Emergence of Divergent Spike Haplotypes in Individuals With Persistent SARS-CoV-2 Infections

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BACKGROUND

- Persistent infection with SARS-CoV-2 is hypothesized to give rise to new variants of concern (VoC).
- However, sequencing methods to investigate intrahost variation are limited: short-read technology precludes haplotyping, and long-read technology has high error rates.
- We describe a new method for investigating SARS-CoV-2 quasispecies and apply it to identify divergent spike haplotypes arising during persistent infection.

METHODS

- A long-read nanopore sequencing workflow was developed to generate high-quality reads (Q30+, 99.9% accuracy) covering the entire spike gene sequence.
- A bioinformatic tool (HaploVar) to identify and quantify unique spike haplotypes at ≥1% frequency in samples.
- Respiratory samples were processed from patients with acute infection and 23 cases of persistent infection lasting longer than 4 weeks.
- Haplotypes were screened for recombination using GARD v0.2.

RESULTS

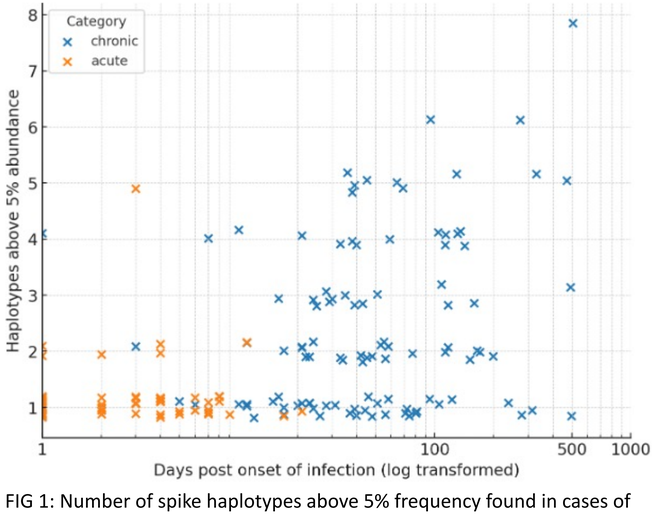
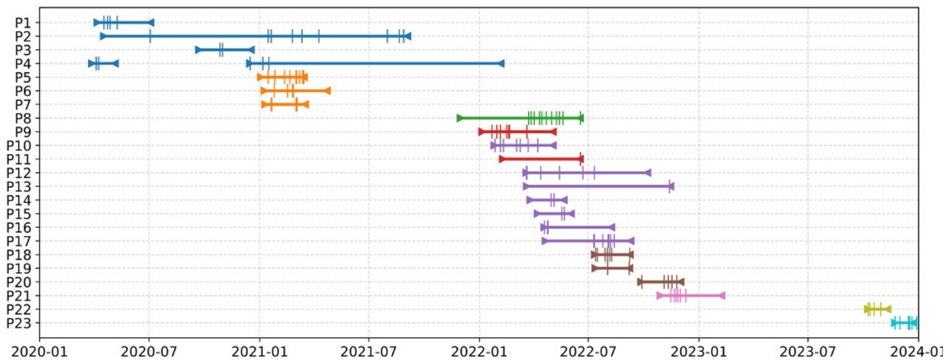


FIG 1: Number of spike haplotypes above 5% frequency found in cases of acute and persistent SARS-CoV-2 infection by days post onset of infection

Key message:

Distinct SARS-CoV-2 spike haplotypes emerge during persistent infection and these share similar mutations to those seen in Variants of Concern (VOC) supporting the hypothesis that VOC emerge during persistent infection.



ID	Age	Sex	Immunocompromise	Vaccination	Days	Samples	Lineage	Severity	Outcome
P1	71	M	Kidney transplant	0	89	4	B.1.2	Critical	Clear
P2	23	M	Inherited immunodeficiency	0	506	9	B.1	Critical	Died
P3	29	M	Primary hypogammaglobulinaemia	0	87	2	B.1.258	Severe	Clear
P4	57	M	Renal transplant, DLBCL	0	39;	2;	B.1.2;	Moderate;	Clear;
					417	3	B.1.177	Moderate	Clear
P5	61	M	Advanced HIV	0	72	8	B.1.1.7	Critical	Died
P6	35	M	Advanced HIV	0	104	4	B.1.1.7	Mild	Clear
P7	79	F	Rheumatoid arthritis,	0	67	3	B.1.1.7	Critical	Died
P8	47	F	Advanced HIV	0	199	11	AY.4	Mild	Clear
P9	37	M	Hodgkin's	3	118	7	BA.1.1	Mild	Clear
P10	67	F	Diffuse large B-cell lymphoma	3	98	7	BA.2	Severe	Clear
P11	52	F	Thymoma	2	129	1	BA.1.1	Moderate	Clear
P12	48	M	Non-Hodgkin's lymphoma, CAR-T	2	202	8	BA.2	Mild	Clear
P13	52	M	Chronic lymphoid leukaemia	4	239	1	BA.2	Moderate	Clear
P14	27	F	Lupus	3	56	2	BA.2	Severe	Clear
P15	36	M	Burkitt's lymphoma	3	56	2	BA.2	Mild	Clear
P16	64	M	DLBCL	3	111	3	BA.2	Mild	Died
P17	62	M	DLBCL	4	142	7	BA.2	Critical	Clear
P18	62	F	Cutaneous T-cell lymphoma	2	59	7	BA.5.2	Mild	Died
P20	51	M	Renal transplant	0	66	5	BA.5.2	Mild	Unknown
P21	77	F	MALT lymphoma	4	102	6	BQ.1.1	Severe	Clear
P22	64	M	DLBCL	3	41	4	GE.1	Moderate	Clear
P23	70	M	Myeloma	8	51	7	JN.1	Critical	Died

FIG 2 & TABLE 1: Timelines for cases of persistent infection displayed with the start of symptoms (>) and last positive sample (<). Successfully sequenced and haplotyped samples are marked (|). Demographic and case details are shown including co-morbidities, length of infection (days), CD20 depletion, severity of infection and outcome.

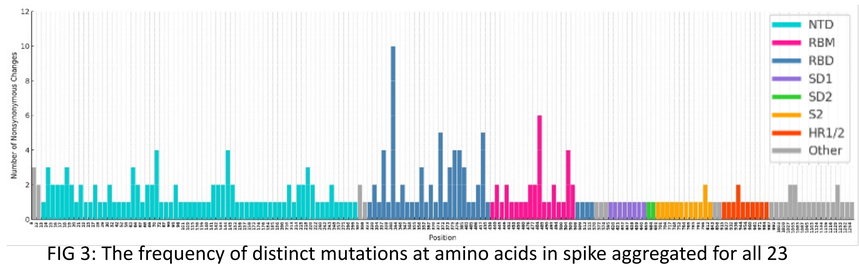


FIG 3: The frequency of distinct mutations at amino acids in spike aggregated for all 23 patients with persistent infection.

ID	Haplos	VOC-like mutations	VOC mutations
P2	32	L8H, T19S, T95S, V213A, D215H, R346I, E484R/T, F490L, Q493E, Q954K	Q52R, 69/70-, 143/144-, 242/243-, L452R, H655Y, T1027I
P3	8	A243V	69/70-, T1027I
P4	9		E484K
P5	19	Y144V, W152L	
P6	8		A67V, T95I, D215G, E484K
P7	5		S371F, N440K, E484Q, A701V
P8	30	L8K, S477I, D796H	K417T, N440K, S477N, Q493E, P621S
P9	18	S371I, N764E	S371L, S365F, K417N, K417T, N460K
P10	10	K417I	K356T
P11	5		S371L, S365F, K417N
P12	12	K356R	
P14	3	D796H	
P15	2	Y248H	
P17	20	R346I, K356R, E484V	
P19	2	A570T	144-
P20	19	E156K, S371-, S373-, S375A, S375S, T376P/T	144-
P21	11		69/70-
			[Legend: Alpha Beta Gamma Delta Eta Mu Omicron KP.3 JN.1]

TABLE 2: The number of unique spike haplotypes identified in each case, and the emergence of VOC-like and VOC mutations in haplotypes identified from cases of persistent infection.

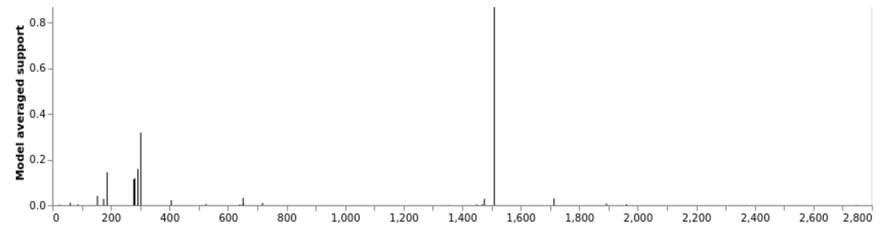


FIG 4: GARD calls recombination between haplotypes identified in one sample from persistent infection with high confidence.

CONCLUSIONS

- This novel workflow identifies intrahost spike haplotypes.
- Provides evidence for the emergence of variants of concern from persistent infection.
- Early evidence demonstrating intrahost recombination occurs within a single individual, however confirmation is needed by other methods.