MJA
The Medical Journal of Australia

Supporting Information 1

Supplementary methods and results

This appendix was part of the submitted manuscript and has been peer reviewed.
It is posted as supplied by the authors.

Appendix to: The Victorian SARS-CoV-2 Reinfection Study Group. Second SARS-CoV-2 infections twelve months after initial infections in Australia, confirmed by genomic analysis. *Med J Aust* 2022; doi: 10.5694/mja2.51352.

Methods

Genomic sequencing and bioinformatic analysis

As previously described,[1,2] RNA was was extracted from samples testing positive for SARS-CoV-2 on the QIAsymphony using the DSP Virus/Pathogen Mini Kit (Qiagen) or using the QIAamp 96 Virus QIAcube HT Kit (Qiagen). Tiled amplicons were prepared from RNA extracts using either ARTIC version 1, 3 or 4 primers,[3] using published protocols.[4] Sequencing libraries were prepared from amplicons using NexteraXT and sequenced on either the NextSeq500/550 or iSeq100 (Illumina) using 150bp paired-end reads as described by the manufacturer. Reads were aligned to the reference genome (Wuhan Hu-1; GenBank MN908947.3) and consensus sequences generated.

Data sharing

Sequence data have been uploaded to the Global Initiative on Sharing All Influenza Data (GISAID). Sequences from patients with second severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) infections are listed in Table 1, and the metadata for all sequences included in analysis are provided in the Supporting Information, file 2.

Table 1. Sequence availability and accession numbers for sequences from patients with identified SARS-CoV-2 reinfection, Victoria

Patient number	Sequence ID	Collection date	GISAID name	GISAID accession number
1	VIC6800	21/07/2020	hCoV-19/Australia/VIC6800/2020	EPI_ISL_564065
	VIC19034	19/07/2021	hCoV-19/Australia/VIC19034/2021	EPI_ISL_5200604
2	VIC6807	21/07/2020	hCoV-19/Australia/VIC6807/2020	EPI_ISL_564069
	VIC19084	22/07/2021	hCoV-19/Australia/VIC19084/2021	EPI_ISL_4761399
3	VIC19042	20/07/2021	hCoV-19/Australia/VIC19042/2021	EPI_ISL_5200602

Table 2. Demographic, clinical and microbiological details of second SARS-CoV-2 infections in three patients, Victoria, 2021

Patient			Clinical details		Microbiological analysis				Serological analysis	
Nr	Sex	Age group (years)*	Onset date	Reported symptoms	Collection date	CT	Sequence ID	Pangolin lineage	Collection date	Result (titre)†
1	F	20-29	13/07/2020	Cough	21/07/2020	19.67	VIC6800	D.2	NA	
			16/07/2021	Cough, runny nose, sore throat, headache	19/07/2021	27.87	VIC19034	B.1.617.2 (Delta)	30/07/2021	Reactive (31.3)
2	M	20-29	-	Asymptomatic	21/07/2020	24.00	VIC6807	D.2	NA	
			20/07/2021	Cough, runny nose, fatigue	22/07/2021	18.24	VIC19084	B.1.617.2 (Delta)	NA	
3	M	20-29	18/07/2020	Runny nose	Sequence NA				NA	
			16/07/2021	Cough, runny nose, sort throat, headache	20/07/2021	29.71	VIC19042	B.1.617.2 (Delta)	30/07/2021	Reactive (22.5)

CT = polymerase chain reaction cycle threshold (E-gene target, in-house assay); NA = not available.

* At initial infection. † Total antibody titre, Wantai SARS-CoV-2 antibody enzyme-linked immunosorbent assay (ELISA).

Table 3. Summary of genetic distances between 2020 and 2021 SARS-CoV-2 infections among patients with second SARS-CoV-2 reinfects, Victoria, 2021, and all Pangolin lineages D.2 and B.1.617.2 included in analysis

The genetic distances below are a numerical representation of those displayed on the phylogenetic tree in the Box in the main text.

The median genetic distance between sequences from the 2020 and 2021 infections of patients 1, 2 and 3, is similar to the median genetic distance between any two samples of these Pangolin lineages, and is substantially higher than the median genetic distance between any two given samples included in analysis. This supports the finding that the 2020 and 2021 infections of these patients were new infections, rather than prolonged viral shedding and within-host evolution.

Compared sequences Pangolin lineage	**Median pairwise genetic distance (IQR), x10^{-4}**	
	Intra-lineage	Inter-lineage
Patients 1, 2, 3		
D.2 (2020 infections)	0.00	15.5 (15.5–15.5)
B.1.617.2* (2021 infections)	0.00 (NA)	
All sequences		
D.2	1.48 (0.74–2.22)	15.2 (14.8–15.9)
B.1.617.2*	1.11 (0.74–3.02)	
All Pangolin lineages	1.48 (0.74–2.22)	10.9 (6.29–15.0)
All possible comparisons	6.29 (2.21, 13.9)	

IQR = interquartile range; NA = not applicable.

* B.1.617.2 includes all AY sub-lineages.

References

1. Lane CR, Sherry NL, Porter AF, et al. Genomics-informed responses in the elimination of COVID-19 in Victoria, Australia: an observational, genomic epidemiological study. *Lancet Public Health* 2021; 6: e547-e556.
2. Seemann T, Lane CR, Sherry NL, et al. Tracking the COVID-19 pandemic in Australia using genomics. *Nature Communications* 2020;11:1-9.
3. ARTIC-nCoV2019 primer schemes (Github). 2020. https://github.com/artic-network/artic-ncov2019/tree/master/primer_schemes/nCoV-2019/V3 (viewed Nov 2021).
4. nCoV-2019 sequencing protocol. 2020. https://dx.doi.org/10.17504/protocols.io.bbmuik6w (viewed Nov 2021).