Article DOI: https://doi.org/10.3201/eid2806.220523

Recombinant BA.1/BA.2 SARS-CoV-2 Virus in Arriving Travelers, Hong Kong, February 2022

Appendix

Additional Methods

Sequencing

SARS-CoV-2 RT-PCR–positive samples with a C_t value <30 were randomly selected for next-generation sequencing (NGS) analysis. RNA samples were sent to a World Health Organization (WHO) reference laboratory at the University of Hong Kong (China) for full genome analyses (IRB no. UW 20–168). We deduced near full-length genomes from the samples using an Illumina (https://www.illumina.com) sequencing protocol previously described by us (*1*,*2*). Briefly, virus genome was reverse transcribed with multiple gene-specific primers targeting different regions of the viral genome. The synthesized cDNA was then subjected to multiple overlapping 2-kb PCRs for full-genome amplification. PCR amplicons obtained from the same specimen were pooled and sequenced using Novaseq or iSeq sequencing platform (Illumina). Specifically, for the sample from case-patient 1 with putative recombinant virus, we additionally performed NGS sequencing with the COVIDSeq kit (Illumina) for cross-validation. Generated sequencing reads were quality-trimmed by fastp (https://github.com/OpenGene/fastp) and mapped to a reference virus genome (Genbank accession no. MN908947.3) by BWA-MEM2 v2.1 (*3*). Potential PCR duplicates were identified and removed by samtools markdup (https://www.htslib.org/doc/samtools-markdup.html). The genome consensus was generated by iVar (*4*) with the PCR primer trimming protocol (minimum sequence depth of 5 for iSeq samples and minimum sequence depth of 10 for Novaseq samples, and minimum Q value of 30). The deduced sequences are available GISAID (https://www.gisaid.org; accession nos. are available at https://github.com/Leo-PoonLab/BA1_BA2_recombinant_HK/blob/main/GISAID_accessions.txt).

The average sequencing depths at the breakpoint region were 1,086 in patient 1 samples and 24,604 in patient 2 samples. We also cloned a ≈2.2 kbp RT-PCR amplicon spanning the putative breakpoint region using patient 2's sample. The 5′ and 3′ end of this clone was subjected to standard Sanger sequencing.

Identification of Putative Recombinants

We scanned all the sequenced samples from imported cases in Hong Kong after November 15, 2021 for putative BA.1/BA.2 recombinants. The lineage defining mutations for BA.1 and BA.2 were curated from Cov-lineages (https://github.com/cov-lineages/pango-designation/issues/361) and CoVariants (https://covariants.org). For defining a sequence as a putative BA.1/BA.2 recombinant, it must have ≥3 BA.1- and BA.2-defining mutations, each with an allele frequency >90%. The statistics of sample's read depth, allele frequency and minor allele frequency were deduced from aligned reads in bam files by using pysamstats (https://github.com/alimanfoo/pysamstats).

Identification of Putative Parental Sequences of the Recombinant

The available public 1,222,642 BA.1 and 767,399 BA.2 sequences from GISAID and GenBank (accessed on March 7, 2022) were downloaded and mapped to the reference sequence (GenBank accession no. MN908947.3) by using minimap2 (https://github.com/lh3/minimap2). The aligned sequences were used as the database for searching putative parental sequences. The leading/tailing partial sequences (positions 1–22005 and 21618–29903) of the recombinant genome were extracted by masking the remainder of the genome with "N". Masking was also performed for the aligned public sequences with letter "?" by using figleaf (https://github.com/Koohoko/figleaf_fasta). After masking, low-quality sequences were dropped (if >300 "N" bases were found within the non-masked regions). The closest matches of the 2 partial recombinant sequences were separately identified from the above aligned public sequences using gofasta (https://github.com/virus-evolution/gofasta).

Phylogenetic Analysis

The consensus sequences deduced from NGS data were aligned to the reference genome (GenBank accession no. MN908947.3) by using MAFFT-add (https://mafft.cbrc.jp/alignment/server/add.html). The representative sequences from SARS-CoV-2 variants of concern Alpha, Beta, Gamma, Delta, and Omicron BA.3 were also included.

The 5′ and 3′ untranslated regions were masked before tree building. The maximum-likelihood phylogenies were estimated by using IQ-TREE version 2.1.3 (*5*), and the best-fit nucleotide substitution models searched by the software by using Wuhan-Hu-1 (GenBank accession no. MN908947.3) as the outgroup. Branch supports are accessed by SH-aLRT and the ultrafast bootstrap, a node is considered supported if SH-aLRT ≥80% and UFboot ≥95% (http://www.iqtree.org/doc/Frequently-Asked-Questions).

Simplot Analysis

The putative BA.1/BA.2 recombinant virus sequence was analyzed in Simplot v3.5.1 (*6*) for the recombination signals. Its similarity was plotted against a smaller group of representative variants of concern (VOCs) including Alpha, Beta, Gamma, Delta, Omicron BA.1, Omicron BA.2, and the prototype Wuhan/WH01/2019 (GenBank accession no. MN908947.3). Due to the relatively large proportion of strictly conserved sites, these sites were excluded from the alignment before subjecting to the similarity plot analysis. The BA.1/BA.2 recombinant, its putative parents from Omicron BA.1 and BA.2, and prototype Wuhan/WH01/2019 (as outgroup) were analyzed for their informative sites of recombination.

The GenBank/GISAID accession numbers of viral sequences used in the Simplot analysis are as follows:

Alpha: OL807059, OU272361, OU052790, OU179605, OL315388, OU208527, OU174622, OU208088, MW933836, MZ280980, MZ296197, MZ077208, OU022681

Beta: OU202380, OU516338, OU136527, OK433425, OM765676, OL779105, OU114765, MW963525, OU233168

Gamma: MW913237, OL803729, MZ414874, MZ217960, MZ536412, OV921949, MZ211976, OM485550, MZ037589

Delta: OK208965, OK258803, MZ988451, OK101403, OK243904, MZ764878, OK160402, OK054978, MZ888548, MZ888540, MZ888535, MZ888534, OU338538, EPI_ISL_8880068

BA.1: EPI_ISL_10273412

BA.1: EPI_ISL_10462716

Code Availability

Detailed analyzing scripts used in the study can be accessed in a GitHub repository (https://github.com/Leo-Poon-Lab/BA1_BA2_recombinant_HK).

Reference

1. Choi EM, Chu DKW, Cheng PKC, Tsang DNC, Peiris M, Bausch DG, et al. In-flight transmission of SARS-CoV-2. Emerg Infect Dis. 2020;26:2713–6. PubMed https://doi.org/10.3201/eid2611.203254

2. Sit THC, Brackman CJ, Ip SM, Tam KWS, Law PYT, To EMW, et al. Infection of dogs with SARS-CoV-2. Nature. 2020;586:776–8. PubMed https://doi.org/10.1038/s41586-020-2334-5

3. Vasimuddin M, Misra S, Li H, Aluru S. Efficient architecture-aware acceleration of BWA-MEM for multicore systems. In: 2019 IEEE International Parallel and Distributed Processing Symposium (IPDPS). Rio de Janeiro, Brazil; May 20–24, 2019; p. 314–24. https://doi.org/10.1109/IPDPS.2019.00041

4. Grubaugh ND, Gangavarapu K, Quick J, Matteson NL, De Jesus JG, Main BJ, et al. An amplicon-based sequencing framework for accurately measuring intrahost virus diversity using PrimalSeq and iVar. Genome Biol. 2019;20:8. PubMed https://doi.org/10.1186/s13059-018-1618-7

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. PubMed https://doi.org/10.1093/molbev/msaa015

6. Lole KS, Bollinger RC, Paranjape RS, Gadkari D, Kulkarni SS, Novak NG, et al. Full-length human immunodeficiency virus type 1 genomes from subtype C-infected seroconverters in India, with evidence of intersubtype recombination. J Virol. 1999;73:152–60. PubMed https://doi.org/10.1128/JVI.73.1.152-160.1999

Appendix Table 1. Number and country of origin of studied imported COVID-19 cases (n = 198), Hong Kong, China, November 15, 2021–February 4, 2022

Country of importation	No. cases
United Kingdom	23
United States of America	22
Philippines	13
Nepal	12
Canada	10
Pakistan	9
Australia	7
Japan	7
Finland	6
France	6
India	6
Germany	5
Italy	5
Spain	5
Thailand	5
Ghana	4
Russia	4
Denmark	3
Ireland	3
Kenya	3
Switzerland	3
Vietnam	3
Brazil	2
Ethiopia	2
Kazakhstan	2
Korea	2
Nigeria	2
Poland	2
Singapore	2
South Africa	2
Sweden	2
Argentina	1
The Bahamas	1
Belgium	1
Chile	1
Cyprus	1
Czech Republic	1
Estonia	1
Lithuania	1
Morocco	1
The Netherlands	1
Papua New Guinea	1
Qatar	1
Republic of Moldova	1
Saudi Arabia	1
Ukraine	1
United Republic of Tanzania	1

Appendix Table 2. GISAID sequences used in a study of SARS-CoV-2 BA.1/BA.2 recombinant variant in arriving travelers, Hong Kong, China, February 2022*

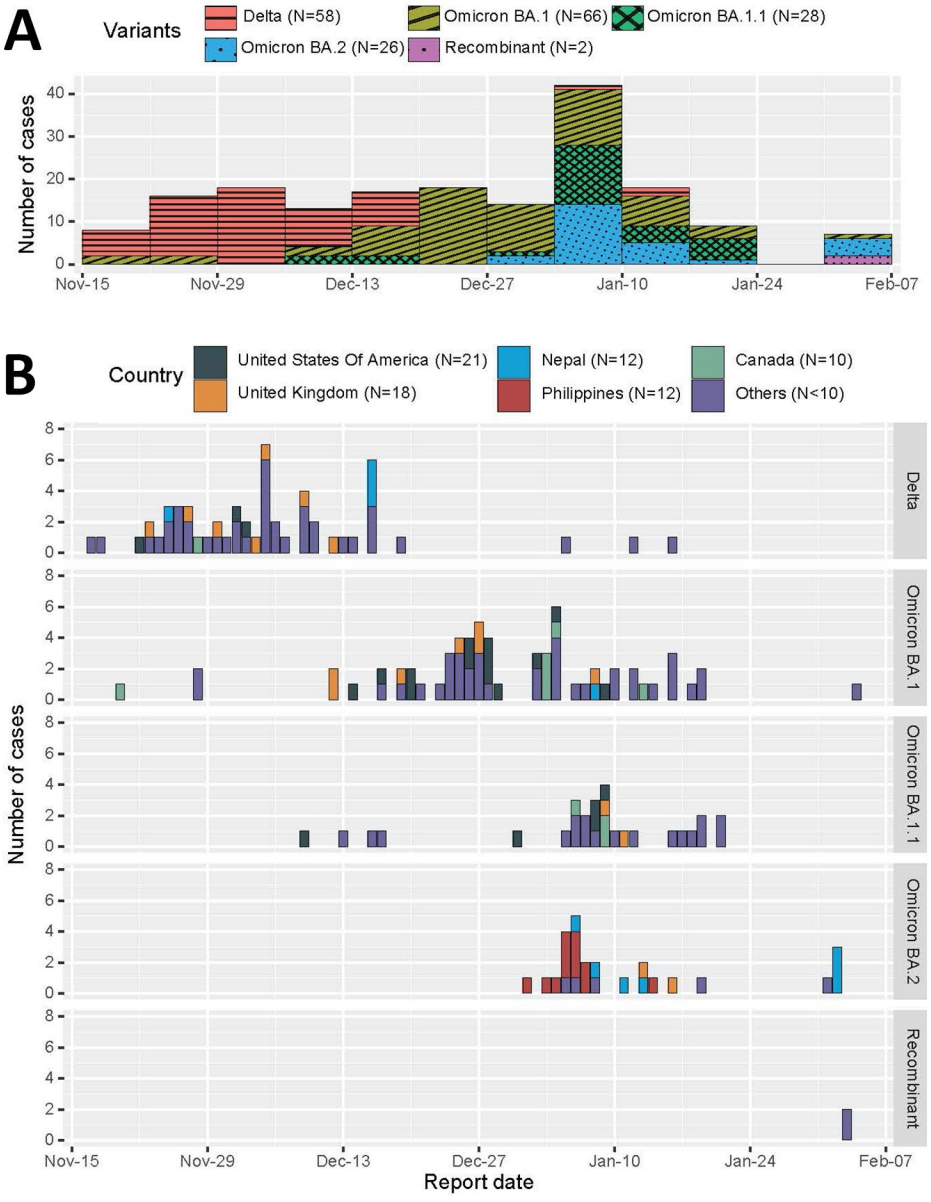
Accession no.	Originating laboratory	Submitting laboratory	Authors
EPI_ISL_1996858, EPI_ISL_2146766	Aegis Sciences Corporation	Centers for Disease Control and Prevention Division of Viral Diseases, Pathogen Discovery	Dakota Howard, Dhwani Batra, Peter W. Cook, Kara Moser, Adrian Paskey, Jason Caravas, Benjamin Rambo-Martin, Shatavia Morrison, Christopher Gulvick, Scott Sammons, Yvette Unoarumhi, Darlene Wagner, Matthew Schmerer, Cyndi Clark, Patrick Campbell, Rob Case, Vikramsinha Ghorpade, Holly Houdeshell, Ola Kvalvaag, Dillon Nall, Ethan Sanders, Alec Vest, Shaun Westlund, Matthew Hardison, Clinton R. Paden, Duncan MacCannell
EPI_ISL_3988521	Colorado Department of Public Health and Environment	Colorado Department of Public Health and Environment	Laura Bankers, Molly C. Hetherington-Rauth, Diana Ir, Alexandria Rossheim, Michael Martin, Mandy Waters, Shannon R. Matzinger, Sarah Elizabeth Totten, Emily A. Travanty
EPI_ISL_3029243	Department of Bacteria, Parasites and Fungi, Statens Serum Institut, Copenhagen, Denmark	Statens Serum Institut Bioinformatics and Microbial Genomics	Danish Covid-19 Genome Consortium
EPI_ISL_9519698	ESPACEBIO	Department of Virology, Henri Mondor University Hospital, Assistance Publique Hôpitaux de Paris, Université Paris-Est Créteil, INSERM U955	Christophe Rodriguez, Slim Fourati, Vanessa Demontant, Guillaume Gricourt, Melissa N'Debi, Alexandre Soulier, Elisabeth Trawinski, Jean-Michel Pawlotsky
EPI_ISL_1557096, EPI_ISL_3348121	Fulgent Genetics	Centers for Disease Control and Prevention Division of Viral Diseases, Pathogen Discovery	Dakota Howard, Dhwani Batra, Peter W. Cook, Kara Moser, Adrian Paskey, Jason Caravas, Benjamin Rambo-Martin, Shatavia Morrison, Christopher Gulvick, Scott Sammons, Yvette Unoarumhi, Darlene Wagner, Matthew Schmerer, Harry Gao, Mickey Li, John Gao, Joseph Fierro, Benafsh Sapra, Becky Tsai, Yan Meng, Doreen Ng, James Xie, Clinton R. Paden, Duncan MacCannell
EPI_ISL_4359169, EPI_ISL_4454918	Fulgent Genetics	Centers for Disease Control and Prevention Division of Viral Diseases, Pathogen Discovery	Dakota Howard, Dhwani Batra, Peter Cook, Jason Caravas, Benjamin Rambo-Martin, Scott Sammons, Yvette Unoarumhi, Matthew Schmerer, Kristine Lacek, Tymeckia Kendall, Victoria Caban Figueroa, Shatavia Morrison, Christopher Gulvick, Erisa Sula, Harry Gao, Mickey Li, John Gao, Joseph Fierro, Benafsh Sapra, Becky Tsai, Yan Meng, Doreen Ng, James Xie, Clinton Paden, Duncan MacCannell
EPI_ISL_4017432	Fulgent Genetics	Centers for Disease Control and Prevention Division of Viral Diseases, Pathogen Discovery	Dakota Howard, Dhwani Batra, Peter Cook, Kara Moser, Adrian Paskey, Jason Caravas, Benjamin Rambo-Martin, Shatavia Morrison, Christopher Gulvick, Scott Sammons, Yvette Unoarumhi, Darlene Wagner, Matthew Schmerer, Harry Gao, Mickey Li, John Gao, Joseph Fierro, Benafsh Sapra, Becky Tsai, Yan Meng, Doreen Ng, James Xie, Clinton Paden, Duncan MacCannell
EPI_ISL_2614026	Gravity Diagnostics, LLC	Gravity Diagnostics, LLC	Gravity Diagnostics
EPI_ISL_9879325	Hospital	National Reference Center for Viruses of Respiratory Infections, Institut Pasteur, Paris	Marion Barbet, Sylvie Behillil, Méline Bizard, Angela Brisebarre, Camille Capel, Vincent Enouf, Louise Lefrançois, Frédéric Lemoine, Christophe Malabat, Corinne Maufrais, Slim El Khiari, Julien Fumey, Etienne Simon-Lorière, Maud Vanpeene, Sylvie Van der Werf, Benedicte LUREAU
EPI_ISL_2282971	Infinity Biologix	Centers for Disease Control and Prevention Division of Viral Diseases, Pathogen Discovery	Dakota Howard, Dhwani Batra, Peter W. Cook, Kara Moser, Adrian Paskey, Jason Caravas, Benjamin Rambo-Martin, Shatavia Morrison, Christopher Gulvick, Scott Sammons, Yvette Unoarumhi, Darlene Wagner, Matthew Schmerer, Christian Bixby, Yihe Wang, Jonathan Schultz, Chirayu Goswami, Russ Hager, Robin Grimwood, Clinton R. Paden, Duncan MacCannell

Accession no.	Originating laboratory	Submitting laboratory	Authors
EPI_ISL_1682200	Laboratory Corporation of America	Centers for Disease Control and Prevention, Division of Viral Diseases, Pathogen Discovery	Dakota Howard, Dhwani Batra, Peter W. Cook, Kara Moser, Adrian Paskey, Jason Caravas, Benjamin Rambo-Martin, Shatavia Morrison, Christopher Gulvick, Scott Sammons, Yvette Unoarumhi, Darlene Wagner, Matthew Schmerer, Minoo Agarwal, Eyad Almasri, Debbie Boles, Ayla Burns, Nuthawin Charoensri, Oren Cohen, Susan Countryman, Mary Ann Cristobal, Bobbi Croy, Suzanne Dale, Hrushikesh Deshmukh, Amanda Douglas, Vincent Drouillon, Marcia Eisenberg, Howard Engler, Rama Ghatti, Prashant Gupta, Susan Hicks, Jake Humphrey, Lax Iyer, Manoj Jain, Mohan Kolli, Brian Krueger, Tim Kuphal, Stanley Letovsky, Michael Levandoski, Craig Lukasik, Jonathan Meltzer, Brian Norvell, Mindy Nye, Scott Parker, Christos Petropoulos, John Pruitt, Steven Ragan, Scott Ryan, Mike Sapeta, Jana Schroth, Suresh Babu Selvaraju, Goran Stevovic, Amanda Suchanek, Andrea Throop, Lyndon Tilson, Thomas Urban, Joe Voshell, Kimberly Wagner, Jonathan Williams, Mary Williamson, Qian Zeng, Tricia Zwiefelhofer, Clinton R. Paden, Duncan MacCannell
EPI_ISL_10273412	Laboratory Corporation of America	Centers for Disease Control and Prevention, Division of Viral Diseases, Pathogen Discovery	Dakota Howard, Dhwani Batra, Peter Cook, Jason Caravas, Benjamin Rambo-Martin, Scott Sammons, Yvette Unoarumhi, Matthew Schmerer, Kristine Lacek, Tymeckia Kendall, Victoria Caban Figueroa, Shatavia Morrison, Christopher Gulvick, Minoo Agarwal, Eyad Almasri, Debbie Boles, Ayla Burns, Nuthawin Charoensri, Oren Cohen, Susan Countryman, Mary Cristobal, Bobbi Croy, Suzanne Dale, Hrushikesh Deshmukh, Amanda Douglas, Vincent Drouillon, Marcia Eisenberg, Howard Engler, Rama Ghatti, Prashant Gupta, Susan Hicks, Jake Humphrey, Lax Iyer, Lisa Pfefferle, Manoj Jain, Matthew Robinson, Mohan Kolli, Brian Krueger, Tim Kuphal, Stanley Letovsky, Michael Levandoski, Craig Lukasik, Jonathan Meltzer, Brian Norvell, Mindy Nye, Scott Parker, Christos Petropoulos, John Pruitt, Steven Ragan, Scott Ryan, Mike Sapeta, Jana Schroth, Suresh Selvaraju, Goran Stevovic, Amanda Suchanek, Andrea Throop, Lyndon Tilson, Thomas Urban, Joe Voshell, Kimberly Wagner, Jonathan Williams, Mary Williamson, Qian Zeng, Tricia Zwiefelhofer, Clinton Paden, Duncan MacCannell
EPI_ISL_3745736, EPI_ISL_4153131	Laboratory Corporation of America	Centers for Disease Control and Prevention, Division of Viral Diseases, Pathogen Discovery	Dakota Howard, Dhwani Batra, Peter Cook, Kara Moser, Adrian Paskey, Jason Caravas, Benjamin Rambo-Martin, Shatavia Morrison, Christopher Gulvick, Scott Sammons, Yvette Unoarumhi, Darlene Wagner, Matthew Schmerer, Minoo Agarwal, Eyad Almasri, Debbie Boles, Ayla Burns, Nuthawin Charoensri, Oren Cohen, Susan Countryman, Mary Cristobal, Bobbi Croy, Suzanne Dale, Hrushikesh Deshmukh, Amanda Douglas ,Vincent Drouillon, Marcia Eisenberg, Howard Engler, Rama Ghatti, Prashant Gupta, Susan Hicks, Jake Humphrey, Lax Iyer, Lisa Pfefferle, Manoj Jain, Matthew Robinson ,Mohan Kolli, Brian Krueger, Tim Kuphal, Stanley Letovsky, Michael Levandoski, Craig Lukasik,Jonathan Meltzer, Brian Norvell, Mindy Nye, Scott Parker, Christos Petropoulos, John Pruitt, Steven Ragan, Scott Ryan, Mike Sapeta, Jana Schroth, Suresh Selvaraju, Goran Stevovic, Amanda Suchanek, Andrea Throop, Lyndon Tilson, Thomas Urban, Joe Voshell, Kimberly Wagner, Jonathan Williams, Mary Williamson, Qian Zeng, Tricia Zwiefelhofer, Clinton Paden, Duncan MacCannell
EPI_ISL_10462716, EPI_ISL_1454544, EPI_ISL_2021385	Lighthouse Lab in Alderley Park	Wellcome Sanger Institute for the COVID-19 Genomics UK (COG-UK) Consortium	Jacquelyn Wynn, Mairead Hyland, The Lighthouse Lab in Alderley Park and Alex Alderton, Roberto Amato, Jeffrey Barrett, Sonia Goncalves, Ewan Harrison, David K. Jackson, Ian Johnston, Dominic Kwiatkowski, Cordelia Langford, John Sillitoe on behalf of the Wellcome Sanger Institute COVID-19 Surveillance Team
EPI_ISL_1518044	Lighthouse Lab in Cambridge	Wellcome Sanger Institute for the COVID-	Rob Howes, The Lighthouse Lab in Cambridge and Alex Alderton, Roberto Amato, Jeffrey Barrett, Sonia Goncalves, Ewan Harrison, David K. Jackson, Ian

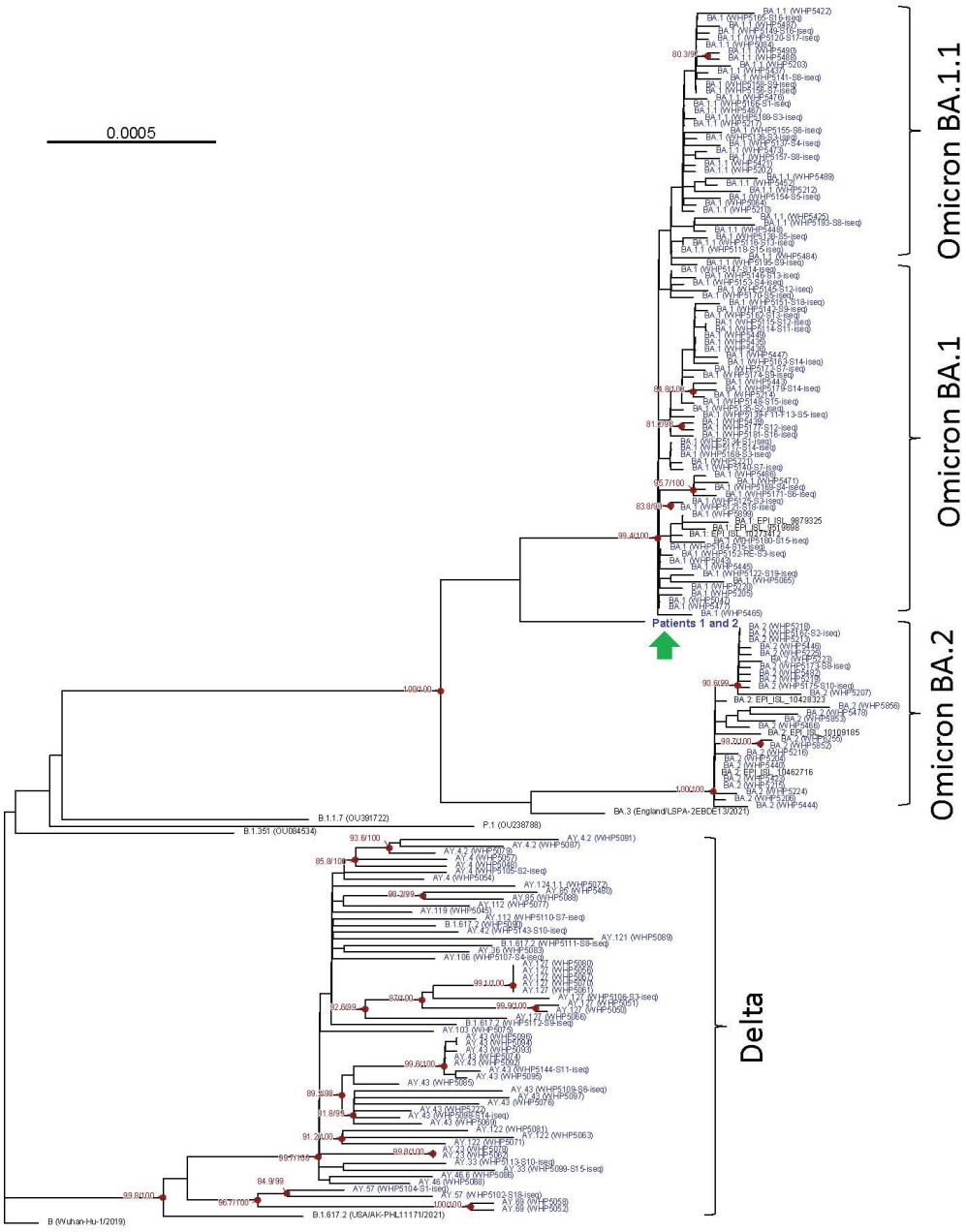
Accession no.	Originating laboratory	Submitting laboratory	Authors
		19 Genomics UK (COG-UK) Consortium	Johnston, Dominic Kwiatkowski, Cordelia Langford, John Sillitoe on behalf of the Wellcome Sanger Institute COVID-19 Surveillance Team
EPI_ISL_1115472, EPI_ISL_1256650, EPI_ISL_1410322, EPI_ISL_1453906, EPI_ISL_2608121	Lighthouse Lab in Glasgow	Wellcome Sanger Institute for the COVID-19 Genomics UK (COG-UK) Consortium	Harper VanSteenhouse, Yumi Kasai, David Gray, Carol Clugston, Anna Dominiczak and Alex Alderton, Roberto Amato, Jeffrey Barrett, Sonia Goncalves, Ewan Harrison, David K. Jackson, Ian Johnston, Dominic Kwiatkowski, Cordelia Langford, John Sillitoe on behalf of the Wellcome Sanger Institute COVID-19 Surveillance Team
EPI_ISL_10109185, EPI_ISL_10428323	Lighthouse Lab in Milton Keynes	Wellcome Sanger Institute for the COVID-19 Genomics UK (COG-UK) Consortium	The Lighthouse Lab in Milton Keynes and Alex Alderton, Roberto Amato, Jeffrey Barrett, Sonia Goncalves, Ewan Harrison, David K. Jackson, Ian Johnston, Dominic Kwiatkowski, Cordelia Langford, John Sillitoe on behalf of the Wellcome Sanger Institute COVID-19 Surveillance Team
EPI_ISL_2959430, EPI_ISL_3129581	Michigan Department of Health and Human Services, Bureau of Laboratories	Michigan Department of Health and Human Services, Bureau of Laboratories	Blankenship HM, Riner D, Soehnlen MK
EPI_ISL_1016848	North Shore Hospital	Institute of Environmental Science and Research (ESR)	Xiaoyun Ren, Matt Storey, Nikki Freed, Muhammad Faisal, Jing Wang, Hermes Perez, Anja Werno, Antje van der Linden, Arlo Upton, Chris Mansell, David Hammer, Dragana Drinkovic, Gary McAuliffe, Hana Sofia Andersson, James Ussher, Jill Sherwood, Josh Freeman, Julia Howard, Juliet Elvy, Mary DeAlmeida, Matt Blakiston, Matthew Rogers, Max Bloomfield, Michael Addidle, Michelle Balm, Sally Roberts, Sarah Jefferies, Sharmini Muttaiyah, Susan Morpeth, Susan Taylor, Timothy Blackmore, Vani Sathyendran, Veronica Playle, Virginia Hope, Erasmus Smit, Lauren Jelly, Olin Silander, Joep de Ligt
EPI_ISL_2876740	Quest Diagnostics Incorporated	Centers for Disease Control and Prevention, Division of Viral Diseases, Pathogen Discovery	Dakota Howard, Dhwani Batra, Peter W. Cook, Kara Moser, Adrian Paskey, Jason Caravas, Benjamin Rambo-Martin, Shatavia Morrison, Christopher Gulvick, Scott Sammons, Yvette Unoarumhi, Darlene Wagner, Matthew Schmerer, S. H. Rosenthal, A. Gerasimova, R. M. Kagan, B. Anderson, M. Hua, Y. Liu, L.E. Bernstein, K.E. Livingston, A. Perez, I. A. Shlyakhter, R. V. Rolando, R. Owen, P. Tanpaiboon, F. Lacbawan, Clinton R. Paden, Duncan MacCannell
EPI_ISL_2133917	Rhode Island Department of Health	Infectious Disease Program, Broad Institute of Harvard and MIT	Siddle,K.J., Azevedo,K., Miller,A., Adams,G., Pearlman,L., Gladden-Young,A., Lagerborg,K., Rudy,M., DeRuff,K., Carter,A., Normandin,E., Bauer,M., Reilly,S., Tomkins-Tinch,C., Loreth,C., Chaluvadi,S., Lemieux,J.E., Birren,B.W., Sabeti,P.C., Huard,R., King,E., Park,D.J., and MacInnis,B.L.
EPI_ISL_8880068	Temporary Specimen Collection Centre at the AsiaWorld-Expo	Hong Kong Department of Health	Alan K.L. Tsang, Peter C.W. Yip, Patricia K. L. Leung, Ken H.L. Ng, Edman T.K. Lam, Rickjason C.W. Chan
EPI_ISL_3695744	TPMG Regional Laboratory	California Department of Public Health	Emily Smith on behalf of CDPH-COVIDNet and UCI Genome Sciences Center/GHTF
EPI_ISL_3409895	UMass Memorial Medical Center	Center for Microbiome Research	Richard T. Ellison III, Karl J. Simon, Doyle V. Ward
EPI_ISL_3506433	Victoria Hospital wc VHW	NHLS/UCT	Arash Iranzadeh, Deelan Doolabh, Lynn Tyers, Bruna Galvao, Innocent Mudau, Marvin Hsiao, Gert Marais, Diana Hardie, Stephen Korsman, Rageema Joseph, Carolyn Williamson
EPI_ISL_2151915	Viollier AG	Department of Biosystems Science and Engineering, ETH Zürich	Chaoran Chen, Sarah Nadeau, Catharine Aquino, Ivan Topolsky, Philipp Jablonski, Lara Fuhrmann, David Dreifuss, Katharina Jahn, Daniel Ehrsam, Isabel Stürmer, Andreia Cabral de Gouvea, Maria Domenica Moccia, Simon Grüter, Timothy Sykes, Lennart Opitz, Griffin White, Laura Neff, Doris Popovic, Andrea Patrignani, Jay Tracy,

Accession no.	Originating laboratory	Submitting laboratory	Authors
			Ralph Schlapbach, Christiane Beckmann, Maurice Redondo, Olivier Kobel, Christoph Noppen, Sophie Seidel, Noemie Santamaria de Souza, Niko Beerenwinkel, Tanja Stadler

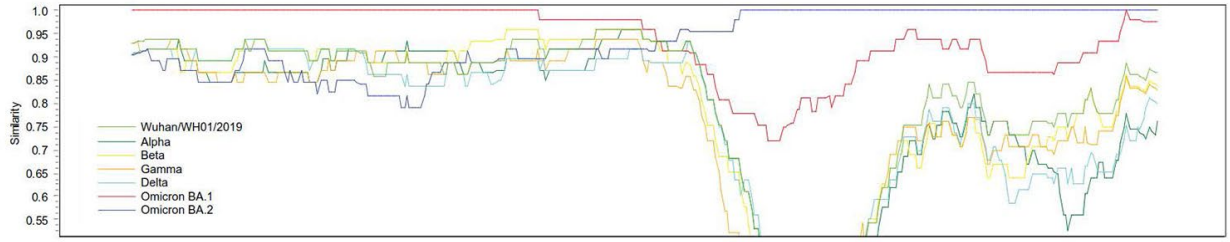
*We gratefully acknowledge the authors from the originating laboratories responsible for obtaining the specimens and the submitting laboratories where genetic sequence data were generated and shared via the GISAID Initiative, on which this research is based.



Appendix Figure 1. Importation of SARS-CoV-2 variants from incoming travelers, Hong Kong, China, November 15, 2021–February 7, 2022. A) Time series of number of patients testing positive for different SARS-CoV-2 variants by RT-PCR. B) Time series divided by SARS-CoV-2 variants, and country of origin. All infections were confirmed by whole-genome sequencing. RT-PCR, reverse transcription PCR.



Appendix Figure 2. Phylogeny of SARS-CoV-2 variants identified in incoming travelers, Hong Kong, China. The maximum-likelihood phylogenetic tree was generated by using IQ-TREE (http://www.iqtree.org) and the GTR+F+I nucleotide substitution model with Wuhan-Hu-1 (GenBank accession no. MN908947.3) as the outgroup. Blue text indicates viral genomes generated from this study and references sequences used in the analysis are shown as indicated. Arrow indicates the recombinant virus detected from patients 1 and 2. Red node points show strongly supported branches by SH-aLRT/ultrafast bootstrap. Scale bar indicates estimated nucleotide substitutions per site.



Appendix Figure 3. Simplot analysis of recombinant BA.1/BA.2 SARS-CoV-2 virus, Hong Kong, China, February 2022. Plot shows similarity between full viral genomes of different variants of concern including Alpha, Beta, Gamma, Delta, Omicron BA.1, Omicron BA.2, as well as the prototype Wuhan/WH01/2019 were used in the analysis.