is 43%–68% more transmissible than the alpha

TABLE 4 List of SARS-CoV-2 containing the spike protein N679–A688 deletion identified before KMUH-9.

Name	Collection date	Accession number	Nextstrain clade	Pangolin lineage
HongKong/XM-PII-S4/2020	January 22, 2020	EPI_ISL_417443	19B	A
HKG/HKU_HK/2020	March 1, 2020	MT621560.1	19B	A
Slovenia/4265/2020	March 17, 2020	EPI_ISL_635205	20B	B.1.1
HKG/Ca-DelMut/2020	June 1, 2020	MT862537.1	19B	A
USA/MO-WUSTL069/2020[a]	June 19, 2020	EPI_ISL_493086	19B	A
USA/MT-Chang-36,929/2020[a]	October 1, 2020	EPI_ISL_9275581.3	19B	A
France/PAC-IHU-3386-1/2020	December 11, 2020	EPI_ISL_1109982	20C	B.1.160
USA/TX-HMH-MCoV-33,204/2020[b]	December 17, 2020	EPI_ISL_2200160	20C	B.1
USA/TX-HMH-MCoV-32,348/2020[c]	December 18, 2020	EPI_ISL_2190982	20G	B.1.2
USA/MT-Chang-E1862/2020[a]	December 21, 2020	EPI_ISL_9275608.3	19B	A
Sweden/DeltaCoV_Isolate_1/2021	January 1, 2021	EPI_ISL_10866183	21J (Delta)	AY.43
Spain/MD-14170721/2021[d]	January 1, 2021	EPI_ISL_1753913	20I (Alpha)	B.1.1.7
Germany/BY-TRES328-escape/2021	January 1, 2021	EPI_ISL_2718549	20A	B.1
England/PHEC-L305L0F9/2021[a]	January 1, 2021	EPI_ISL_2749072	19B	A
India/MH-ICMR-MCL-21-221-3733-Plaque Purified-1-P1/2021	January 10, 2021	EPI_ISL_13857846	20B	P.2
India/MH-ICMR-MCL-21-221-3735-Plaque_Purified-3-P1/2021[e]	January 10, 2021	EPI_ISL_13857848	20B	P.2
South Korea/KDCA595s/2021	March 10, 2021	EPI_ISL_1646760	19B	A.18
Canada/BC-BCCDC-92432/2021	May 20, 2021	EPI_ISL_3486876	20I (Alpha)	B.1.1.7
Taiwan/KMUH-9/2021	August 21, 2021	OM373110	21J (Delta)	AY.4.5

Abbreviation: SARS-CoV-2, severe acute respiratory syndrome coronavirus-2.
[a]Spike T678-S689.
[b]Spike N679-I693.
[c]Spike N679–A694.
[d]Spike G667–L699.
[e]Spike A668–N703.

variant[25] and that the delta variant is associated with higher infectious virus loads than the alpha variant in both unvaccinated and vaccinated people.[26] Twohig et al.[27] recently reported "a higher hospital admission and hospital admission/emergency care attendance risk for patients with COVID-19 infected with the delta variant compared with the alpha variant", at 2.26 (adjusted hazard ratio; 95% CI: 1.32–3.89) and 1.45 (95% CI: 1.08–1.95), respectively. Furthermore, Ong et al.[28] suggested that COVID-19 patients infected with the delta variant have higher risk for ICU admission and mortality than those infected with the alpha variant when compared with those infected with wild-type, at 1.88 (adjusted odds ratio [aOR]; 95% CI: 0.30–14.76) and 4.90 (95% CI: 1.43–30.78), respectively (Table S4). However, the delta variant did not cause the expected large outbreak in Taiwan, and the CFR of patients infected with delta variants was relatively low compared with that of those infected with alpha variants. This difference might be attributable to the following reasons in addition to the measures taken before the first autochthonous delta variant cluster infection event occurred (e.g., intensive and immediate contact tracing, mobile application help in deploying masks, wearing facemasks, alcohol-based hand hygiene, and social distancing). First, hybrid immunity is acquired from infection[29,30] and rapid propagation of vaccination programs. The outbreak of alpha variants that started in May 2021 enhanced people's willingness to be vaccinated, with the vaccination rate increasing from 6.9% (June 21, 2021) to 79.99% for the first dose and 69.07% for the second dose (December 31, 2021).[9] Second, many lessons were learned from the alpha variant outbreak. The official clinical guidelines were modified to involve triage of mild or asymptomatic cases in centralized quarantine centers rather than admittance to hospitals. This action avoided crowding-out effects on patients with severe COVID-19 by patients with mild COVID-19 who were previously mandatorily admitted to the hospital. Previously insufficient antiviral drugs (e.g., remdesivir and anti-SARS-CoV-2 monoclonal antibody drugs) and medical equipment (e.g., high-flow nasal cannula oxygen therapy) were replenished promptly. Third, quick responses were taken against the cluster infections initiated by the delta variant. The government stopped the delta-SARS-CoV-2 outbreak through rapid epidemic investigations, expanding quarantine, and screening of SARS-CoV-2 antigen.[5]

Omicron has been the most prevalent VOC since late December 2021. Approximately 390 omicron sublineages have been documented since then.[6] Recent studies suggest that COVID-19 patients infected

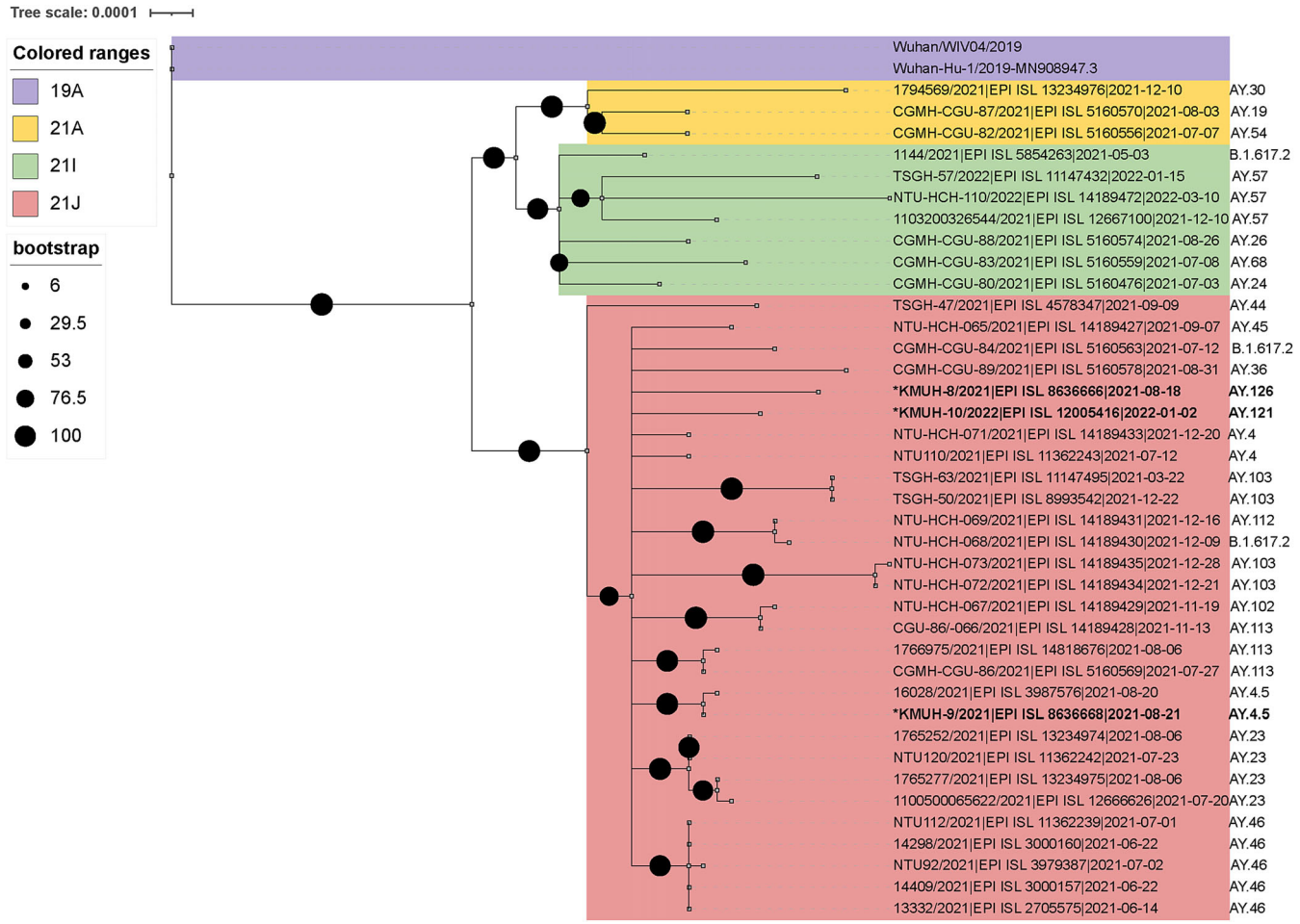

FIGURE 2 Phylogenetic tree of all 39 delta variant sequences deposited in GISAID EpiCoV from samples collected in Taiwan between January 2020 and December 2022. Phylogenetic analysis was inferred by using the maximum likelihood and fits of 484 different nucleotide substitution models, and the results suggest TIM + F + I as the best-fitting model with the lowest Bayesian information criterion scores of 86,380.234 among the 484 models tested. Tree topology was automatically computed to estimate maximum likelihood values. The optimal log-likelihood for this computation was −42,825.299. There were a total of 29,723 positions in the final dataset. The original tree is displayed using iTOL[12] with an indicator of bootstrap values and a scale bar. Viruses are shown as the virus name/sample collection date.

with the delta variant have a higher rate of hospitalization, ICU admission, and COVID-19-related death than those infected with the omicron variant, with a trend of Delta>BA.1 ≥ BA.2 (Table S4).[31–34] Nonetheless, the severity of COVID-19 appears to be decreasing after accounting for various factors, including therapeutics, vaccinations, previous infections,[34] and altered type II transmembrane serine protease (TMPRSS2) usage by SARS-CoV-2.[35] Several sublineages circulated in Taiwan in 2022, such as BA.1, BA.2, BA.4, BA.5, BA.2.75, BF.7, BQ.1, and XBB.[9] Delta-omicron hybrids resulting from interlineage recombination were detected in early 2021,[36] and the list is growing (e.g., XBC.1 and XAY.2).[6] The backbone of the delta variant genome possibly spread worldwide in the form of deltacron, which may be just as lethal as delta and transmit as rapidly as omicron.[27,37] Although delta variants are relatively rare in the omicron era, they still existed in North America, South America, Europe, and Africa until December 2022 (data were accessed from GISAID on January 1, 2023). In addition, in the second half of 2022, there were 6.7 times as many newly discovered delta variants deposited in GISIAD as alpha variants, even though both variants were discovered at almost the same time in 2020. Furthermore, SARS-CoV-2 has been found in animals,[38–40] including the delta variant.[40] SARS-CoV-2-susceptible animal species represent niches for viral reservoirs, and these viruses that exist in animals may someday return to humans.

There are some limitations to this study. First, the SARS-CoV-2 sequences used for sequence alignment analysis and phylogenetic tree reconstruction were downloaded from freely accessed public sequence repositories and may not fully represent all epidemic delta SARS-CoV-2 variants during the time investigated. Second, how *ORF7a*Δ91 and *Spike*Δ30 affect the function of viral proteins, behavior of the delta variant, and/or relevance to infectivity/pathogenesis might be important but are unclear at present and warrant additional research.

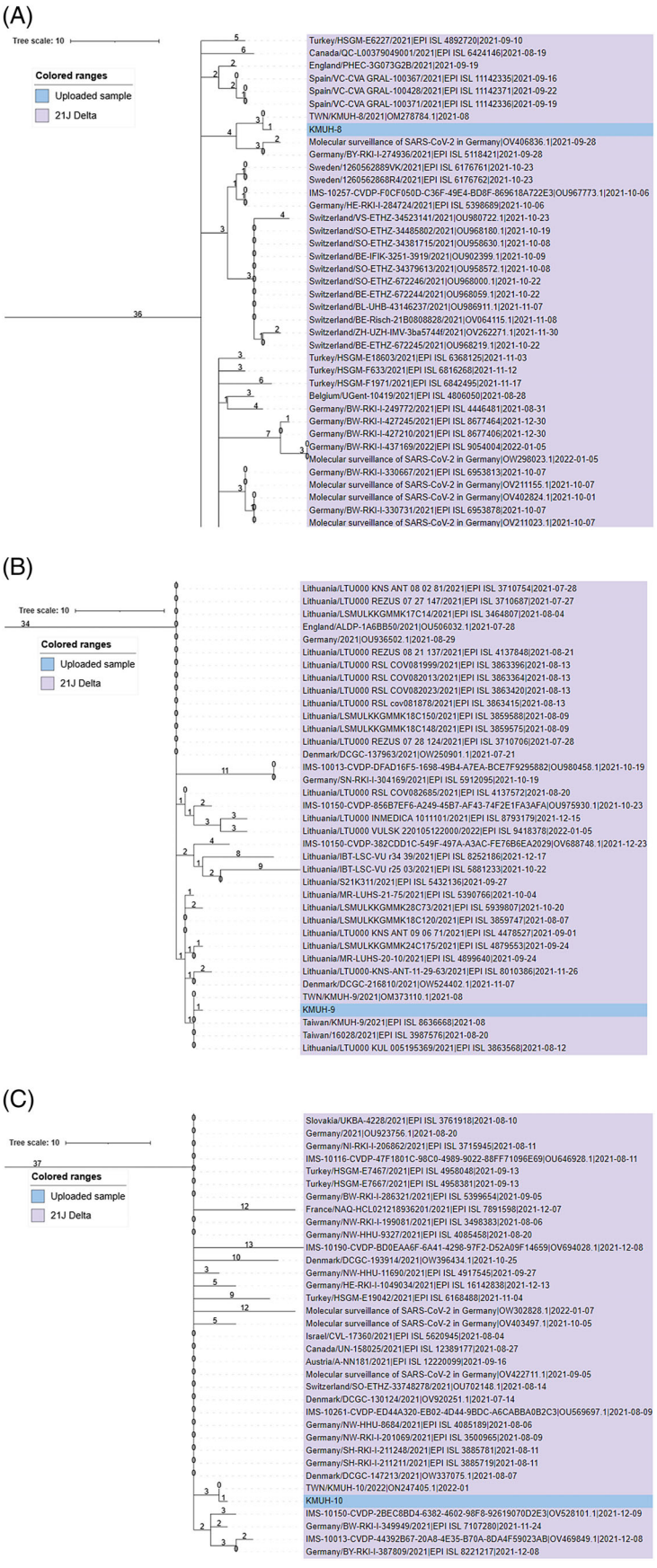

FIGURE 3 Phylogenetic analysis of KMUH-8, KMUH-9, and KMUH-10 using UShER. UShER enables real-time sequence placement for the severe acute respiratory syndrome coronavirus-2 pandemic using an existing phylogenetic tree generated by the sarscov2phylo pipeline, containing 13,419,668 genomes from GISAID, GenBank, COG-UK, and CNCB (December 31, 2022). The phylogenetic tree data were visualized using iTOL.[12] The 60 nearest neighboring SARS-CoV-2 sequences, including the sequence uploaded for analysis, already in the existing phylogenetic tree comprised the output for visualization for each input sequence. (A) Partial tree of the 60 nearest neighboring sequences to KMUH-8. (B) Partial tree of the 60 nearest neighboring sequences to KMUH-9. (C) Partial tree of the 60 nearest neighboring sequences to KMUH-10.

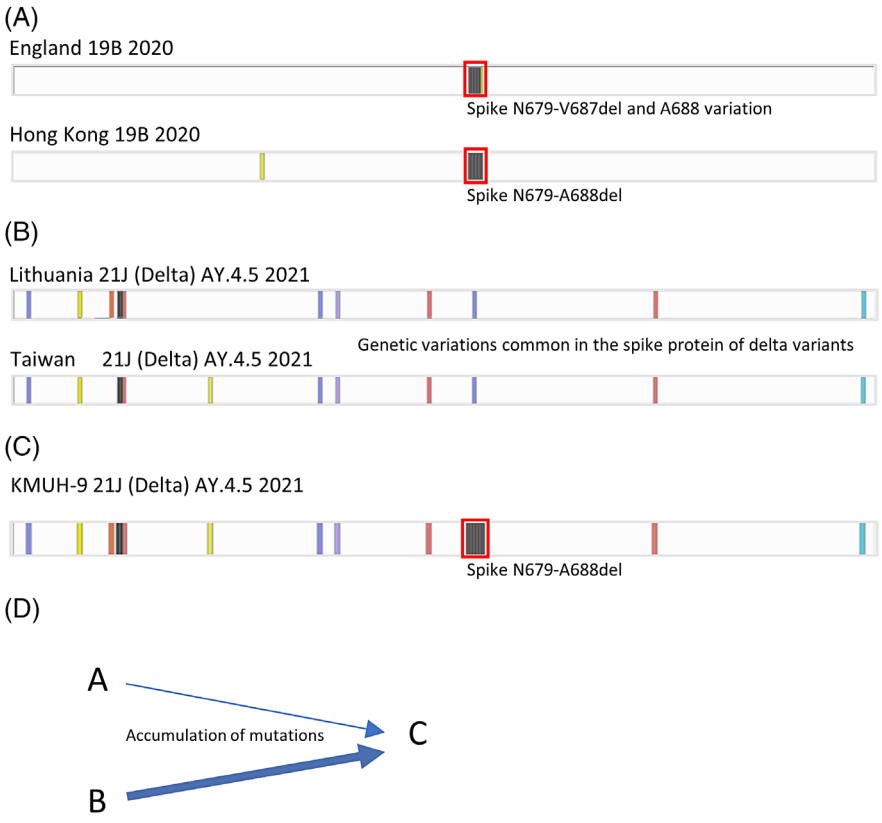

FIGURE 4 Emergence of KMUH-9. (A) Severe acute respiratory syndrome coronavirus-2 (SARS-CoV-2) variants with spike N697–A698del or similar genetic variations in the early stage of the coronavirus disease 2019 pandemic. (B) SARS-CoV-2 variants possess genetic variations common in the spike protein of delta variants. (C) KMUH-9 contains common genetic variations and an additional N679–A688 deletion in spike. (D) The possible process by which KMUH-9 arose. Other colored bars indicate amino acid substitutions in the spike protein.

In summary, we isolated three SARS-CoV-2 delta variants and identified two long gene deletions: *ORF7a*Δ91 in KMUH-8 and *Spike*Δ30 in KMUH-9. The impact of these two deletions on SARS-CoV-2-associated pathogenesis deserves further investigation. The delta variants did not cause the expected large outbreak in Taiwan, and the CFR of patients infected with delta variants was relatively low (0.94%) compared with that of those infected with alpha variants (5.95%). Although delta variants are relatively rare in the omicron era, they still exist on many continents, and the backbone of the delta variant genome may spread worldwide in the form of deltacrons (e.g., XBC.1 and XAY.2), potentially threatening public health. Our study highlights the importance of a better understanding of delta variants.

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CONFLICT OF INTEREST STATEMENT

All authors declare no conflict of interest.

ORCID

Li-Teh Liu https://orcid.org/0000-0002-7993-7263
Jih-Jin Tsai https://orcid.org/0000-0003-2226-8916
Shang-Jyh Hwang https://orcid.org/0000-0002-9404-3305

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SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

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