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Original Article

Genetic characterization of respiratory syncytial virus surface glycoproteins F and G in Taiwan, 2017—2021

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Abstract *Background:* Respiratory syncytial virus (RSV) infection imposes substantial health burden and disproportionally affects young infants, elderly, and immunocompromised hosts. RSV harbors key surface glycoproteins F and G, both crucial for viral infection and evolution.
Methods: In this study, we examined the genetic characteaistics of 179 RSV isolates collected between 2017 and 2021 in Taiwan. G ectodomain and whole F gene were sequenced and aligned with available references from GenBank.
Results: RSV ON1 and BA9 were two predominant genotypes throughout the study period. Genetic variations of G protein accumulated over time. New ON1 strains containing E257K and K204R-V225A-T238I-Y280H in combination emerged in 2019 and contributed to a local endemic in 2020. RSV-B strain with A131T and T137I substitution in G protein emerged in 2018. On the other hand, F protein of both RSV genotypes was generally conserved but some feature changes should be noted: RSV-B in Taiwan harbored 100% of I206M and Q209R in site Ø, and L172Q and S173L in site V. These amino acid changes do not affect the susceptibility of Nirsevimab but imply no effectiveness of Suptavumab.
Conclusion: RSV continuously evolves in Taiwan and accumulated signature genetic changes over time. Vigilant RSV genomic surveillance is important to monitor the viral evolution in the upcoming future of new RSV vaccines and prophylaxis.

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Introduction

Respiratory syncytial virus (RSV) is a worldwide leading cause of lower respiratory tract infection and has great impact on young children, elderly, and immunocompromised patients, with over 33 million pediatric cases aged <5 years and 1.5 million episodes of acute respiratory tract infection among the elderly population over 65 years of age.[1—3]

Antivirals against RSV infection are still ineffective; a monoclonal antibody, Palivizumab, has been used for years to protect high-risk infants.[4] In 2023, another extended half-life monoclonal antibody (Nirsevimab) and several vaccines were approved for infants and elderly populations.[5]

RSV is a negative sense, single stranded negative RNA virus. Two surface glycoproteins, G and F, are the keys for viral attachment, for viral pathogenesis,[6] and the major neutralizing sites. RSV-F protein is highly conserved and crucial for viral viability, host cell entry, and formation of syncytia. The mature F protein contains homotrimer (F1 and F2 subunits), linked by two disulfide bonds. Conformation transition from prefusion to post-fusion form is essential for RSV attachment to infected cells; six antigenic sites are found in F protein (Ø and I-V): Ø and V are only found in prefusion, whilst antigenic sites I-IV are present in both conformations.[7] RSV infection induces a protective neutralizing response against the F protein. Notably, the prefusion conformation of F elicits a more potent neutralizing activity compared to the post-fusion state. Consequently, the primary approaches in pharmaceutical strategies to prevent RSV infections focus on monoclonal antibodies and vaccines designed to target prefusion forms.[8]

G protein regulates host responses and disease[9]; it is responsible for cell surface binding and cellular infection. RSV is divided into A and B groups, circulating interchangeably and resulting in epidemics. RSV genotype is generally determined by the high diversity of G gene sequences. Nowadays, at least 14 genotypes of RSV-A and 26 genotypes of RSV-B have been identified.[10] RSV-A ON1 and RSV-B BA genotypes are the main circulating strains worldwide since they emerged in 1999 and 2010,[11,12] and keep evolving nationally and globally.[13—17]

Our previous study on 10-year-stored RSV isolates revealed how in Taiwan BA9 became the predominant genotype of RSV-B, and RSV-A ON1 prevailed on NA1 genotype in 2012.[18] In order to understand the molecular epidemiology and genomic characters of RSV in Taiwan before the advent of new RSV vaccines and therapeutics, in this study, we further investigated the genetic evolution of F and G isolated between 2017 and 2021.

Materials and methods

Source of stocked RSV isolates

RSV isolates collected between 2017 and 2021 were retrieved from the Taichung Veterans General hospital, one of the 9 Taiwan CDC contrast virology laboratories. Generally, nasopharyngeal swabs collected by collaborating sentinel clinics and hospitals in central Taiwan were sent to this laboratory; they were screened for the presence of 7 common respiratory viruses, including RSV, influenza A and B, adenovirus, and parainfluenza virus I-III. All identified respiratory viruses were stored at −80c before further analysis. The COVID-19 pandemic interrupted the routine collection of nasopharyngeal samples from sentinel clinics, hence, only 7 RSVs were isolated in 2021. We retrieved 5 RSV samples in 2021 from our study targeting the viral etiologies of childhood lower respiratory tract infection, which was approved by the SCMH institute review board (IRB-1081002).

RNA extraction, cDNA synthesis, and G and F protein sequencing

Viral RNA was extracted using QIAamp Viral RNA Mini Kit (QIAGEN, Hilden, Germany) following manufacturer's instructions. RNA was reverse transcribed to cDNA using UltraScript 2.0 cDNA Synthesis Kit (PCR Biosystems, Wayne, PA). The primers used for F and G gene amplifications and the thermocycling condition were performed as prescribed (Supplementary Table S1). All PCR reactions were conducted in a 25 μL reaction volume, containing 40 ng of cDNA, 10 μM of each primer, and 2 × PCRBIO Ultra Mix Red (PCR Biosystems, UK). The thermal profile was 2 min at 95 °C, and 40 cycles of 95 °C for 15 s, 55 °C for 15 s and 72 °C for 60 s. The final amplicon size of F gene was 1725bps, and G gene of RSV-A and RSV-B were 669 and 684bps, respectively. The purified products were subjected to Sanger sequencing by using an ABI 3730 automated sequencer (Applied Biosystems).

Phylogenetic analysis by maximum likelihood method

We obtained 72 representative RSV-G gene sequences (RSV-A: 33, RSV-B: 39) and 70 F gene representative sequences (RSV A: 41, RSV B: 29) from GenBank, spanning from 1997 to 2021. Before conducting phylogenetic tree analysis, all the F and G gene sequences were proven non-recombined, by using a genetic algorithm for recombination-detection, from the Datamonkey website (https://www.datamonkey.

org/). The phylogenetic trees analysis of RSV-A and RSV-B were conducted by the MEGA11 software, generated using the Maximum Likelihood (ML) method based on TN93+G and HKY + G models respectively. Bootstrap of 1000 replicates was set for the evaluation of confidence estimates. Phylogenetic trees were visualized and annotated using ITOL V6.

Deduced amino acid analysis of the F and G proteins

The translated F gene amino acid (AA)sequences were aligned with the reference sequences derived from Netherlands RSVA/13—005275 — GenBank accession number (AC) KX858757 — and Netherlands RSVB/13-001273 (AC KX858756). Moreover, the G protein AA sequences were aligned with references ON67-1210A (AC JN257693) and BA/100/04 (AC DQ227395.1). Amino acid variations analysis was conducted using CLC Genomics Workbench 20 and presented in percentages determined from pairwise alignments between sample sequences and references. The potential N and O-linked glycosylation sites were analyzed using NetNGlyc 1.0 and NetOGlyc 4.0 (https://services.healthtech.dtu.dk/services/NetNGlyc-1.0/ and https://services.healthtech.dtu.dk/services/NetOGlyc-4.0/) respectively. Amino acid motif of N-X-S/T (where X is not proline) is defined as the potential N linked glycosylation site, and the S/T residues is the potential O-linked ones.

Mapping of F protein amino acids variation

The prefusion and post-fusion forms of three-dimensional (3D) structures of F protein were adapting two files (IDs: 5UDE and 3RRR) from protein data bank (PDB) and further visualized with PyMOL, version 2.5 (Schrödinger, LLC). Epitope sites on the F protein are shown by colors, and black ones annotated amino acid variations with frequencies >1% in the antigenic sites.

Results

Distribution of RSV-A and RSV-B genotypes

In total, 179 RSV positive samples were analyzed in this study (RSV-A: 140, RSV-B: 39). Throughout the study period, RSV-A largely prevailed every year, except 2018. RSV-A and B circulated equally in the 2018 season, and the detail yearcase distribution is shown in Table 1.

Table 1 Yearly distribution of RSV in Taiwan, 2017—2021.

Year	RSVA	RSVB	Total RSV isolates
2017	20	5	25
2018	20	23	43
2019	41	9	50
2020	48	1	49
2021	11	1	12
Total	140	39	179

Annual viral isolates from TCVGH are 744 (2017); 853(2018); 1067(2019); 253 (2020), and 149 (2021)

Deduced amino acid substitutions and phylogenetic analysis of G protein

G protein genetic variation of the circulating RSV-B viruses in Taiwan did not evolve quickly over time. Compared to the BA reference (DQ227395.1), twelve major amino acids substitutions were identified among the RSV-B sequences: T107A (97%), A131T (56%), R136T (100%), T137I (56%), I198T (100%), T252I (82%), P262T (100%), L265S (100%), I279T (100%), T288I (87%), F295S (100%), and T310I (67%). A131T and T137I substitutions were especially found among the isolates after 2018, genetically close to the following strains: MT107528-G-B-CHE-2019/MT373705-G-B-RUS-2019/MW020595-G-B-AUS-2018/MZ515553-G-B-GBR-2019/MZ516101-G-B-GBR-2018/OM857371-G-B-AUS-2019/OM857373-G-B-AUS-2019/OM857377-G-B-AUS-2020.

On the other hand, G-protein genetic variation of RSV-A evolved significantly over time. Referenced to ON1 prototype (JN257693), the rates of shared amino acid substitutions on aa113, 131, 178, 258, and 266 increased drastically, beginning in 2019 (Table 2); strains with L274P and L298P waned yearly. Additional aa changes of H266L, Y280H, Y304H increased gradually and reached their highest in year 2020—2021. Moreover, K204R, V225A, T238I, and E257K substitutions were particularly found in the RSV-A in year 2020—2021. Noteworthy, RSV-A in 2020—2021 season could be divided into two clades, harboring their own characteristic amino acid substitution of E257K and K204R-V225A-T238I-Y280H in combination, respectively. All studied RSV-A phylogenetic tree and amino acid variations are summarized in Fig. 1; RSV-A G, OP690299- OP690402; RSV-B G, OP690260- OP690298, derived from this study, have been deposited in GenBank

The phylogenetic tree analysis was conducted using archived G ectodomain sequences of RSV-A and B, and 72 reference sequences. All the analyzed RSV-A sequences harbored characteristic 72-nts duplications and belonged to ON1 genotype. Similarly, all RSV-B sequences had the specific 60-nts duplications in C-terminal of the hypervariable region 2 and were classified as BA9 genotype. Plots of amino acids variations >10% of G proteins and phylogenetic trees of RSV-A and B are shown in Fig. 1A and B.

RSV-F protein amino acid substitutions and phylogenetic analysis

To assess the evolution of F protein, a total of 96 RSV-A and 39 RSV-B F protein sequences were compared to the reference sequences of Netherland RSV-A/13-005275 (AC KX858757) and RSV-B/13-001273 (AC KX858756). The amino acid variation and phylogenetic analysis are summarized in Fig. 2. Overall, the genetic diversity of F protein is relatively conservative between 2017 and 2021, but there were more amino acid variations of F protein in RSV-B than RSV-A. Of 2017-2019 RSV-A strains, T12I is the only variant with frequency >10%, whilst F15L (100%), A103V (97.3%), L172Q (100%), S173L (100%), K191R (86.5%), L206M(86.5%), and Q209R (86.5%) were shared amino acid substitutions in 2017—2019 RSV-B strains. Furthermore, all RSV-B strains in 2020—2021 harbored 100% of these aforementioned amino acid changes and gained additional variations of S380N

Table 2 Change of signature amino acid polymorphisms of RSV G protein during the study period.

Prototype/JN257693	113	131	178	204	225	238	257	258	266	274	280	298	304	319
	T	V	N	K	V	T	E	H	H	L	Y	L	Y	T
MG971431.1/A/NLD/2015	.	D	.	.	.	.	.	Q	.	.	.	.	.	.
MW260584.1/CHN/2017	I	D	G	.	.	.	K	Q	L	.	.	.	.	.
MT422269/A/RUS/2019	I	D	G	.	.	.	.	Q	L	.	.	.	.	.
MN306017/A/USA/2019	I	D	G	.	.	.	.	Q	L	.	.	.	.	.
OM062719/A/CHN/2019	I	D	G	.	A	.	.	Q	L	.	.	.	.	.
MZ515626/A/GBR/2019	I	D	G	.	A	.	.	Q	L	.	.	.	.	.
MW678547/A/THA/2020	I	D	G	.	.	.	.	Q	L	P	H	.	.	.
2017 (N = 20)	I	D	G	.	.	.	.	Q	L	P	.	P	H	I
	10%	10%	10%					10%	10%	50%		45%	25%	10%
2018 (N = 20)	I	D	G	.	.	.	.	Q	L	P	H	P	H	Q/K
	25%	25%	25%					25%	25%	30%	10%	25%	20%	10%
2019 (N = 41)	I	D	G	.	A	.	.	Q	L	P	H	P	H	K/I
	73.2%	68.3%	73.2%		14.6%			73.2%	73.2%	21.9%	4.9%	17%	12.2%	17%
2020—2021 (N = 59)	I	D	G	R	A	I	K	Q	L	P	H	P	H	.
	98.3%	98.3%	96.6%	42.3%	42.3%	42.3%	54.2%	98.3%	98.3%	6.8%	42.3%	3.4%	96.6%	

The percentage presented in each cell indicates the rate of polymorphism of amino acid at each site.

(50%) and V531I (50%). For 2020-2021 RSV-A strains, the frequency of T12I reached 100%, and the substitution rates of another three substitutions of S362L, S466N, and H514N were 18.6%, 10.1%, and 54.2%, respectively (Fig. 2); RSV-A F, OP690164- OP690259; RSV-B F, OP661286- OP661324, derived from this study, have been deposited in GenBank.

Amino acid variations among major six epitopes (Ø and I to V) of F protein were further examined (Fig. 3A). In total, 12 amino acid changes were seen in 5 out of 6 antigenic sites for RSV-A, and the frequencies ranged from 1.2 to 10.2%. Notably, the amino acid changes were distributed unevenly between the strains of season 2017-2019 and 2020-2021, and eight of 12 AA variations were occasionally seen in season 2017-2019 strains. S466N (10.2%) in site IV, the only amino acid change in 2020—2021 RSV-A F, was highly polymorphic. For RSV-B, there were 13 amino acid variations in the 6 major epitopes with frequencies from 2.5 to 100%. Five of them were highly polymorphic: I206M (87.2%) and Q209R (87.2%) in site Ø, and L172Q (100%), S173L (100%), and K191R (100%) in site V. All major identified AA changes with frequencies >10% were labeled on prefusion and postfusion F protein trimer structures (Fig. 3B).

Putative glycosylation sites of RSV G and F proteins

In this study, four N-glycosylation sites (103, 135, 237, and 318) were predicted in ectodomain of G protein among the RSV-A ON1 strains. For RSV-B BA9 strains, there were 2 predicted N glycosylation sites in G protein (298 and 310). There are numerous serines and threonines across the G protein, which are potentially O-glycosylated. In estimate, 65-78 predicated O-glycosylation sites were found in ON1 genotype strains, and 72-75 ones found in BA9 strains. Most predicted O-glycosylation sites locate in the two hypervariable regions. Respective to F protein, each of five predicted N glycosylation sites were found in ON1 and BA9 strains, which were located at sites 27, 70, 116, 120, and 126 of ON1 F protein, and at sites 27, 70, 116, 120, and 126 of BA 9 F protein (Table 3).

Discussion

The study found that RSV-A ON1 and RSV-B BA9 genotypes were consistently present in Taiwan throughout the study period. In 2018, RSV-B BA9 genotype predominated over RSV ON1. RSV ON1 was first identified in 2010, while RSV-B BA-like genotypes were discovered in 1999. Both genotypes rapidly spread globally and continued to evolve at local and global levels. Our previous research indicated that RSV-B BA9 emerged in Taiwan in 2008 and became the predominant RSV-B genotype from 2012 onward. RSV ON1 was first observed in Taiwan in 2011 and replaced the NA1 genotype as the prevailing strain from 2012.

The high genetic diversity of G protein is fundamental to define RSV genotype and trace the evolution. The second hypervariable region is commonly studied elsewhere, but the whole sequence analysis of G ectodomain is recommended, which is comparable to whole genome sequencing in phylogenetic analysis.[10] Our previous study showed I236V, I243S, E262K, Y273H, L274P, Y297H, L298P/S, P300S, and Y304H aa substitutions were identified diversely in the Taiwan RSV ON1 isolates.[18] Notably, E262K, L274P, L298P, Y304H, and L310P were worldwide described amino acid substitutions of ON1 isolates, before 2017.[14,15,17] Interesting, we observed a local evolutionary trend of G protein in Taiwan: RSV with aa changes of L274P and L298P declined to <7% in the end of study year. By contrast, the proportions of RSV containing T113I, V131D, N178G, H258Q, and H266L increased dramatically through the study period and reached >96% in year 2020/2021. Moreover, additional K204R, V225A, T238I, and E257K substitutions were found among RSV isolates in year 2020/2021, which were divided into harbors K204R, V225A, T238I, and Y280H in combination, and in E257K substitution. These two ON1 variants accounted for a significant outbreak in Taiwan, 2020.[19]

This phenomenon was also found in the studies from Beijing and Xiamen, China.[20,21] Both studies disclosed an RSV ON1 subtype, containing aa changes of T113I, V131D, N178G, H258Q, and H266L, prevailing in 2019. In line with

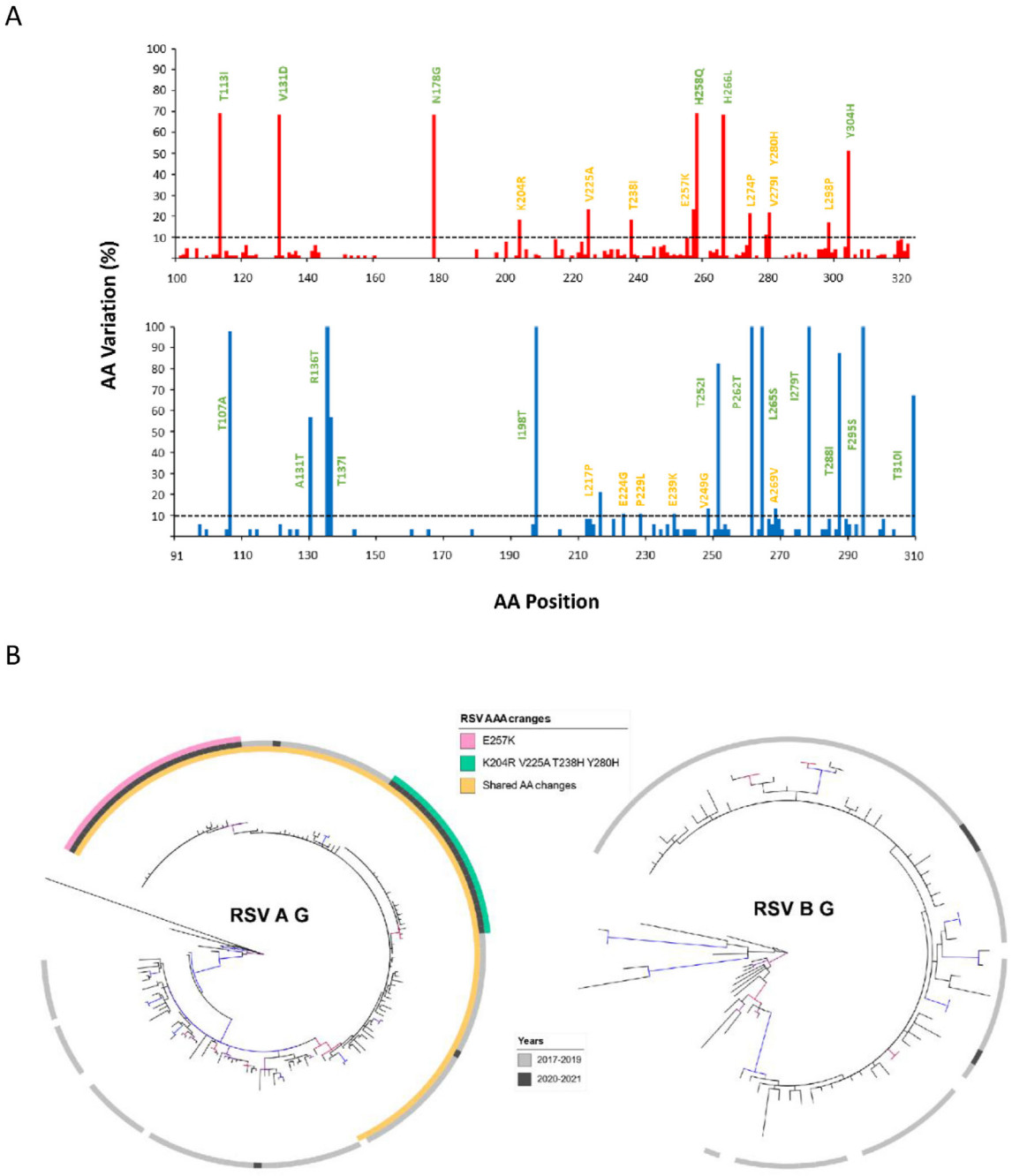
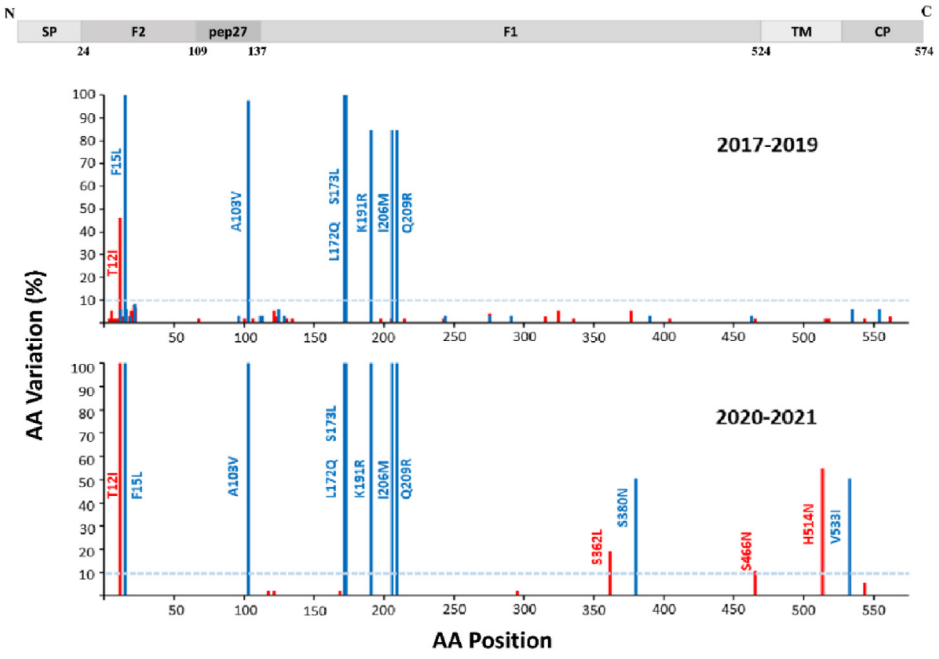


Figure 1. Amino acid substitutions spanning on G ectodomain and phylogenetic trees of RSV A and B (A) The variation rate of amino acids of RSV G ectodomain: only aa variation rate >10% was denoted, and red bar chart is for RSV A and blue bar chart is for RSV B. Sequences JN257693.1and DQ227395.1 were used as reference for RSV A and B, respectively. (B) ML Phylogenetic trees of RSV A and B were generated by using reference sequences JN257693.1and DQ227395.1 to root the trees. Year distribution is presented by light and dark gray colors, and signature amino acid substitutions of RSV A were denoted with green, pink, and yellow colors.

our study, they found this ON1 variant emerged in 2016-17 and progressively prevailed. These amino acid variants spread globally: they could also be found in Russia 2019 strains (MT422269) and United States 2018-2019 strains (MN306017, MN306048). Compared to the old RSV-A ON1 virus before 2017, this ON1 subvariant increased the need for oxygen, steroid, and antimicrobial therapy, but not the clinical symptoms' severity.[19,20] Instead, another new ON1 variant has been discovered in the 2022-2023 outbreak in Washington, USA.[22] We should keep monitoring whether this new one will be introduced into Taiwan. For RSV-B, our study, together with studies from Xiamen and Beijing,[20,21] discovered that RSV BA9 strains circulating after 2018 contained A131T and T137I substitutions in combination.

A



B

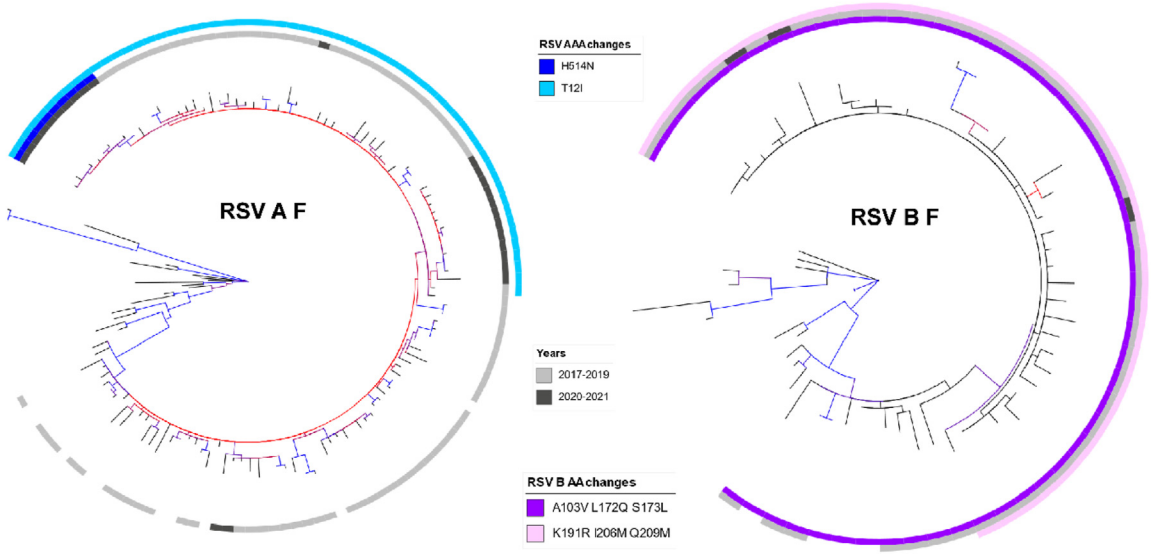


Figure 2. Amino acid substitutions spanning on whole F protein and phylogenetic trees of RSV A and B (A) Plots of AA variation frequency by position of F protein (1—574) to the reference sequences KX858757.1 and KX858756.1. The functional domain structures presented on the top (SP, signal peptide; TM, transmembrane domain, CT, cytoplasmic tail). RSV samples from 2017 to 2019 and 2020-2021 are plotted separately. Only Variations >10% (dash lines) in each season are labelled, Red for RSV A, and Blue for RSV B. (B) ML Phylogenetic trees of RSV A and B were constructed with reference to sequences KX858757.1 and KX858756.1 for RSV A and RSV B. Metadata including years of isolation and signature AA substitutions for the major branches are labeled in colors.

F protein is generally conserved, whilst the gain and loss of genetic variations occurred in F continuously and varied geographically. RSV-A and B have their own antigenic variations in F protein, but a changes occur more frequently in RSV-B than A.[13,16,23,24] In Taiwan, no single a substitution was found >10% in RSV-A strains 2017-19 and only four aa substitutions >10% were found in RSV-A 2020-2021 including T12I, S362L, S466N (site IV), and H514N. In the USA, RSV-B composed A103V, L172Q, S173L substitutions at episode V have emerged since 2014—15, and those having I206M: Q209R substitution combination appeared in 2016-17 with the rate of 18.6%.[13] Position mutation rate of K191R was

B cell epitope	Amino acid Substitution(s)	RSV A 2017-2019 N=81	RSV A 2020-2021 N=59	RSV B 2017-2021 N=39	
ϕ 62–69, 196–210	K68N	(1/81,1.2%)	0	I206M	(34/39,87.2%)
	I206T	(1/81,1.2%)	0	Q209R	(34/39,87.2%)
	Y198H	(1/81,1.2%)			
p27 109–136	T118I	0	(1/59,1.7%)	P112S	(1/39,2.5%)
	T122A	(4/81,4.9%)	(1/59,1.7%)	Q113K	(1/39,2.5%)
	N124T	(2/81,2.5%)	0	L125P	(2/39,5.1%)
	K131R	(1/81,1.2%)	0	I129M	(1/39,2.5%)
	R135K	(1/81, 1.2%)	0		
I (380-400)	-	-	-	N380S	(1/39,2.5%)
				K390R	(1/39,2.5%)
II 254–277	S276N	(3/81,3.7%)	0	S276N	(1/39,2.5%)
III	S377N	(4/81,4.9%)	0	-	-
IV 422-471	S466N	(1/81,1.2%)	(6/59,10.2%)	E463D	(1/39,2.5%)
AM14/V 148–194	S169N	0	(1/59,1.7%)	L172Q	(39/39,100%)
				S173L	(39/39,100%)
				K191R	(34/39,100%)
Undefined site	T12I	(37/81,45.7%)	(59/59,100%)		
	H514N	0	(32/59,54.2%)		

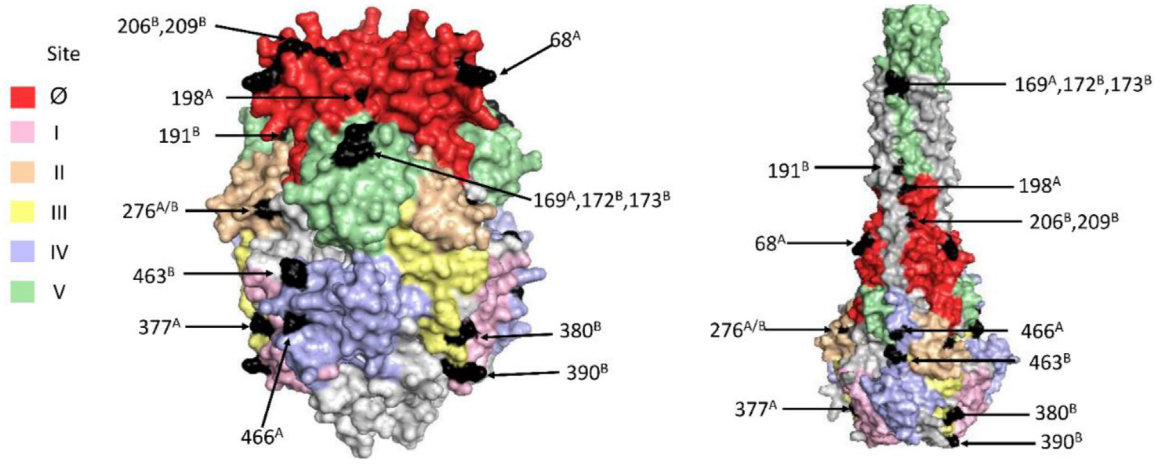


Figure 3. Amino acid mutations within each epitope of RSV F protein (A) Frequency of polymorphisms in antigenic sites of RSV A and B F Protein. (B) Annotation o acid variation on each epitope of RSV F protein: The pre-fusion and post-fusion 3D conformations were based on the PDF files of 5UDE and 3RRR, respectively. Antigenic sites (Ø, I, II, III, IV and V) are denoted in colors. red: site Ø, lightpink: I, wheat: II, paleyellow: III, lightblue: IV, palegreen: V. Only sites with variations >1.0% are labeled on the 3D structures. Upper cases of A and B indicates variation only presents in RSV A or B.

3.0—18.6% in 2016-17[13,16] and increased to nearly >95% after 2018.[23,25] These mutations have highly accumulated and globally prevailed in current circulating RSV-B.[21,23,25]

Understanding F conformation and relative immune epitopes takes treatment and prophylaxis against RSV into a new era.[5] Until 2023, there have been two approved prophylactic monoclonal antibodies and at least 2 approved RSV vaccines for adults, all targeting the F protein. Palivizumab is the first approved monoclonal antibody used in high-risk infants since 1998. Palivizumab targeted the episode II in F, but the escape mutations (K272M/T and S275F) were sparsely reported.[26,27] In our study, none of

Table 3 Putative N-linked sites at G and F genes of circulating RSV ON1 and BA9 strains in Taiwan.

Genotype	Putative N-Glycosylation Site % (n/N)			
ON1	G gene		F gene	
	N103	96.4% (135/140)	N27	100% (140/140)
	N135	97.1% (136/140)	N70	100% (140/140)
	N237	98.6% (138/140)	N116	99.3% (139/140)
	N318	7.9% (11/140)	N120	96.4% (135/140)
			N126	100% (140/140)
BA9	N298	100% (39/39)	N27	100% (39/39)
	N310	33.3% (13/39)	N70	100% (39/39)
			N116	100% (39/39)
			N120	100% (39/39)
			N126	100% (39/39)

the above-mentioned mutations were found. Nirsevimab is a new prophylactic antibody targeting epitope Ø in F and approved by FDA in 2023. According to a global based study analyzing >8000 RSV isolates collected from 1956 to 2021, the amino acid mutation rates within nirsevimab binding site are 0% in RSV-A and 12% in RSV-B[24]. The major two aa variants are I206M and Q209R in RSV B F protein, which emerged in 2014—2016 and highly prevailed in current RSV BA9 strains. In Taiwan, the rate of these two polymorphisms was 86.5% in 2017-19 and reached 100% in 2020—21. In the USA, the update prevalence rate of I206M: Q209R in 2018-20 was 93%.[25] However, I206M: Q209R substitution combination did not affect the susceptibility to nirsevimab.[24,28] K68N and N201S are two nirsevimab escaping mutants,[24] which were rarely reported (<1%) between 2015 and 2021 and not seen in our study.

Nearly all current circulating RSV-B across the world have two combined substitutions of L172Q and L173L in epitope V, compromising suptavumab's neutralizing activity.[29] Clesrovimab (MK-1654) is another monoclonal antibody in phase III development and targets episode IV of F protein. Neither K445T (RSV A) nor V447I (RSV B), two putative immune escape mutants of Clesrovimab, were detected in this study.

We analyzed the dynamics of RSV circulation and genetic diversity in Taiwan from 2017 to 2021. RSV ON1 and BA9 genotypes consistently dominated, showing ongoing evolution. One limitation is the study's local scope and modest sample size. RSV ON1 predominated, with BA9 accounting for only one-fifth of cases during the study period. Nevertheless, this study, along with our previous work, contributes to a reference database for monitoring post-pandemic RSV circulation changes and the selection pressure driven by new prophylactic antibodies and vaccines in Taiwan's future.

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Conflict of interest

All authors have nothing to be declared.

Ethics approval statement

This study was waived because non-involvement of human materials.

Patient consent statement

This study was waived because non-involvement of human materials.

CRediT authorship contribution statement

Yu Ping Fang: Data curation, Formal analysis, Methodology, Visualization, Writing — original draft. **Chun Chin Chang:** Resources. **De Wei Lai:** Methodology, Visualization. **Chun Yi Lee:** Conceptualization, Formal analysis, Funding acquisition, Investigation, Methodology, Project administration, Writing — review & editing.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.jmii.2024.06.003.