6. Katoh K, Standley DM. MAFFT multiple sequence alignment software version 7: improvements in performance and usability. Mol Biol Evol. 2013;30:772–80. https://doi.org/10.1093/molbev/mst010
7. Minh BQ, Schmidt HA, Chernomor O, Schrempf D, Woodhams MD, von Haeseler A, et al. IQ-TREE 2: new models and efficient methods for phylogenetic inference in the genomic era. Mol Biol Evol. 2020;37:1530–4. https://doi.org/10.1093/molbev/msaa015
8. Sagulenko P, Puller V, Neher RA. TreeTime: maximum-likelihood phylodynamic analysis. Virus Evol. 2018;4:vex042. https://doi.org/10.1093/ve/vex042
9. Giovanetti M, Slavov SN, Fonseca V, Wilkinson E, Tegally H, Patane JSL, et al. Genomic epidemiology of the SARS-CoV-2 epidemic in Brazil. Nat Microbiol. 2022;7:1490–1500.
10. Xu S, Dai Z, Guo P, Fu X, Liu S, Zhou L, et al. ggtreeExtra: compact visualization of richly annotated phylogenetic data. Mol Biol Evol. 2021;38:4039–42. https://doi.org/10.1093/molbev/msab166

Address for correspondence: Bright Adu, Department of Immunology, Noguchi Memorial Institute for Medical Research, College of Health Sciences, University of Ghana, PO Box LG 581, Legon, Ghana; email: badu@noguchi.ug.edu.gh

Genomic Characterization of Respiratory Syncytial Virus during 2022–23 Outbreak, Washington, USA

Stephanie Goya, Jaydee Sereewit, Daniel Pfalmer, Tien V. Nguyen, Shah A.K. Mohamed Bakhash, Elizabeth B. Sobolik, Alexander L. Greninger

Author affiliations: University of Washington, Seattle, Washington, USA (S. Goya, J. Sereewit, D. Pfalmer, T.V. Nguyen, S.A.K. Mohamed Bakhash, E.B. Sobolik, A.L. Greninger); Fred Hutchinson Cancer Research Center, Seattle (A.L. Greninger)

DOI: https://doi.org/10.3201/eid2904.221834

We sequenced 54 respiratory syncytial virus (RSV) genomes collected during 2021–22 and 2022–23 outbreaks in Washington, USA, to determine the origin of increased RSV cases. Detected RSV strains have been spreading for >10 years, suggesting a role for diminished population immunity from low RSV exposure during the COVID-19 pandemic.

Annual seasonality of respiratory syncytial virus (RSV) in Washington, USA, has been limited primarily to late autumn and winter (*1*). However, an RSV outbreak was not detected during the 2020–21 season because of the COVID-19 pandemic. After lockdowns were relaxed in the summer of 2021, an early RSV season began in August (Figure, panel A). The 2022–23 outbreak also began earlier, but the number of RSV cases was unexpectedly higher than in 2021, alarming public health authorities and the general community (*2*).

Increased severity of the 2022–23 RSV outbreak might have been caused by diminished protective immunity in the population from prolonged low exposure to this virus (*3*). Furthermore, selective pressure because of low transmission in 2020 might have caused emergence of new viral strains with improved fitness. We evaluated whether RSV causing the 2022–23 outbreak had genomic characteristics different from strains from previous seasons.

We performed hybridization capture-based, metagenomic next-generation sequencing of 54 RSV genomes (14 RSV strains from 2021–22 and 40 from 2022–23) isolated during outbreaks in King County, Washington. In brief, we extracted virus RNA from excess nasal or nasopharyngeal swab specimens collected from persons seeking care at University of Washington Medicine COVID-19 collection sites, clinics, emergency rooms, and inpatient facilities who tested positive for RSV by PCR with a cycle threshold <30 (Table) (*4*). All persons were outpatients except for 2 hospitalized patients from 2021. For phylogenetic analyses, we downloaded complete genomes of RSV-A and RSV-B subtypes from GenBank and GISAID (https://www.gisaid.org) databases. We performed genome alignments by using MAFFT software (https://mafft.cbrc.jp/alignment/software) and constructed phylogenetic trees by using IQ-TREE (*5*) (Appendix, https://wwwnc.cdc.gov/EID/article/29/4/22-1834-App1.pdf).

Among sequenced specimens, we detected 1 RSV-A and 13 RSV-B subtypes from 2021–22 and 30 RSV-A and 10 RSV-B subtypes from 2022–23 (Table). We did not detect co-infections with other respiratory viruses (Appendix) or differences in subtype predominance by patient age group or sex during the 2022–23 outbreak ($p>0.1$ by Fisher exact test). We genotyped the RSV *G* gene and found that 7 RSV-A sequences were GA2.3.5 and 24 were GA2.3.6b genotypes (both comprising ON1 strains), and all RSV-B sequences were the GB5.0.5.a genotype (BA strains) (*6*) (Appendix). We found that Washington RSV (WA-RSV) sequences were closely related to contemporary viruses by

using complete genome phylogenetic analysis with all historical and recent RSV sequences in public databases up to December 2022 (Appendix). We then constructed reduced phylogenetic trees with RSV genomes from public databases collected during 2017–2022 (Figure, panels B, C; Appendix Figures 1, 2); the trees showed the WA-RSV sequences from 2021–22 and 2022–23 outbreaks were closely related to those genomes. However, WA-RSV sequences from 2018 and 2019 were not phylogenetically close to database-derived RSV genomes collected during 2017–2022. Some WA-RSVs from 2022 were individually associated with viruses from France, Spain, Argentina, Brazil, Netherlands, Israel, Australia, and northern Macedonia, isolated in 2019, 2021, or early 2022, suggesting multiple viral introductions within Washington. Nevertheless, most WA-RSVs were within statistically supported monophyletic clades (Figure, panels B, C; Appendix Figures 1, 2), indicating the 2022–23 outbreak in King County has been mainly caused by the same RSV-A and RSV-B lineages observed globally for ≈1 decade. We observed no phylogenetic relationship between clade and patient age.

Analysis of all viral genes from 2022–23 WA-RSVs showed no specific nonsynonymous changes compared with other RSV strains collected globally since 2017. Furthermore, WA-RSVs contained amino acid changes previously identified in sequences isolated before the COVID-19 pandemic. For example, the amino acid constellation A103T and T122A in the RSV-A fusion protein was also detected in 14 other RSV genomes, including a 2019 sequence from the

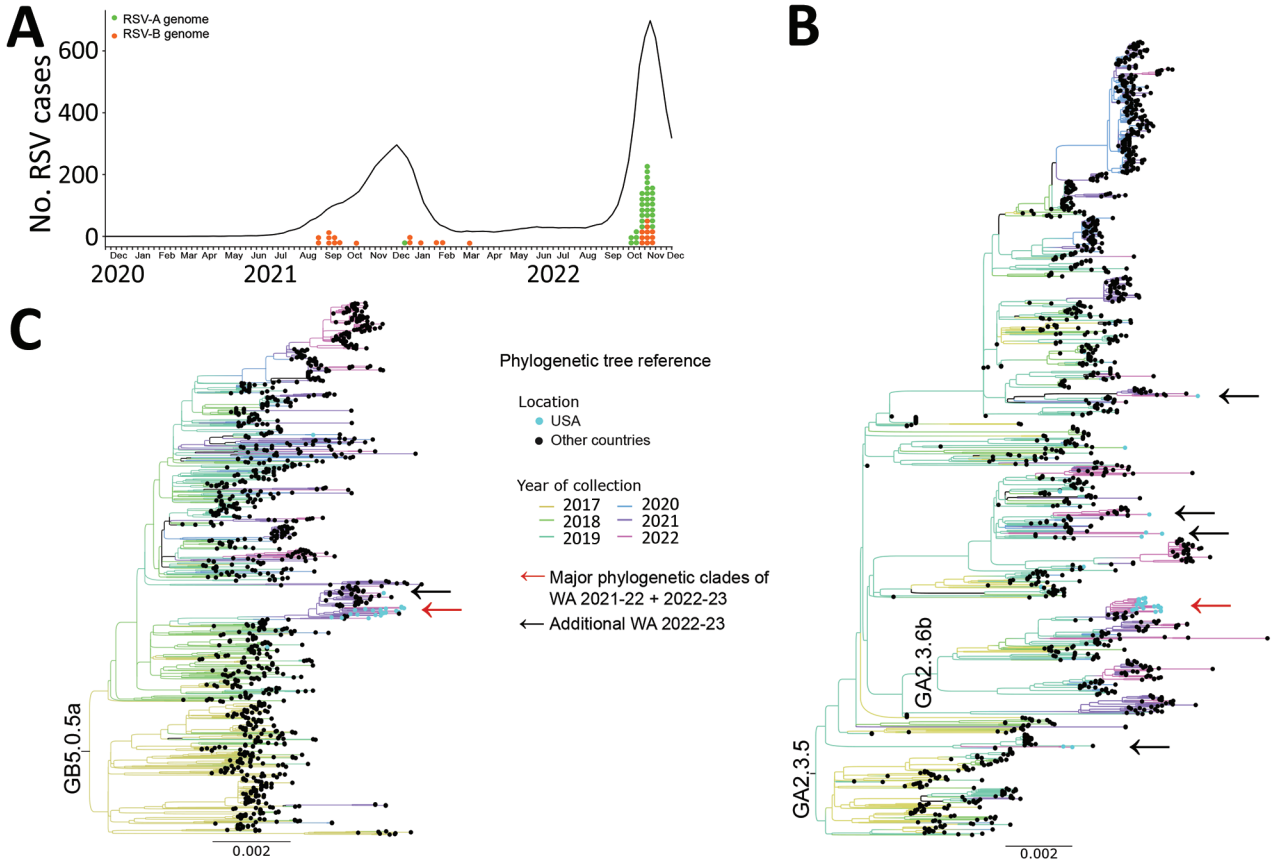


Figure. Molecular epidemiology and genomic characterization of RSV during 2021–22 and 2022–23 outbreaks, Washington, USA. A) Number of patients positive for RSV-A and RSV-B during 2021–22 and 2022–23 outbreaks. Graph shows 5-week averages of RSV-positive cases in Washington detected by PCR; data were taken from The National Respiratory and Enteric Virus Surveillance System (https://www.cdc.gov/surveillance/nrevss/index.html) through December 7, 2022. Tick marks indicate weeks for each month beginning on November 28, 2020, and ending on December 3, 2022. Orange and green dots show collection dates for RSV genomes analyzed in this study. B, C) Maximum-likelihood phylogenetic trees of complete genomes of RSV-A (B) and RSV-B (C) collected during 2017–2022. Collection years for specimens are depicted by tree branch color. RSV genomes from the United States are highlighted with light blue circles at branch tips. Red arrow indicates the location of the major phylogenetic clade comprising most of the sequences from Washington during 2021–22 and 2022–23; black arrows indicate locations of other sequences from Washington during 2022–23. Scale bar indicates nucleotide substitutions per site. Complete phylogenetic trees are provided in the Appendix (https://wwwnc.cdc.gov/EID/article/29/4/22-1834-App1.pdf). RSV, respiratory syncytial virus; RSV-A, RSV subtype A; RSV-B, RSV subtype B.

Table. Number of sequenced respiratory syncytial virus genomes according to different patient characteristics during 2021–22 and 2022–23 virus outbreaks in Washington, USA*

Characteristics	2021–22 outbreak			2022–23 outbreak		
	RSV-A	RSV-B	Total	RSV-A	RSV-B	Total
No. complete genomes	1	13	14	30	10	40
Patient sex						
M	0	6	6	13	7	20
F	1	7	8	16	4	20
Clinical status						
Inpatient	1	1	2	0	0	0
Outpatient	0	12	12	30	10	40
Patient age, y						
<3	0	5	5	10	6	16
3–18	0	3	3	10	2	12
19–65	1	4	5	8	1	9
>65	0	2	2	2	1	3

*RSV, respiratory syncytial virus; RSV-A, RSV subtype A; RSV-B, RSV subtype B.

Netherlands (GenBank accession no. MZ515825.1), suggesting a bottleneck effect caused by low transmission during 2020 that reduced virus diversity (*7*). Alternating prevalence of RSV subtypes between outbreaks might also lead to high levels of RSV spread (Table). Further analyses of RSV sequences from Washington and globally are needed to confirm those hypotheses.

The first limitation of our study is that few RSV genomes from Washington were available before the COVID-19 pandemic. Second, we conducted convenience sampling from excess clinical specimens and had limited access to clinical metadata. Nonetheless, Washington is comparatively a well-sampled state for RSV sequences, because only 2 other RSV genomes have been isolated from the rest of the United States since 2017. RSV genomics is also currently limited by a lack of consensus on genotyping classification.

In conclusion, effects of COVID-19 pandemic lockdown measures on the RSV ecosystem have been reported (*8*–*10*). Real-time genomic surveillance of RSV outbreaks in Washington did not reveal specific changes in RSV since the COVID-19 pandemic began that would account for increased viral spread. Our data suggest that RSV reemergence in King County is likely because of diminished protective immunity in the population from low RSV exposure, a consequence of pandemic mitigation measures. With likely future widespread availability of RSV vaccines, continued real-time RSV genomic surveillance will be required to monitor the evolution and emergence of new viral strains.

This study was approved by the University of Washington Institutional Review Board with a consent waiver (protocol no. STUDY00000408).

A.L.G. reports contract testing for Abbott, Cepheid, Novavax, Pfizer, Janssen, and Hologic and research support from Gilead and Merck, outside of the described work.

About the Author

Dr. Goya is a postdoctoral researcher in the Department Laboratory of Medicine and Pathology at the University of Washington Medical Center. Her work focuses on respiratory virus evolution and interactions with the immune system.

References

1. Jackson ML, Scott E, Kuypers J, Nalla AK, Roychoudury P, Chu HY. Epidemiology of respiratory syncytial virus across five influenza seasons among adults and children one year of age and older—Washington state, 2011/2012–2015/2016. J Infect Dis. 2021;223:147–56. https://doi.org/10.1093/infdis/jiaa331
2. Schrier K, Sperring J. The Seattle Times. Declaration of public health emergency is urgently needed for RSV. December 4, 2022 [cited 2022 Dec 7]. https://www.seattletimes.com/opinion/declaration-of-public-health-emergency-is-urgently-needed-for-rsv
3. Hatter L, Eathorne A, Hills T, Bruce P, Beasley R. Respiratory syncytial virus: paying the immunity debt with interest. Lancet Child Adolesc Health. 2021;5:e44–5. https://doi.org/10.1016/S2352-4642(21)00333-3
4. Greninger AL, Waghmare A, Adler A, Qin X, Crowley JL, Englund JA, et al. Rule-out outbreak: 24-hour metagenomic next-generation sequencing for characterizing respiratory virus source for infection prevention. J Pediatric Infect Dis Soc. 2017;6:168–72. https://doi.org/10.1093/jpids/pix019
5. Minh BQ, Schmidt HA, Chernomor O, Schrempf D, Woodhams MD, von Haeseler A, et al. IQ-TREE 2: new models and efficient methods for phylogenetic inference in the genomic era. Mol Biol Evol. 2020;37:1530–4. https://doi.org/10.1093/molbev/msaa015
6. Goya S, Galiano M, Nauwelaers I, Trento A, Openshaw PJ, Mistchenko AS, et al. Toward unified molecular surveillance of RSV: a proposal for genotype definition. Influenza Other Respir Viruses. 2020;14:274–85. https://doi.org/10.1111/irv.12715
7. Sanjuán R, Domingo-Calap P. Genetic diversity and evolution of viral populations. In: Bamford D, Zuckerman M, editors. Encyclopedia of virology. 4th ed. Cambridge: Elsevier Academic Press; 2021. p. 53–61.
8. Dolores A, Stephanie G, Mercedes S NJ, Érica G, Mistchenko AS, Mariana V. RSV reemergence in Argentina since the SARS-CoV-2 pandemic. J Clin Virol. 2022;149:105126. https://doi.org/10.1016/j.jcv.2022.105126

9. Fourgeaud J, Toubiana J, Chappuy H, Delacourt C, Moulin F, Parize P, et al. Impact of public health measures on the post-COVID-19 respiratory syncytial virus epidemics in France. Eur J Clin Microbiol Infect Dis. 2021;40:2389–95. https://doi.org/10.1007/s10096-021-04323-1
10. Foley DA, Yeoh DK, Minney-Smith CA, Martin AC, Mace AO, Sikazwe CT, et al. The interseasonal resurgence of respiratory syncytial virus in Australian children following the reduction of coronavirus disease 2019-related public health measures. Clin Infect Dis. 2021;73:e2829–30. https://doi.org/10.1093/cid/ciaa1906

Address for correspondence: Alexander L. Greninger, Department of Laboratory Medicine and Pathology, University of Washington, 850 Republican St, Seattle, WA 98109, USA; email: agrening@uw.edu

Article DOI: https://doi.org/10.3201/eid2904.221834

Genomic Characterization of Respiratory Syncytial Virus during 2022–23 Outbreak, Washington, USA

Appendix

Material and methods

RSV genome sequencing

We extracted virus RNA by using the Quick-RNA Viral Kit (Zymo Research, https://www.zymoresearch.com). For samples with RSV PCR cycle threshold counts <25, virus genome sequencing was performed as previously described (*1*). In brief, we used the TURBO DNA-free Kit (ThermoFisher Scientific, https://www.thermofisher.com) to remove genomic DNA from extracted RNA. We performed first-strand cDNA synthesis by using random hexamers and SuperScript IV Reverse Transcriptase (ThermoFisher Scientific) and second-strand synthesis by using Sequenase Version 2.0 DNA Polymerase (ThermoFisher Scientific). We purified double-stranded cDNA by using AMPure XP Magnetic Beads (Beckman Coulter, https://www.beckman.com) before proceeding to tagmentation and library preparation by using the Illumina DNA Prep, (S) Tagmentation kit (Illumina, https://www.illumina.com). For samples with RSV PCR threshold counts of 25–30, we used the RNA Prep with Enrichment, (L) Tagmentation and Respiratory Virus Oligos Panel v2 (Illumina) for library generation. DNA libraries were sequenced as 2 × 100-bp and 2 × 250-bp runs in a NextSeq500 sequencer (Illumina).

Bioinformatic analysis

Quality of FastQ files was analyzed by using FastQC software (http://www.bioinformatics.babraham.ac.uk/projects/fastqc). RSV genome assembly was generated by using the Revica pipeline (https://github.com/greninger-lab/revica). Briefly, adaptor trimming and quality filtering was performed with Trimmomatic v0.39 (*2*). Mapping against a

viral genome reference database was performed, followed by one round of mapping against the virus reference sequence with the highest median coverage (RSV-A reference, GenBank accession no. MZ516076.1; RSV-B reference, GenBank accession no. OK649754.1) and 2 iterations of mapping against the consensus reconstruction. Consensus genomes were generated by using a minimum base quality of 20, minimum depth of coverage of 5 times, and 60% allele frequency. Co-infection in a sample was indicated when 2 complete or partial consensus genomes of different virus species resulted from the Revica analysis.

Comparative analysis of nonsynonymous changes was performed by using consensus genome alignments that included genomes published in GenBank and GISAID (https://www.gisaid.org) with collection dates during January 2017–December 2022 (2,481 total genomes: 1,320 RSV-A and 1,161 RSV-B subtypes). Inclusion criteria comprised complete genomes from clinical isolates with >95% sequence coverage. Alignments were trimmed according to the open reading frame of the virus genes and translated with standard amino acid codes by using Aliview (*3*).

Phylogenetic analysis

RSV genome alignments were built for each RSV subtype by using MAFFT software and visualized with Aliview (*3*,*4*). RSV genotyping was based on the *G* gene (*5*). The sequenced genomes were trimmed to the ectodomain of the *G* gene and analyzed with ReSVidex (https://cacciabue.shinyapps.io/resvidex) and corroborated by maximum-likelihood inference by using RSV-A and RSV-B reference alignments (*5*). *G* gene genotyping trees are available at https://github.com/greninger-lab/RSV-WA-2022. Genotype classification and sequences analyzed in this study are also available on the Nextstrain platform (https://nextstrain.org/rsv/a/G, https://nextstrain.org/rsv/b/G) (*6*).

In accordance with other RSV genotyping schemes, the sequences in this study would be classified as follows: lineage GA2.3.5 would be equivalent to ON1 (*7*), A23 (*8*), A.5.9 (*9*), and NA1 (*10*); lineage GA2.3.6b would be equivalent to ON1 (*7*), A23 (*8*), A.5.11 (*9*), and NA1 (*10*); lineage GB5.0.5a would be equivalent to BA9 (*11*), B6 (*8*), B.5.8 (*9*), and BA (*12*).

For comprehensive phylogenetic analyses, RSV genomes from clinical samples were downloaded from GenBank and GISAID databases if they had <5% N bases. Two phylogenetic analyses were performed: 1 analysis using all available RSV genomes (mentioned in the main

text as historical and recent RSV: 2,195 RSV-A and 1,711 RSV-B) and a reduced analysis that included RSV genomes with collection dates since 2017 (1,320 RSV-A and 1,161 RSV-B). Maximum-likelihood trees were inferred by using IQ-TREE v2.1 (*13*). The molecular evolution model was estimated by using ModelFinder (*14*), and the reliability of sequences clusters was evaluated by using UFBoot2 (10,000 replicates) (*15*). Complete RSV-A and RSV-B tree files and extended versions of the reduced trees (Figure, main text) are available at https://github.com/greninger-lab/RSV-WA-2022.

Data availability

RSV consensus genomes are available in GenBank (accession nos. OP890312–50 and OP965698–712). Sequencing reads are available in NCBI BioProject no. PRJNA907066 (https://www.ncbi.nlm.nih.gov/bioproject). Line-item specimen data are available in the Appendix Table.

GISAID acknowledgment

We thank the authors from the originating laboratories responsible for obtaining the specimens and the submitting laboratories where genetic sequence data were generated and shared via the GISAID Initiative (Authors acknowledgment table available at https://github.com/greninger-lab/RSV-WA-2022).

References

1. Goya S, Valinotto LE, Tittarelli E, Rojo GL, Nabaes Jodar MS, Greninger AL, et al. An optimized methodology for whole genome sequencing of RNA respiratory viruses from nasopharyngeal aspirates. PLoS One. 2018;13:e0199714. PubMed https://doi.org/10.1371/journal.pone.0199714

2. Bolger AM, Lohse M, Usadel B. Trimmomatic: a flexible trimmer for Illumina sequence data. Bioinformatics. 2014;30:2114–20. PubMed https://doi.org/10.1093/bioinformatics/btu170

3. Larsson A. AliView: a fast and lightweight alignment viewer and editor for large datasets. Bioinformatics. 2014;30:3276–8. PubMed https://doi.org/10.1093/bioinformatics/btu531

4. Katoh K, Standley DM. MAFFT multiple sequence alignment software version 7: improvements in performance and usability. Mol Biol Evol. 2013;30:772–80. PubMed https://doi.org/10.1093/molbev/mst010

5. Goya S, Galiano M, Nauwelaers I, Trento A, Openshaw PJ, Mistchenko AS, et al. Toward unified molecular surveillance of RSV: a proposal for genotype definition. Influenza Other Respir Viruses. 2020;14:274–85. PubMed https://doi.org/10.1111/irv.12715

6. Hadfield J, Megill C, Bell SM, Huddleston J, Potter B, Callender C, et al. Nextstrain: real-time tracking of pathogen evolution. Bioinformatics. 2018;34:4121–3. PubMed https://doi.org/10.1093/bioinformatics/bty407

7. Eshaghi A, Duvvuri VR, Lai R, Nadarajah JT, Li A, Patel SN, et al. Genetic variability of human respiratory syncytial virus A strains circulating in Ontario: a novel genotype with a 72 nucleotide G gene duplication. PLoS One. 2012;7:e32807. PubMed https://doi.org/10.1371/journal.pone.0032807

8. Ramaekers K, Rector A, Cuypers L, Lemey P, Keyaerts E, Van Ranst M. Towards a unified classification for human respiratory syncytial virus genotypes. Virus Evol. 2020;6:veaa052. PubMed https://doi.org/10.1093/ve/veaa052

9. Chen J, Qiu X, Avadhanula V, Shepard SS, Kim DK, Hixson J, et al. Novel and extendable genotyping system for human respiratory syncytial virus based on whole-genome sequence analysis. Influenza Other Respir Viruses. 2022;16:492–500. PubMed https://doi.org/10.1111/irv.12936

10. Muñoz-Escalante JC, Comas-García A, Bernal-Silva S, Robles-Espinoza CD, Gómez-Leal G, Noyola DE. Respiratory syncytial virus A genotype classification based on systematic intergenotypic and intragenotypic sequence analysis. Sci Rep. 2019;9:20097. PubMed https://doi.org/10.1038/s41598-019-56552-2

11. Trento A, Viegas M, Galiano M, Videla C, Carballal G, Mistchenko AS, et al. Natural history of human respiratory syncytial virus inferred from phylogenetic analysis of the attachment (G) glycoprotein with a 60-nucleotide duplication. J Virol. 2006;80:975–84. PubMed https://doi.org/10.1128/JVI.80.2.975-984.2006

12. Muñoz-Escalante JC, Comas-García A, Bernal-Silva S, Noyola DE. Respiratory syncytial virus B sequence analysis reveals a novel early genotype. Sci Rep. 2021;11:3452. PubMed https://doi.org/10.1038/s41598-021-83079-2

13. Minh BQ, Schmidt HA, Chernomor O, Schrempf D, Woodhams MD, von Haeseler A, et al. IQ-TREE 2: new models and efficient methods for phylogenetic inference in the genomic era. Mol Biol Evol. 2020;37:1530–4. PubMed https://doi.org/10.1093/molbev/msaa015

14. Kalyaanamoorthy S, Minh BQ, Wong TKF, von Haeseler A, Jermiin LS. ModelFinder: fast model selection for accurate phylogenetic estimates. Nat Methods. 2017;14:587–9. PubMed https://doi.org/10.1038/nmeth.4285

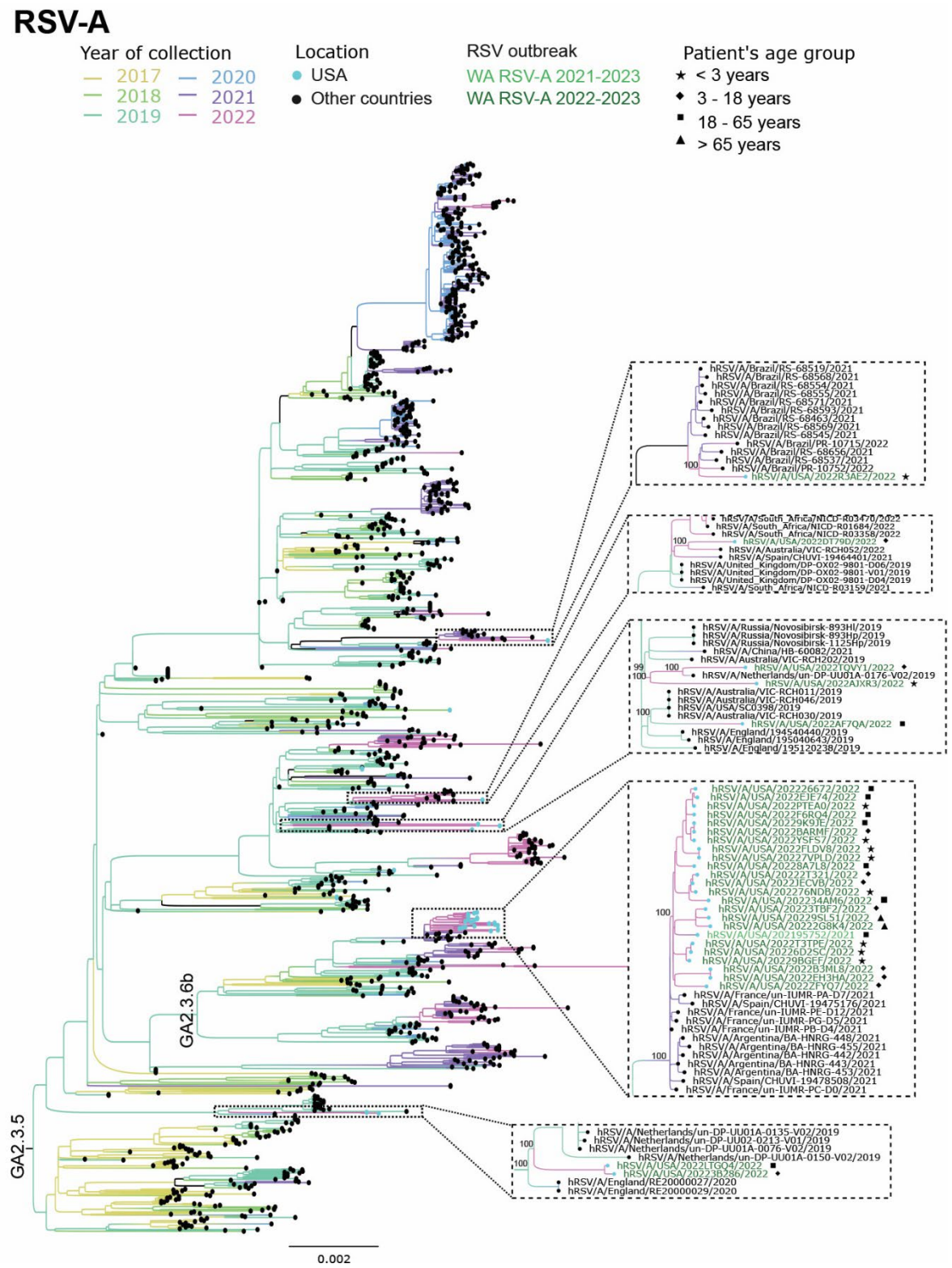
15. Hoang DT, Chernomor O, von Haeseler A, Minh BQ, Vinh LS. UFBoot2: improving the ultrafast bootstrap approximation. Mol Biol Evol. 2018;35:518–22. PubMed https://doi.org/10.1093/molbev/msx281

Appendix Table. Metadata of sequenced RSV specimens*

Sequence name	Collection date	RSV subtype	Ct	Coverage†	Genbank no.	BioProject no.‡	BioSample no.‡	SRA fastq file‡
hRSV/A/USA/202276NDB/2022	10–2022	A	19.20	120.86	OP890312	PRJNA907066	SAMN32118079	SRR22580785
hRSV/A/USA/2022FLDV8/2022	10–2022	A	18.46	87.38	OP890313	PRJNA907066	SAMN32118080	SRR22580784
hRSV/A/USA/2022R3AE2/2022	10–2022	A	18.65	147.96	OP890314	PRJNA907066	SAMN32118081	SRR22580773
hRSV/A/USA/2022AF7QA/2022	10–2022	A	19.08	114.32	OP890315	PRJNA907066	SAMN32118082	SRR22580762
hRSV/A/USA/2022LTGQ4/2022	10–2022	A	19.27	119.10	OP890316	PRJNA907066	SAMN32118083	SRR22580751
hRSV/A/USA/202226672/2022	10–2022	A	20.46	130.82	OP890317	PRJNA907066	SAMN32118084	SRR22580740
hRSV/B/USA/20229B2FA/2022	11–2022	B	19.33	138.62	OP890341	PRJNA907066	SAMN32118085	SRR22580735
hRSV/A/USA/20223TBF2/2022	10–2022	A	19.19	141.67	OP890318	PRJNA907066	SAMN32118086	SRR22580734
hRSV/A/USA/2022TQVY1/2022	11–2022	A	18.78	148.55	OP890319	PRJNA907066	SAMN32118087	SRR22580733
hRSV/A/USA/20229K9JE/2022	11–2022	A	19.78	125.66	OP890320	PRJNA907066	SAMN32118088	SRR22580732
hRSV/A/USA/20222G8K4/2022	10–2022	A	17.13	65.64	OP890321	PRJNA907066	SAMN32118089	SRR22580783
hRSV/A/USA/2022DT79D/2022	10–2022	A	18.84	147.88	OP890322	PRJNA907066	SAMN32118090	SRR22580782
hRSV/B/USA/20229BJQ7/2022	11–2022	B	18.79	126.65	OP890342	PRJNA907066	SAMN32118091	SRR22580781
hRSV/A/USA/202234AM6/2022	11–2022	A	18.82	54.28	OP890323	PRJNA907066	SAMN32118092	SRR22580780
hRSV/A/USA/2022YSFS7/2022	10–2022	A	18.47	53.97	OP890324	PRJNA907066	SAMN32118093	SRR22580779
hRSV/B/USA/2022A7421/2022	10–2022	B	19.68	93.93	OP890343	PRJNA907066	SAMN32118094	SRR22580778
hRSV/A/USA/2022PTEA0/2022	10–2022	A	19.11	123.60	OP890325	PRJNA907066	SAMN32118095	SRR22580777
hRSV/B/USA/2022KH4F2/2022	10–2022	B	19.98	112.33	OP890344	PRJNA907066	SAMN32118096	SRR22580776
hRSV/B/USA/2022RWWYB/2022	10–2022	B	18.43	115.10	OP890345	PRJNA907066	SAMN32118097	SRR22580775
hRSV/A/USA/20227VPLD/2022	10–2022	A	18.83	86.18	OP890326	PRJNA907066	SAMN32118098	SRR22580774
hRSV/A/USA/2022T3TPE/2022	10–2022	A	19.26	85.36	OP890327	PRJNA907066	SAMN32118099	SRR22580772
hRSV/A/USA/2022BARMF/2022	11–2022	A	17.97	126.93	OP890328	PRJNA907066	SAMN32118100	SRR22580771
hRSV/A/USA/20222T321/2022	11–2022	A	17.36	138.17	OP890329	PRJNA907066	SAMN32118101	SRR22580770
hRSV/B/USA/20228BLN9/2022	10–2022	B	20.3	125.53	OP890346	PRJNA907066	SAMN32118102	SRR22580769
hRSV/B/USA/2022RFHA8/2022	11–2022	B	19.59	121.36	OP890347	PRJNA907066	SAMN32118103	SRR22580768
hRSV/A/USA/2022ZFYQ7/2022	11–2022	A	22.53	130.05	OP890330	PRJNA907066	SAMN32118104	SRR22580767
hRSV/A/USA/20226D2SC/2022	10–2022	A	17.85	139.53	OP890331	PRJNA907066	SAMN32118105	SRR22580766
hRSV/A/USA/20228A7L8/2022	10–2022	A	19.74	21.53	OP890332	PRJNA907066	SAMN32118106	SRR22580765
hRSV/A/USA/2022B3ML8/2022	11–2022	A	18.1	10.49	OP965712	PRJNA907066	SAMN32118107	SRR22580764
hRSV/A/USA/2022EH3HA/2022	11–2022	A	20.2	105.85	OP890333	PRJNA907066	SAMN32118108	SRR22580763
hRSV/A/USA/20229BGEF/2022	11–2022	A	20.26	22.35	OP890334	PRJNA907066	SAMN32118109	SRR22580761
hRSV/A/USA/2022EJE74/2022	11–2022	A	17.89	74.50	OP890335	PRJNA907066	SAMN32118110	SRR22580760
hRSV/A/USA/2022F6RQ4/2022	11–2022	A	17.88	101.39	OP890336	PRJNA907066	SAMN32118111	SRR22580759
hRSV/A/USA/20223B286/2022	11–2022	A	19.46	18.19	OP890337	PRJNA907066	SAMN32118112	SRR22580758
hRSV/B/USA/2022YVED3/2022	11–2022	B	19.46	139.35	OP890348	PRJNA907066	SAMN32118113	SRR22580757
hRSV/A/USA/20229SL51/2022	10–2022	A	19.80	138.35	OP890338	PRJNA907066	SAMN32118114	SRR22580756
hRSV/A/USA/2022JECVB/2022	10–2022	A	19.58	79.68	OP890339	PRJNA907066	SAMN32118115	SRR22580755
hRSV/B/USA/2022YFP3F/2022	10–2022	B	19.09	111.2	OP890349	PRJNA907066	SAMN32118116	SRR22580754
hRSV/B/USA/2022M4SWF/2022	10–2022	B	18.65	103.76	OP890350	PRJNA907066	SAMN32118117	SRR22580753
hRSV/A/USA/2022AJXR3/2022	10–2022	A	18.58	95.86	OP890340	PRJNA907066	SAMN32118118	SRR22580752
hRSV/B/USA/202177756/2021	08–2021	B	19.4	355.53	OP965698	PRJNA907066	SAMN32118119	SRR22580750
hRSV/B/USA/202196775/2021	09–2021	B	22.9	19.97	OP965699	PRJNA907066	SAMN32118120	SRR22580749
hRSV/B/USA/202118974/2021	09–2021	B	21.1	140.21	OP965700	PRJNA907066	SAMN32118121	SRR22580748
hRSV/B/USA/202131818/2021	09–2021	B	22.5	43.97	OP965701	PRJNA907066	SAMN32118122	SRR22580747
hRSV/B/USA/202134981/2021	09–2021	B	23.9	238.93	OP965702	PRJNA907066	SAMN32118123	SRR22580746
hRSV/B/USA/202179926/2021	10–2021	B	20.4	350.92	OP965703	PRJNA907066	SAMN32118124	SRR22580745
hRSV/A/USA/202195752/2021	12–2021	A	18.6	115.71	OP965711	PRJNA907066	SAMN32118125	SRR22580744
hRSV/B/USA/202221067/2022	03–2022	B	21.6	40.88	OP965704	PRJNA907066	SAMN32118126	SRR22580743

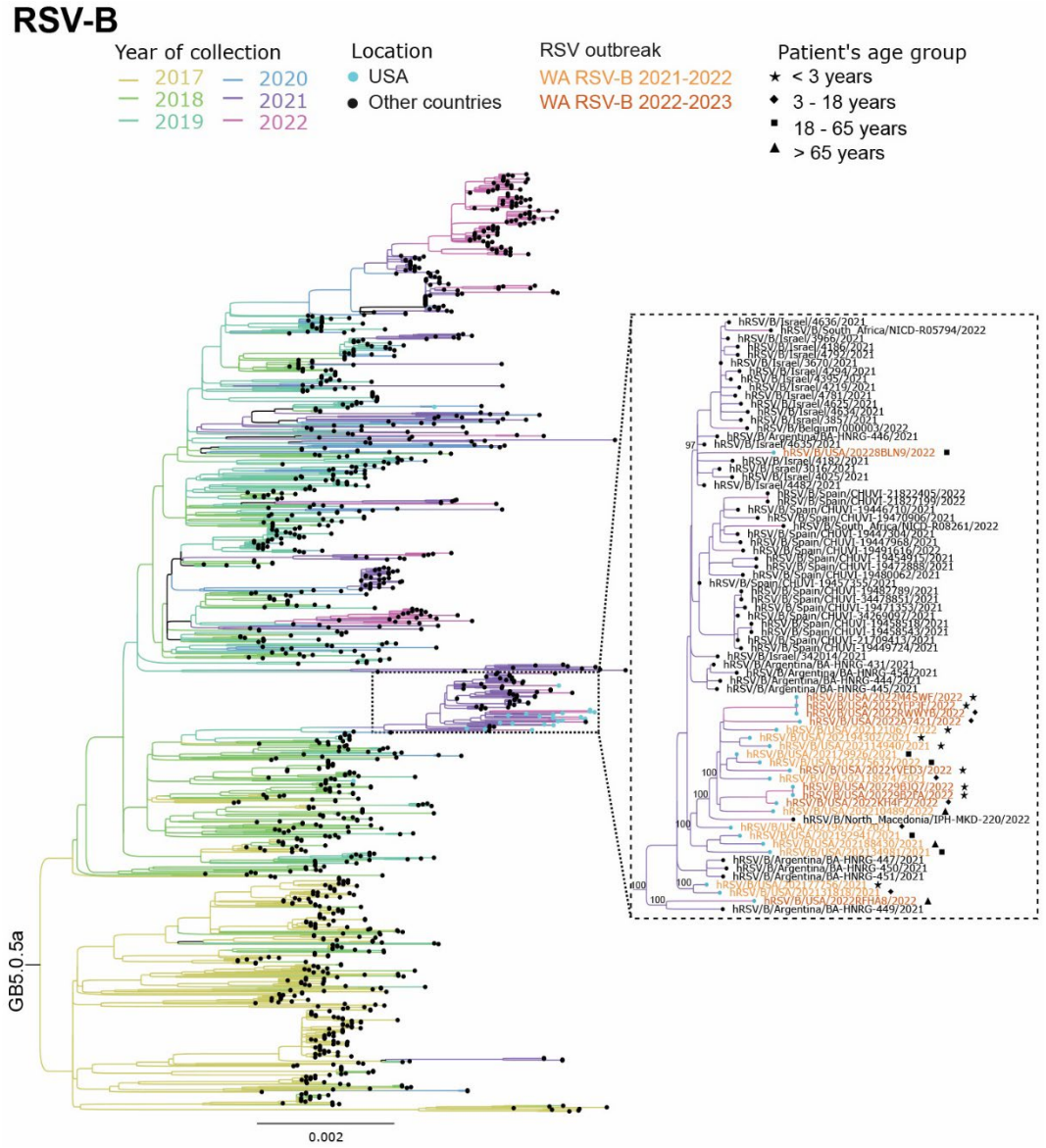
Sequence name	Collection date	RSV subtype	Ct	Coverage†	Genbank no.	BioProject no.‡	BioSample no.‡	SRA fastq file‡
hRSV/B/USA/202210489/2022	02–2022	B	28.3	5,147.37	OP965708	PRJNA907066	SAMN32118130	SRR22580738
hRSV/B/USA/202275637/2022	02–2022	B	26.5	2,371.03	OP965709	PRJNA907066	SAMN32118131	SRR22580737
hRSV/B/USA/202188430/2021	12–2021	B	30	241.61	OP965710	PRJNA907066	SAMN32118132	SRR22580736
hRSV/B/USA/202194302/2021	08-2021	B	27.2	2,773.8	OP965705	PRJNA907066	SAMN32118127	SRR22580742
hRSV/B/USA/202114940/2021	09-2021	B	25.8	1,3972.6	OP965707	PRJNA907066	SAMN32118129	SRR22580739
hRSV/B/USA/202192941/2021	09-2021	B	30	2,652.03	OP965706	PRJNA907066	SAMN32118128	SRR22580741

*Ct, PCR cycle threshold; RSV, respiratory syncytial virus.
†Average depth of sequencing coverage (x-fold).
‡NCBI BioProject, BioSample, and SRA (sequence read archive) accession numbers (https://www.ncbi.nlm.nih.gov).



Appendix Figure 1. Complete phylogenetic analysis of respiratory syncytial virus, subtype A. Maximum-likelihood trees were constructed by using complete genomes of RSV-A (collected during 2017–2022) downloaded from GenBank and GISAID (https://www.gisaid.org) databases. Phylogenetic associations of

RSV-A genomes from our study are shown in boxes, which include bootstrap values for the main phylogenetic clades. Lineages GA2.3.5 and GA2.3.6b are labeled within their ancestral nodes. Collection years for specimens are depicted by tree branch color. RSV-A genomes from the United States are highlighted with light blue circles at branch tips. Washington RSV-A genomes from 2021–22 and 2022–23 outbreak seasons are highlighted in shades of green, and patient age groups are indicated by symbols. Scale bar indicates nucleotide substitutions per site. RSV-A, respiratory syncytial virus, subtype A.



Appendix Figure 2. Complete phylogenetic analysis of respiratory syncytial virus, subtype B. Maximum-likelihood trees were constructed by using complete genomes of RSV-B (collected during 2017–2022) downloaded from GenBank and GISAID (https://www.gisaid.org) databases. Phylogenetic association of RSV-B genomes from our study are shown in the box inset, which includes bootstrap values for the main

phylogenetic clades. Lineage GB5.0.5a is labeled within its ancestral node. Collection years for specimens are depicted by tree branch color. RSV-B genomes from the United States are highlighted with light blue circles at branch tips. In the box inset, Washington RSV-B genomes from 2021–22 and 2022–23 outbreak seasons are highlighted in shades of orange, and patient age groups are indicated by symbols. Scale bar indicates nucleotide substitutions per site. RSV-B, respiratory syncytial virus, subtype B.