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# Genotype Analysis of Respiratory Syncytial Virus Before and After the COVID-19 Pandemic Using Whole-Genome Sequencing: A Prospective, Single-Center Study in Korea From 2019 to 2022

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## ABSTRACT

**Background:** Respiratory syncytial virus (RSV), a highly transmissible virus, is the leading cause of lower respiratory tract infections. We examined molecular changes in the RSV genome before and after the coronavirus disease 2019 (COVID-19) pandemic in Korea, and investigated whether drug-resistant mutations were present.
**Methods:** In this prospective, single-center study, RSV-positive respiratory samples were collected between September 2019 and December 2022. Long-read whole-genome sequencing (WGS) was performed, and the presence of known drug-resistant substitutions for palivizumab, nirsevimab, and suptavumab was investigated.
**Results:** Overall, 288 respiratory samples were collected from 276 children. WGS data were available for 133 samples (71 and 62 samples from the pre- and post-pandemic periods, respectively). All RSV-A strains (n = 56) belonged to the GA2.3.5 (ON1) genotype, whereas all RSV-B strains (n = 77) belonged to the GB5.0.5a (BA) genotype. No significant differences in genotypes were observed between the pre- and post-pandemic periods. In addition, no notable mutations related to nirsevimab or palivizumab resistance were detected in the F gene. However, the L172Q and S173L substitutions, which are known to confer resistance to suptavumab, were present in all RSV-B samples.
**Conclusion:** Despite the unprecedented interruption of RSV seasonality, there were no significant molecular changes in circulating RSV strains in Korea related to nirsevimab or palivizumab resistance before and after the COVID-19 pandemic. However, RSV-specific drug-resistance substitutions for suptavumab were identified.

**Keywords:** RSV; Genotype; Whole-Genome Sequencing; Resistance; Palivizumab; Nirsevimab; Suptavumab; COVID-19 Pandemic; Children; Korea

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**Disclosure**
The authors have no potential conflicts of interest to disclose.

**Data Availability Statement**
The respiratory syncytial virus whole-genome sequencing data has been deposited in GenBank. Deidentified data licensed for this analysis will be made available upon reasonable request to the corresponding authors.

**Author Contributions**
Conceptualization: Park M, Lee ST, Kang JM; Data curation: Na B, Park YJ, Seo J, Kim M; Investigation: Lee JY; Methodology: Park YJ, Baek JY; Software: Park YJ; Supervision: Ahn JG, Lee ST, Kang JM; Writing - original draft: Na B, Park YJ, Kang JM; Writing - review & editing: Kang JM.

# INTRODUCTION

Respiratory syncytial virus (RSV) is a highly transmissible virus that is the leading cause of lower respiratory tract infections (LRTI) in infants and young children.[1] Globally, approximately 94.6 infants per 1,000 person-years (uncertainty range [UR]; 70.8–131.6) experience RSV-associated LRTI. Furthermore, 15.9 infants per 1,000 person-years (UR; 12.6–21.2) require hospitalization, even though a majority of them were previously healthy without underlying illnesses.[1,2] Moreover, this virus is recognized as life-threatening in the elderly population.[3]

RSV is an enveloped, negative-sense, single-stranded RNA virus that contains 10 genes: nonstructural protein 1 (NS1), NS2, nucleoprotein (N), phosphoprotein (P), matrix (M), short hydrophobic protein (SH), glycoprotein (G), fusion protein (F), transcription processivity factor (M2), and large polymerase complex (L).[4] Traditionally, RSV genotypes (strain or clade) have been determined based on the genetic characteristics of the hypervariable region 2 within the G gene.[5] Recently, several RSV-specific antiviral drugs have been developed, which mostly target the F protein; however, they can also target the NS2 and L proteins, and the selective pressures imparted by these drugs can result in mutations in non-G genes.[6-8] Therefore, genetic surveillance based on whole-genome sequencing (WGS) has become increasingly important.[9]

The coronavirus disease 2019 (COVID-19) pandemic led to an abrupt interruption of seasonal RSV outbreaks in Korea.[10] The RSV epidemic in Korea during the 2019–2020 period terminated early in February 2020, and there were almost no RSV outbreaks for approximately 1.5 years (until October 2021), when domestic non-pharmaceutical interventions (NPIs) were eased.[11,12] This phenomenon provided a serendipitous opportunity to investigate the impact of the COVID-19 pandemic and the corresponding effects of NPIs on the molecular trends of RSV in Korea. Furthermore, we investigated the presence of resistance-associated substitutions against RSV-specific monoclonal antibodies (palivizumab, nirsevimab, and suptavumab).

# METHODS

## Study design and population

This prospective study was conducted at Severance Children's Hospital in Seoul, Korea. RSV-positive (detected through real-time multiplex respiratory virus polymerase chain reaction [Anyplex™ II RV16 Detection; Seegene, Seoul, Korea]) residual respiratory samples were collected from September 2019 to December 2022. Samples from February 20, 2020 to May 31, 2020 were not collected during the early stages of the COVID-19 pandemic. Initially, we planned to only collect RSV-positive samples from children aged < 3-years; however, since July 2022, RSV-positive samples were collected from children aged < 19 years to broaden the sample pool. The collected samples were stored at −72°C in a deep freezer until further analyses. To verify whether our hospital's RSV detection status aligned with the overall RSV epidemic in Korea, we utilized nationwide clinical surveillance data provided by the Korea Disease Control and Prevention Agency (KDCA) through an infectious disease portal (https://dportal.kdca.go.kr/pot/is/st/ari.do).

### Preparations for WGS

RNA extraction and amplification process are described in **Supplementary Method 1**. The RSV genome is 15-kb in size, and six primer sets were designed to generate six amplicons (**Supplementary Table 1**) To develop a WGS approach on the PacBio circular consensus sequencing (CCS) platform, we modified six previously described primer sets to generate overlapping amplicons across the entire RSV-A and RSV-B genome segments (**Supplementary Fig. 1A**). Total RNA was extracted and approximately 3-Mb sized amplicons (which covered the full-length RSV-A and RSV-B genomes) were generated using reverse transcription polymerase chain reaction (**Supplementary Fig. 1B**). The amplicons were pooled for library preparation using the M13 barcode and sequenced using a PacBio single molecule real-time (SMRT)bell CCS platform (**Supplementary Fig. 1C**).

### SMRTbell platform library construction and sequencing

SMRTbell libraries were prepared using a SMRTbell Express Template Prep Kit 2.0 (PN:101-938-900). All incubations were performed according to the manufacturers' recommendations. Amplicon genomic DNA sequencing data were collected on a PacBio Sequel system (Pacific Biosciences, Menlo Park, CA, USA) using high fidelity (HiFi) sequencing protocols and a sequencing kit (PN:101-597-900). Sequence data collection was standardized to 10 hours to allow ample time for multiple-pass sequencing around the SMRTbell template molecules, yielding high-quality CCS (HiFi) results. Raw base-called data were extracted from the sequencing instrument and imported into SMRTLink (version 10.2) to generate HiFi reads using the CCS algorithm. HiFi reads were aligned using pbmm2 v1.9.0 (https://github.com/PacificBiosciences/pbmm2) to the RSV-A (GenBank: FJ948820.1) and RSV-B (GenBank: JQ582843.1) reference genomes. After sorting by virus group (RSV-A or RSV-B), sequences from RSV open reading frames (NS1, NS2, N, P, M, SH, G, F, M2-1, M2-2, and L) were selected using Python scripts. The quality control data are presented in **Supplementary Table 2**.

### Phylogenetic analysis

All obtained nucleotide sequences in both genomic regions were aligned with reference strains obtained from GenBank using ClustalW in MEGA 11 software. Known public RSV sequences representing each subtype were retrieved from GenBank (**Supplementary Table 3**).
After alignment, sequences were trimmed, and final fragments of 14,792 bp and 15,001 bp for RSV-A and RSV-B, respectively, were used for the final phylogenetic analysis. Phylogenetic reconstruction was performed using the maximum likelihood method implemented in MEGA 11. The detailed parameter settings are listed in **Supplementary Table 4**. Furthermore, regarding the F gene, we investigated the presence of known substitutions against currently approved or in-development RSV-specific monoclonal antibodies.[13,14] The RSV genomic data analyzed in this study has been deposited in NCBI GenBank (PP785426-PP785558).

### Evolutionary rate estimation

The Bayesian Markov Chain Monte Carlo (MCMC) approach was implemented using the BEAST software (version 1.10.4) to estimate the substitution rates of full genome and for each individual open reading frames.[15] The GTR Gamma model with Gamma value 4 was used for nucleotide substitution model in all runs along with Relaxed Clock Log Normal and Coalescent Constant Size. The MCMC analyses were carried out using 500 million chain length with sampling every 50 thousand chain length. Tracer software was used to calculate mean evolutionary rate.

### Ethics statement

This study was conducted in accordance with the World Medical Association guidelines and the Declaration of Helsinki. This study protocol was reviewed and approved by our Institutional Review Board at Yonsei University, Seoul, Korea (No. 4-2019-0723). The requirement for participants' consent was waived by the board as only residual specimens were collected.

## RESULTS

During the study period, 276 children with RSV infections were hospitalized and 288 respiratory samples were collected. Among these, 133 samples (46.2%) were available for amplification: 71 (53.4%) from the pre-COVID-19 period and 62 (46.6%) from the COVID-19 period (**Fig. 1A**). Among the 133 samples included in the WGS, 6 cases are samples obtained from three patients with an interval of more than a week between collections. The male to female ratio was 1.1:1, and infants accounted for 47.5% of the study population. The detailed demographics are presented in **Supplementary Table 5**.

### RSV epidemics and molecular epidemiology in Korea from 2019 to 2022

The data on monthly RSV hospitalizations at Severance Children's Hospital closely aligned with the RSV trends in the clinical surveillance operated by the KDCA (**Fig. 1B**). RSV-B was predominant during the 2019–2020 season; however, the RSV epidemic ended abruptly in February 2020. A resurgence in RSV cases began in November 2021; RSV-B was initially predominant; however, RSV-A became predominant after February 2022 (**Fig. 1B**).

RSV-A was dominated by the GA2.3.5 (ON1) strain, whereas RSV-B was dominated by the GB5.0.5a (BA) strain. No significant changes were observed before or after the pandemic (**Fig. 2**). The *P*-distance, which represents the degree of nucleotide differences by the total number of nucleotides between seasons (i.e., 2019–2020, 2020–2021, 2021–2022), was not substantial (ranging from 0.008 to 0.011). Additionally, within each season, the degree of simple variation between strains was not significant (ranging from 0.005 to 0.010) (**Table 1**). An examination of the degree of variation in the 10 RSV genes through WGS revealed that the G protein had the highest nucleotide substitution rate with $2.53 \times 10^{-3}$ substitutions per site per year (s/s/y) (95% highest posterior density [HPD]: $2.05–3.05 \times 10^{-3}$ s/s/y) and $4.31 \times 10^{-3}$ (95% HPD: $3.35–5.36 \times 10^{-3}$ s/s/y) for RSV-A and RSV-B, respectively. Regarding the F gene, the nucleotide substitution rate was low (ranging from $6.34–8.65 \times 10^{-4}$ s/s/y in RSV-A and $8.41 \times 10^{-4}–1.30 \times 10^{-3}$ s/s/y in RSV-B) (**Fig. 3**). RSVs with identical G regions were identified, and the number of changes outside the G region were determined (**Supplementary Figs. 2 and 3**). In all determined identical G regions, at least 1 nucleotide difference was noted in all panels; notably, more gaps in genome were more prevalent in post-pandemic samples. Tracking WGS nucleotide differences rather than G region alone may be beneficial in tracking short-term transmission patterns.

### Site-specific substitution analysis of the RSV F gene

**Table 2**[16,17] shows the site-specific substitutions in the RSV F gene. I206M:Q209R and I206M:Q209R:S211N substitutions were observed in all RSV-B strains (n = 77). I206M:Q209R accounted for 100% (n = 43) of substitutions identified in the pre-pandemic period, whereas the I206M:Q209R:S211N combined substitution accounted for 63.9% (n = 22) of substitutions in the post-pandemic period (I206M:Q209R accounted for 36.1% (n = 12)).

A

Children (< 36 months of age) who were hospitalized and underwent real-time respiratory multiplex PCR testing between September 2019 and December 2022
N = 1,753 (No. of samples = 4,728)

Cases in which RSV has been detected
n = 283 (No. of samples = 295)

No residual samples,
n = 21 (No. of samples = 21)

Cases in which residual samples were obtained
n = 262 (No. of samples = 274)

Addition of cases of RSV detection in children ≥ 3 years of age
n = 14 (No. of samples = 14)

Pre-COVID-19 period
(between September 2019 and February 20, 2020)
n = 163 (No. of samples = 168)

COVID-19 period
(between June 2020 and December 2022)
n = 113 (No. of samples = 120)

Not amplified
n = 146 (No. of samples = 155)

Cases in which WGS was available
n = 69 (42.3%)
(No. of samples = 71, 42.3%)

Cases in which WGS was available
n = 61 (54.0%)
(No. of samples = 62, 51.7%)

B

Monthly RSV hospitalization report cases by KDCA surveillance, No.
4,000
3,000
2,000
1,000
0
RSV B
RSV A
KDCA
Monthly No. of RSV hospitalization at SCH
60
50
40
30
20
10
0
Sep Oct Nov Dec Jan Feb Mar Apr May Jun Jul Aug Sep Oct Nov Dec Jan Feb Mar Apr May Jun Jul Aug Sep Oct Nov Dec Jan Feb Mar Apr May Jun Jul Aug Sep Oct Nov Dec
2019 2020 2021 2022

**Fig. 1.** Flowchart of study population selection and monthly trend of RSV hospitalizations. (**A**) Selection schematic of study population and samples. (**B**) Monthly RSV hospitalization data at SCH during the study period. The shaded portion represents clinical surveillance data on RSV hospitalizations from the KDCA.
PCR = polymerase chain reaction, RSV = respiratory syncytial virus, COVID-19 = coronavirus disease 2019, WGS = whole-genome sequencing, SCH = Severance Children's Hospital, KDCA = Korea Disease Control and Prevention Agency.

There were no known reported major mutations that confer resistance to nirsevimab in the F2 and F1 regions of either the RSV-A or RSV-B strains. Mutations conferring resistance to palivizumab, which is currently available in Korea, were not observed in any of the analyzed RSV strains. Notably, mutations known to confer resistance to suptavumab, such as L172Q and S173L, were observed in 100% of the analyzed RSV-B strains (n = 77).

A

B

C

D

**Fig. 2.** Phylogenetic tree of RSV-A and RSV-B before and after the coronavirus disease 2019 pandemic based on whole-genome sequencing. (**A**, **C**) depict the phylogenetic tree of pre-pandemic RSV (from September 2019 to February 2020), whereas (**B**, **D**) represent the phylogenetic tree of post-pandemic RSV (from November 2021 to December 2022). The red circles represent RSV samples from the 2019–2020 season, the blue circles represent samples from the 2021–2022 season, and the circles with blue borders represent samples from the 2022–2023 season.
RSV = respiratory syncytial virus.

**Table 1.** Estimation of average *P*-distances between and within RSV seasons in Korea

| Epidemic season | *P*-distance within season | Epidemic seasons | *P*-distance between seasons |
|---|---|---|---|
| RSV-A | | | |
| 2019–2020 | 0.00960 | 2019–2020 vs. 2021–2022 | 0.01074 |
| 2021–2022 | 0.00674 | 2019–2020 vs. 2022–2023 | 0.01149 |
| 2022–2023 | 0.00948 | 2021–2022 vs. 2022–2023 | 0.00837 |
| RSV-B | | | |
| 2019–2020 | 0.00499 | 2019–2020 vs. 2021–2022 | 0.00754 |
| 2021–2022 | 0.00550 | | |

The average *P*-distances among the nucleotide sequences of epidemic seasons were calculated by pairwise comparison using the Tamura-Nei model (Gamma Distributed), with transitions and transversions, in MEGA 11.
RSV = respiratory syncytial virus.

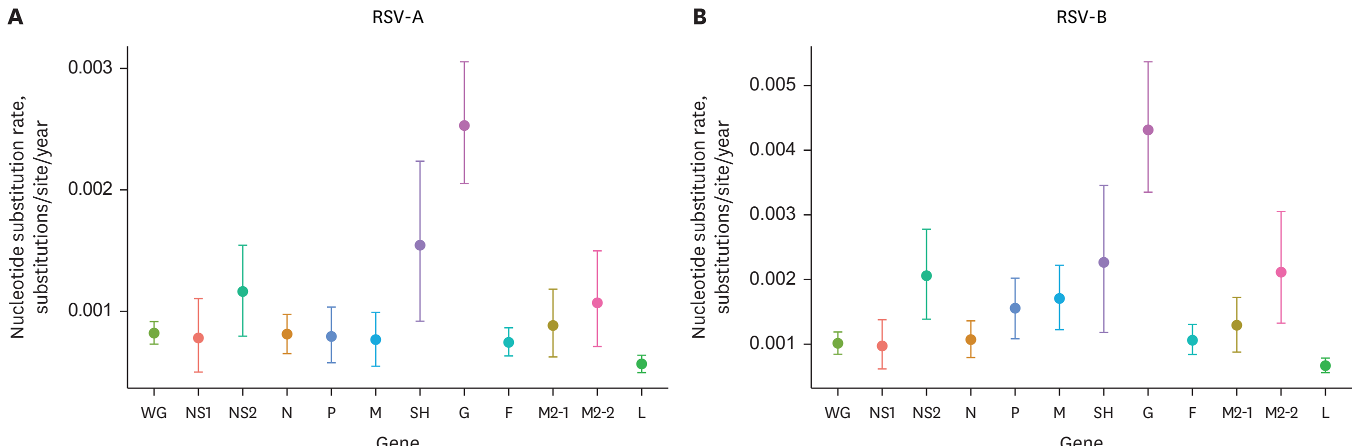


**Fig. 3.** Estimates of nucleotide substitution rates for RSV-A and RSV-B using whole-genome sequencing. The analysis was conducted using the search-thinned datasets and was carried out using the BEAST program.
RSV = respiratory syncytial virus.

## DISCUSSION

In this prospective surveillance study, we assessed changes in the molecular epidemiology of RSV before and after the COVID-19 pandemic in Korea using WGS for the first time. Despite the unprecedented disruption of the seasonal RSV epidemic, there were no significant genotypic changes in the circulating RSV strains in Korea between the pre- and post-pandemic periods, and their genotypic trends aligned with global patterns. No substitutions in the F gene, which is known to confer resistance to RSV-specific monoclonal antibodies nirsevimab and palivizumab, but not suptavumab, were identified.

Our findings revealed that all the analyzed RSV-A strains belonged to GA2.3.5 (ON1), and all the analyzed RSV-B strains were GB5.0.5a (BA). According to the INFORM-RSV study conducted in eight countries (the United Kingdom, Spain, the Netherlands, Finland, Japan, Brazil, South Africa, and Australia) during the pre-pandemic season (2017–2018), the BA9 genotype of RSV B and ON1 genotype of RSV-A were predominant in all countries except South Africa.[5] Similar findings were reported during the pre-pandemic period (2017–2018) in Korea.[18,19] Yun et al.[18] reported that in Korea, the ON1 genotype of RSV-A had replaced all other RSV-A genotypes since 2012, whereas the BA genotype of RSV-B (mainly BA9) had replaced all other RSV-B genotypes since 2007. The minimal changes in the RSV genotypes in the post-pandemic period could be explained by a genetic bottleneck. Recent studies comparing the prevalent RSV genotypes pre- and post-pandemic have reported similar


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**Table 2.** Substitution analysis of binding sites on F protein-targeting monoclonal antibodies and comparisons with previous studies

| Frequency in our surveillance | | | | | | The US-based OUTSMART-RSV, the global INFORM-RSV, and a pilot study in South Africa, 1956–2021 (%) | | | Choi et al.,[13] Korea, 2009–2015 |
|---|---|---|---|---|---|---|---|---|---|
| Drug binding site | RSV fusion substitution | RSV-A | | RSV-B | | Frequency (n = 2,800, %) | IC50 fold reduction | | Frequency (n = 60, %) |
| | | Pre-COVID (n = 28, %) | COVID (n = 28, %) | Pre-COVID (n = 43, %) | COVID (n = 34, %) | | Nirsevimab | Palivizumab | |
| F2 | | | | | | | | | |
| Nirsevimab (AA residues 62–69) | S62G | 0.00 | 0.00 | | | 0.03 | NA | NA | 0.00 |
| | T63N | 100.00 | 100.00 | | | NA | NA | S | 0.00 |
| | N63S | | | 0.00 | 0.00 | 0.04 | 2.80 | 8.20 | 0.00 |
| | I64T | 0.00 | 0.00 | 0.00 | 0.00 | 1* (case) | > 496.30 | 5.20 | 0.00 |
| | I64V | 0.00 | 0.00 | | | 0.03 | 1.70 | 1.80 | 0.00 |
| | K65E | | | 0.00 | 0.00 | NA | NA | NA | 3.30 |
| | K65Q | 0.00 | 0.00 | | | 0.03 | NA | NA | 0.00 |
| | K65R | 0.00 | 0.00 | 0.00 | 0.00 | 0.10/0.11 | 3.90/0.80 | 2.20/1.50 | 0.00 |
| | E66D | | | 0.00 | 0.00 | 0.04 | 2.00 | 1.70 | 0.00 |
| | T67A | | | 0.00 | 0.00 | 0.04 | 1.20 | 3.00 | 0.00 |
| | K68E | 0.00 | 0.00 | | | 0.07 | 12.60 | 1.90 | 0.00 |
| | K68N | 0.00 | 0.00 | 0.00 | 0.00 | 0.56/0.32 | 5.10/29.90 | 2.10/2.00 | 0.00 |
| | K68Q | | | 0.00 | 0.00 | 0.04 | > 369.50 | 2.70 | 0.00 |
| | K68R | 0.00 | 0.00 | 0.00 | 0.00 | 0.03/0.07 | 1.40/0.80 | 1.30/1.90 | 0.00 |
| F1 | | | | | | | | | |
| Nirsevimab (AA residues 196–212) | N197D | 0.00 | 0.00 | 0.00 | 0.00 | 0.03/0.39 | 2.50/QNS | 1.20/QNS | 0.00 |
| | N197H | 0.00 | 0.00 | | | 0.03 | 1.10 | 1.00 | 0.00 |
| | N197K | 0.00 | 0.00 | | | 0.14 | 1.50 | 1.80 | 0.00 |
| | N197S | | | 0.00 | 0.00 | 0.04 | 1.60 | 2.50 | 0.00 |
| | I199M | 0.00 | 0.00 | | | 0.03 | 1.70 | 1.50 | 0.00 |
| | D200N | 0.00 | 0.00 | | | 0.03 | NA | NA | 0.00 |
| | N201S | | | 0.00 | 0.00 | 0.21 | 126.70 | 3.10 | 16.70 |
| | N201T | | | 0.00 | 0.00 | 0.04 | > 405.70 | 2.80 | 0.00 |
| | L204I | 0.00 | 0.00 | 0.00 | 0.00 | 0.03 | 2.00 | 4.20 | 0.00 |
| | I206M | | | 100.00 | 100.00 | 68.89 | 5.00 | 2.00 | 0.00 |
| | I206T | 0.00 | 0.00 | | | 0.17 | 1.60 | 1.00 | 0.00 |
| | I206V | 0.00 | 0.00 | | | 0.03 | 3.20 | 2.30 | 0.00 |
| | V207I | 0.00 | 0.00 | | | 0.03 | 2.50 | 1.50 | 0.00 |
| | N208S | 0.00 | 0.00 | 0.00 | 0.00 | 1* (case) | > 386.60 | 1.80 | 0.00 |
| | Q209K | | | 0.00 | 0.00 | 0.21 | 0.50 | 1.80 | 23.30 |
| | Q209L | | | 0.00 | 0.00 | 0.11 | 0.40 | 0.80 | 0.00 |
| | Q209R | | | 100.00 | 100.00 | 68.18 | 0.50 | 3.10 | 0.00 |
| | Q210H | | | 0.00 | 0.00 | 0.07 | 2.30 | 2.50 | 0.00 |
| | Q210L | 0.00 | 0.00 | | | 0.03 | 2.20 | 1.70 | 0.00 |
| | S211I | | | 0.00 | 0.00 | 0.04 | 1.90 | 1.90 | 0.00 |
| | S211N | | | 0.00 | 63.90 | 1.14 | 1.20 | 1.90 | 0.00 |
| | I206M: Q209R | | | 100.00 | 36.10 | 65.80 | 0.20 | 1.30 | 0.00 |
| | I206M: Q209R: S211N | | | 0.00 | 63.90 | 1.10 | 0.50 | 3.70 | 0.00 |
| Palivizumab (AA residues 262–275) | D263Y | 0.00 | 0.00 | | | 0.03 | 0.60 | 0.20 | 0.00 |
| | M264I | | | 0.00 | 0.00 | 0.04 | 0.60 | 2.10 | 0.00 |
| | K272N | | | 0.00 | 0.00 | 0.04 | 1.20 | > 213.60 | 0.00 |
| | K272Q | | | 0.00 | 0.00 | 0.04 | 1.40 | > 226.40 | 0.00 |
| | K272R | | | 0.00 | 0.00 | 0.04 | ND | 41.80 | 0.00 |
| | K272T | 0.00 | 0.00 | | | 0.03 | 1.10 | > 213.90 | 0.00 |
| | K272M | 0.00 | 0.00 | | | 0.03 | 2.70 | > 179.90 | 0.00 |
| | K272E | 0.00 | 0.00 | 0.00 | 0.00 | NA | NA | NA | 0.00 |
| | L273I | | | 0.00 | 0.00 | 0.07 | QNS | QNS | 0.00 |
| | S275F | 0.00 | 0.00 | | | 0.03 | 6.40 | > 356.10 | 0.00 |
| | N276S | 90.30 | 39.30 | | | NA | NA | S[16] | 0.00 |
| | S276N | | | 0.00 | 11.10 | NA | NA | S[16,17] | 0.00 |
| Suptavumab | L172Q | 0.00 | 0.00 | 100.00 | 100.00 | NA | NA | NA | 66.70 |
| | S173L | 0.00 | 0.00 | 100.00 | 100.00 | NA | NA | NA | 3.30 |

RSV = respiratory syncytial virus, COVID = coronavirus disease, AA = amino acid, NA = not available, ND = not detected, QNS = quality not sufficient, S = susceptible.

results to ours, indicating that there were no significant genotypic changes. According to recent research by the Australian RSV study group, there was no seasonal RSV outbreak in the winter of 2020. However, in late 2020, there was an out-of-season RSV-A outbreak of the previously prevalent ON1 genotype, which showed limited genetic diversity based on WGS analysis.[20] Therefore, it is possible that in Korea, the RSV strain that was prevalent prior to the pandemic might have been circulating at undetectable levels locally and re-emerged after NPIs were lifted.

However, the limitation of strain diversity by NPIs does not necessarily guarantee the resurgence of previously dominant strains. Lin et al.[21] conducted a genetic analysis of an RSV-A outbreak in Taiwan, and targeted the G and F genes. They reported the prevalence of the RSV-A ON1 strain in the second half of 2020, which exhibited distinct features from the predominant ON1 strains during the 2013–2016 epidemics. Pierangeli et al.[22] exclusively analyzed the G gene, revealing that the RSV-A ON1 strain was predominant in Rome from October to December 2021, and closely resembled strains from the pre-pandemic RSV season. However, the RSV-B BA10 subtype, which dominated the 2022–2023 season, was different from the predominant RSV-B strains during the 2018–2019 season in Rome.

Our findings underscore the high degree of conservation in the F gene, which enhances the likelihood of stable drug efficacy for RSV-specific drugs. In an international study on nirsevimab, which recently received Food and Drug Administration approval in 2023 for pregnant women and older individuals, the authors also reported that a prevalence of < 1% of substitutions would affect the RSV neutralization capacities of both nirsevimab and palivizumab.[5,14] However, suptavumab, a fully human monoclonal antibody targeting site V of the pre-F protein, failed to meet the primary endpoint in a recent clinical trial due to high levels of resistance against RSV-B strains with L172Q/S173L variants.[23] Moreover, these RSV-specific monoclonal antibodies can potentially exert selective pressure after their widespread use in clinical practice, even though their prevalence is currently extremely low. In a study analyzing the F gene of RSV samples collected from 2009 to 2015 in Korea, Choi et al.[13] reported the prevalence of the N201S substitution (16.7%), which reduced the neutralization capacity of nirsevimab. Therefore, continuous monitoring of RSV genotypes at the national level is imperative. Continued genetic surveillance is crucial because there's a possibility that some rare strains or variants could emerge as predominant strains during the post-pandemic period.

The correlation between RSV genotype and disease severity has been suggested in the literature but with limitations. Li et al.[24] analyzed the G gene in a study conducted during an RSV-A ON1 epidemic in Guangzhou, China during the 2018–2019 season, reporting a cluster with a novel set of five amino acid substitutions that exhibited milder symptoms such as reduced wheezing and shorter symptom duration. Additionally, Midulla et al.[25] reported in a study conducted in Italy on hospitalized RSV-infected infants under one year old that RSV-A NA1 showed more severe chest retractions and a higher likelihood of hypoxia compared to RSV-A ON1 or RSV B BA. However, these studies require careful interpretation due to risk factors such as older siblings and smoke exposure not being fully controlled, and variations in the extra-G regions were not evaluated. Therefore, more well-designed clinical studies using the WGS method are necessary for a precise analysis.

This study also has limitations. First, this was a single-center study, which may not represent the overall Korean pediatric population. Second, as this study used residual samples, we were unable to gather clinical information on disease severity in the participants. However,

regardless of the severity, the genotypes of all specimens analyzed in this study were similar to those of ON1 and BA. Moreover, because factors such as immunological debt due to the absence of natural boosting during the NPI period can also influence severity, there may be limitations in explaining virulence based solely on viral genotypes. To address this issue, future studies that include both clinical and immunological information are needed.[26] Third, we were unable to analyze the genotypes of unamplified samples, which accounted for approximately 53.8% of collected specimens. However, positive rates were also observed in other studies and were higher than the historical RSV culture rate of 10–30%.[18,27] Finally, we were unable to conduct neutralization tests to directly assess drug resistance. To address this, we are developing a microneutralization assay for RSV to evaluate the effectiveness of RSV-specific drugs against domestic RSV variants.

Nevertheless, our study results can help to elucidate the molecular epidemiology of RSV using WGS. The long-read next-generation sequencing technology and interpretation pipeline established in this study are expected to play a significant role in the molecular surveillance of RSV in Korea. In conclusion, our WGS analysis revealed no significant genetic changes in RSV genomes related to nirsevimab or palivizumab resistance when comparing the pre- and post-pandemic periods in South Korea. Furthermore, the unpredictability of RSV seasonality after the pandemic highlights the importance of year-round surveillance, rather than relying solely on traditional seasonal monitoring.

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## SUPPLEMENTARY MATERIALS

**Supplementary Method 1**
RNA extraction and amplification

**Supplementary Table 1**
Primer sets used in this study

**Supplementary Table 2**
Quality control of SMRTbell run summary

**Supplementary Table 3**
GenBank accession number, country, genotype, year of RSV-A and RSV-B reference sequences

**Supplementary Table 4**
The parameter settings for phylogenetic analysis

**Supplementary Table 5**
Baseline characteristics of study subjects and samples

**Supplementary Fig. 1**

Schematic diagram of long-reads whole-genome sequencing of RSV.

**Supplementary Fig. 2**

Comparison of RSV-A genomes with identical G regions. Each panel represents RSVs with identical G gene sequences highlighted in gray. Orange vertical lines are for nucleotide substitutions, blue lines are for deletions, and red lines are for insertions.

**Supplementary Fig. 3**

Comparison of RSV-B genomes with identical G regions. Each panel represents RSVs with identical G gene sequences highlighted in gray. Orange vertical lines are for nucleotide substitutions, blue lines are for deletions, and red lines are for insertions.

## REFERENCES


1. Hall CB, Weinberg GA, Iwane MK, Blumkin AK, Edwards KM, Staat MA, et al. The burden of respiratory syncytial virus infection in young children. *N Engl J Med* 2009;360(6):588-98. PUBMED | CROSSREF
2. Li Y, Wang X, Blau DM, Caballero MT, Feikin DR, Gill CJ, et al. Global, regional, and national disease burden estimates of acute lower respiratory infections due to respiratory syncytial virus in children younger than 5 years in 2019: a systematic analysis. *Lancet* 2022;399(10340):2047-64. PUBMED | CROSSREF
3. Savic M, Penders Y, Shi T, Branche A, Pirçon JY. Respiratory syncytial virus disease burden in adults aged 60 years and older in high-income countries: a systematic literature review and meta-analysis. *Influenza Other Respi Viruses* 2023;17(1):e13031. PUBMED | CROSSREF
4. Mejias A, Garcia-Maurino C, Rodriguez-Fernandez R, Peeples ME, Ramilo O. Development and clinical applications of novel antibodies for prevention and treatment of respiratory syncytial virus infection. *Vaccine* 2017;35(3):496-502. PUBMED | CROSSREF
5. Tabor DE, Fernandes F, Langedijk AC, Wilkins D, Lebbink RJ, Tovchigrechko A, et al. Global molecular epidemiology of respiratory syncytial virus from the 2017-2018 INFORM-RSV study. *J Clin Microbiol* 2020;59(1):e01828-20. PUBMED | CROSSREF
6. Zhu Q, Patel NK, McAuliffe JM, Zhu W, Wachter L, McCarthy MP, et al. Natural polymorphisms and resistance-associated mutations in the fusion protein of respiratory syncytial virus (RSV): effects on RSV susceptibility to palivizumab. *J Infect Dis* 2012;205(4):635-8. PUBMED | CROSSREF
7. Yu JM, Fu YH, Peng XL, Zheng YP, He JS. Genetic diversity and molecular evolution of human respiratory syncytial virus A and B. *Sci Rep* 2021;11(1):12941. PUBMED | CROSSREF
8. Ramilo O, Rodriguez-Fernandez R, Mejias A. Respiratory syncytial virus infection: old challenges and new approaches. *J Infect Dis* 2023;228(1):4-7. PUBMED | CROSSREF
9. Teirlinck AC, Johannesen CK, Broberg EK, Penttinen P, Campbell H, Nair H, et al. New perspectives on respiratory syncytial virus surveillance at the national level: lessons from the COVID-19 pandemic. *Eur Respir J* 2023;61(4):2201569. PUBMED | CROSSREF
10. Huh K, Jung J, Hong J, Kim M, Ahn JG, Kim JH, et al. Impact of nonpharmaceutical interventions on the incidence of respiratory infections during the coronavirus disease 2019 (COVID-19) outbreak in Korea: a nationwide surveillance study. *Clin Infect Dis* 2021;72(7):e184-91. PUBMED | CROSSREF
11. Kim JH, Roh YH, Ahn JG, Kim MY, Huh K, Jung J, et al. Respiratory syncytial virus and influenza epidemics disappearance in Korea during the 2020-2021 season of COVID-19. *Int J Infect Dis* 2021;110:29-35. PUBMED | CROSSREF
12. Kim JH, Kim HY, Lee M, Ahn JG, Baek JY, Kim MY, et al. Respiratory syncytial virus outbreak without influenza in the second year of the coronavirus disease 2019 pandemic: a national sentinel surveillance in Korea, 2021-2022 season. *J Korean Med Sci* 2022;37(34):e258. PUBMED | CROSSREF
13. Choi SH, Park KS, Kim YJ. Analysis of respiratory syncytial virus fusion protein from clinical isolates of Korean children in palivizumab era, 2009-2015. *J Infect Chemother* 2019;25(7):514-9. PUBMED | CROSSREF
14. Wilkins D, Langedijk AC, Lebbink RJ, Morehouse C, Abram ME, Ahani B, et al. Nirsevimab binding-site conservation in respiratory syncytial virus fusion glycoprotein worldwide between 1956 and 2021: an analysis of observational study sequencing data. *Lancet Infect Dis* 2023;23(7):856-66. PUBMED | CROSSREF

15. Drummond AJ, Suchard MA, Xie D, Rambaut A. Bayesian phylogenetics with BEAUti and the BEAST 1.7. *Mol Biol Evol* 2012;29(8):1969-73. PUBMED | CROSSREF
16. Ahani B, Tuffy KM, Aksyuk AA, Wilkins D, Abram ME, Dagan R, et al. Molecular and phenotypic characteristics of RSV infections in infants during two nirsevimab randomized clinical trials. *Nat Commun* 2023;14(1):4347. PUBMED | CROSSREF
17. Yasui Y, Yamaji Y, Sawada A, Ito T, Nakayama T. Cell fusion assay by expression of respiratory syncytial virus (RSV) fusion protein to analyze the mutation of palivizumab-resistant strains. *J Virol Methods* 2016;231:48-55. PUBMED | CROSSREF
18. Yun KW, Choi EH, Lee HJ. Molecular epidemiology of respiratory syncytial virus for 28 consecutive seasons (1990-2018) and genetic variability of the duplication region in the G gene of genotypes ON1 and BA in South Korea. *Arch Virol* 2020;165(5):1069-77. PUBMED | CROSSREF
19. Kim HN, Hwang J, Yoon SY, Lim CS, Cho Y, Lee CK, et al. Molecular characterization of human respiratory syncytial virus in Seoul, South Korea, during 10 consecutive years, 2010-2019. *PLoS One* 2023;18(4):e0283873. PUBMED | CROSSREF
20. Eden JS, Sikazwe C, Xie R, Deng YM, Sullivan SG, Michie A, et al. Off-season RSV epidemics in Australia after easing of COVID-19 restrictions. *Nat Commun* 2022;13(1):2884. PUBMED | CROSSREF
21. Lin TY, Chi H, Kuo CY, Tsai HP, Wang JR, Liu CC, et al. Outbreak of respiratory syncytial virus subtype ON1 among children during COVID-19 pandemic in Southern Taiwan. *J Microbiol Immunol Infect* 2022;55(6 Pt 2):1168-79. PUBMED | CROSSREF
22. Pierangeli A, Nenna R, Fracella M, Scagnolari C, Oliveto G, Sorrentino L, et al. Genetic diversity and its impact on disease severity in respiratory syncytial virus subtype-A and -B bronchiolitis before and after pandemic restrictions in Rome. *J Infect* 2023;87(4):305-14. PUBMED | CROSSREF
23. Simões EA, Forleo-Neto E, Geba GP, Kamal M, Yang F, Cicirello H, et al. Suptavumab for the prevention of medically attended respiratory syncytial virus infection in preterm infants. *Clin Infect Dis* 2021;73(11):e4400-8. PUBMED | CROSSREF
24. Li W, Wang Y, Yu B, Tan Q, Zhou J, Hu J, et al. Disease severity of respiratory syncytial virus (RSV) infection correlate to a novel set of five amino acid substitutions in the RSV attachment glycoprotein (G) in China. *Virus Res* 2020;281:197937. PUBMED | CROSSREF
25. Midulla F, Nenna R, Scagnolari C, Petrarca L, Frassanito A, Viscido A, et al. How respiratory syncytial virus genotypes influence the clinical course in infants hospitalized for bronchiolitis. *J Infect Dis* 2019;219(4):526-34. PUBMED | CROSSREF
26. Abu-Raya B, Viñeta Paramo M, Reicherz F, Lavoie PM. Why has the epidemiology of RSV changed during the COVID-19 pandemic? *EClinicalMedicine* 2023;61:102089. PUBMED | CROSSREF
27. Gueudin M, Vabret A, Petitjean J, Gouarin S, Brouard J, Freymuth F. Quantitation of respiratory syncytial virus RNA in nasal aspirates of children by real-time RT-PCR assay. *J Virol Methods* 2003;109(1):39-45. PUBMED | CROSSREF



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