DOI: 10.1002/jmv.27667

LETTER TO THE EDITOR

JOURNAL OF MEDICAL VIROLOGY WILEY

Omicron variant losing its critical mutations in the receptor-binding domain

Dear Editor,

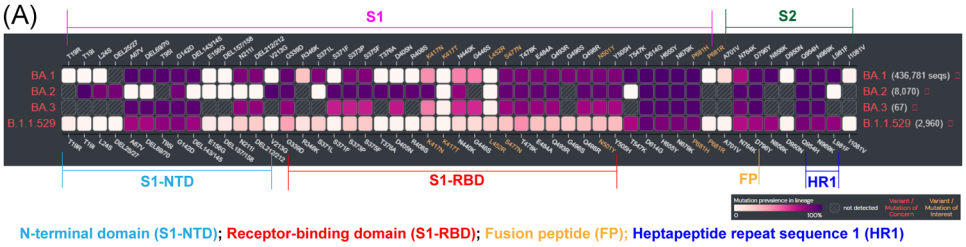
Recently the Omicron variant has been divided into four lineages: B.1.1.529, BA.1, BA.2, and BA.3 lineages.[1] However, it was noted that the Omicron variant was referred to as the B.1.1.529 lineage during the period when the Omicron variant was first discovered.[2,3] Here, we observed that the B.1.1.529 lineage, which is such a concerning lineage, is not widely dominating, but BA.1 is widely dominating and spread throughout the world that if it were an Omicron variant, it would mainly represent the BA.1 lineage. It can be speculated that this B.1.1.529 lineage has lost its dominance as the lineage continues to evolve and replace mutations in the unique spike protein for the Omicron variant.

As of January 22, 2022, B.1.1.529 is 0.63% (3,268/520,021), BA.1 is 97.3% (505,978/520,021), BA.2 is 2.05% (10,703/520,021), and BA.3 is 0.014% (72/520,021) in the sequences submitted to GISAID. Our previous study reported the difference in mutations in spike protein between the BA.1, BA.2, and BA.3 lineages.[4] Similarly, we examined whether there is any difference between the BA.1 and B.1.1.529 lineages of mutations in spike protein. However, we did not find any significant difference in mutations in spike protein between the BA.1 and B.1.1.529 lineages (Figure 1A,B). Following this, we examined whether there were any differences between the BA.1 and B.1.1.529 lineages in mutations in other proteins. SARS-CoV-2 is a virus that contains 12 proteins such as ORF1a, ORF1b, spike, ORF3a, Envelope (E), Membrane (M), ORF6, ORF7a, ORF7b, ORF8, Nucleocapsid (N), and ORF10. Of these, proteins such as ORF6, ORF7a, and ORF10 have no mutations in the BA.1 and B.1.1.529 lineages. However, we did not find any significant difference between the BA.1 and B.1.1.529 lineages in mutations in the ORF1a, ORF1b, ORF3a, Envelope (E), Membrane (M), ORF7b, ORF8, and Nucleocapsid (N) proteins (Figure S1A–H). Although no significant difference was found in all protein mutations between the BA.1 and B.1.1.529 lineages, we observed that there were significant differences in the percentage of specific mutations in the total GISAID sequences of a particular lineage (Figures 1A,B, S1A–H). The importance of mutations in the Omicron variant, immune escape from neutralizing antibodies, evasion of the protection provided by mAbs, antiviral drugs, the effectiveness of existing vaccines, booster vaccine, prevention/control strategies, and policy formulation has been intensively reviewed in previous studies.[5–10]

In the B.1.1.529 lineage, the percentage of mutations in a particular amino acid position, especially in the receptor-binding domain, decreases significantly (Figure 1A,B). It should be noted that the G339D, S371L, S373P, S375F, K417N, N440K, G446S, S477N, T478K, E484A, Q493R, G496S, Q498R, N501Y, and Y505H mutations in the receptor-binding domain corresponding to the B.1.1.529 lineage of the Omicron variant[2] are present in only 35.2%, 20.2%, 22.5%, 21.8%, 14.9%, 15.6%, 14.6%, 24.8%, 41.5%, 25.5%, 26.8%, 22.9%, 26.5%, 27.3%, and 27.6% of the sequences, as of January 22, 2022, respectively (Figure 1A,B). All of this suggests that the B.1.1.529 lineage has largely lost mutations within its receptor-binding domain. In other words, the B.1.1.529 lineage continues to evolve by adding or replacing new mutations in its receptor-binding domain. Thus almost every mutation in the receptor-binding domain of the B.1.1.529 lineage is modified by approximately 60%–85% (Figure 1A,B), and it is not known whether these mutations increase or decrease the binding with ACE2. The great beauty of this is that it retains 87.4%–88.9% of the H655Y, N679K, and P681H mutations (Figure 1A,B) in or around the furin cleavage site. It should be noted that, like the B.1.1.529 lineage, each mutation in the BA.1lineage receptor-binding domain is approximately 7%–85% altered/changed/replaced (Figure 1A,B). However, it should be noted that BA.1 lineage retains 99% of the H655Y, N679K, and P681H mutations at or near the furin cleavage site (Figure 1A,B). In particular, K417N mutation is present only 14.9% of the sequences in BA.1lineage (Figure 1B), with studies suggesting that K417N mutation alone may reduce binding to ACE2 while increasing binding with ACE2 when combined with E484K and N501Y mutations.[11,12] In particular, as of January 22, 2022, the mutation R346K has newly appeared in 25.2% (110 115 sequences) of the BA.1 lineage sequences (Figure 1B). Studies have shown that this mutation on the yeast-display platform mildly increases the surface expression of RBD[13] and is resistant to neutralizing antibodies.[14] Furthermore, the N440K and G446S mutations contain only BA.1lineage in the 44.4% and 45.4% sequences, respectively (Figure 1B); in other words, altered/changed/replaced approximately 55% of the sequences. Studies show that these two mutations are resistant to convalescent sera or neutralizing antibodies.[15–17]

In particular, other mutations in the receptor-binding domain are modified in approximately 12% of the sequences in BA.1 lineage (Figure 1B). As of January 22, 2022, 12% of the total 436 781 BA.1 lineage sequences indicate that these mutations have changed over approximately 50 000 sequences (Figure 1A,B). Similarly, on the S1/S2 cleavage site, BA.1lineage contains the rest in around 1% of the sequences, and this 1% represents approximately 4400 sequences (Figure 1B). When mutations in the Omicron variant RBD are

(A)



(B)

	Mutation	BA.2 8070 seqs No. Seqs	%	BA.3 67 seqs No. Seqs	%	BA.1 436,781 seqs No. Seqs	%	B.1.1.529 2,960 seqs No. Seqs	%
N-terminal domain (S1-NTD)	T19I	7958	98.6						
	L24S	7175	88.9						
	DEL25/27	7175	88.9						
	A67V			64	95.5	429,175	98.3	2,292	77.4
	Del69/70			64	95.5	423,867	97	2,272	76.8
	T95I			64	95.5	425,404	97.4	2,451	82.8
	G142D	7190	89.1	64	95.5	416,466	95.3	2,527	85.4
	DEL143/145			64	95.5	416,227	95.3	2,341	79.1
	E156G							248	8.4
	DEL157/158							247	8.3
	N211I			55	85.1	379,321	86.8	615	20.8
	DELL212			55	85.1	381,371	87.3	616	20.8
	V213G	7883	97.7						
Receptor-binding domain (S1-RBD)	G339D	7739	95.9	55	85.1	406,251	93	1042	35.2
	R346K					110,115	25.2	325	11
	S371L					374,728	85.8	597	20.2
	S371F	7732	95.8	46	68.7				
	S373P	7747	96	46	68.7	376,354	86.2	666	22.5
	S375F	7742	95.9	46	68.7	376,581	86.2	646	21.8
	T376A	7730	95.8						
	D405N	7727	95.7	47	70.1				
	R408S	7647	94.8						
	K417N	7579	93.9	31	46.3	179,081	14.9	442	14.9
	N440K	6515	80.7	44	65.7	193,943	44.4	463	15.6
	G446S			44	65.7	198,392	45.4	432	14.6
	L452R							609	20.6
	S477N	7474	92.6	53	79.1	384,258	88	734	24.8
	T478K	7501	92.9	57	85.1	385,362	88.2	1227	41.5
	E484A	7514	93.1	54	80.6	383,477	87.8	755	25.5
	Q493R	7569	93.8	54	80.6	387,417	88.7	793	26.8
	G496S					384,337	88	679	22.9
	Q498R	7577	93.9	52	77.6	383,705	88.7	785	26.5
	N501Y	7560	93.7	51	76.1	385,138	88.2	808	27.3
	Y505H	7550	93.6	51	76.1	383,041	87.7	817	27.6
In and around cleavage site	T547K					432,855	99.1	2252	76.1
	D614G	8025	99.4	66	98.5	433,742	99.3	2781	94
	H655Y	8019	99.4	66	98.5	433,302	99.2	2588	87.4
	N679K	8020	99.4	66	98.5	433,157	99.2	2616	88.4
	P681H	8019	99.4	66	98.5	432,976	99.1	2631	88.9
S2	A701V					61,843	14.2	69	2.3
	N764K	7818	96.9	60	89.6	328,405	75.2	2169	73.3
	D796Y	8004	99.2	66	98.5	422,821	96.8	2304	77.8
	N856K					427,339	97.8	2361	79.8
	Q954H	8021	99.4	66	98.5	426,560	97.7	2607	88.1
	N969K	8023	99.4	65	97	428,197	98	2522	85.2
	L981F					427,899	98	2417	81.7

S1

S2

FIGURE 1 Comparison of mutations in spike protein of the four lineages B.1.1.529, BA.1, BA.2, and BA.3 lineages in the Omicron variant. (A) Depicting the percentage of specific mutations in a specific amino acid position in the total number of particular lineage sequences submitted to GISAID, as of January 21, 2022 (https://outbreak.info/compare-lineages). The color assigned to each amino acid position indicates the percentage of specific mutations in the total sequences of each lineage submitted to GISAID. The scale bar depicts the percentage that this color represents. (B) The table represents the total number of sequences available for different lineages in the GISAID as of January 21, 2022, and particular mutations present in the number of sequences and their percentage (https://outbreak.info/compare-lineages).

individually assayed using a high-throughput assay in yeast-display platform, S371L, S373P, S375F, K417N, G446S, E484A, Q493R, G496S, Q498R, and Y505H mutations have been found to reduce binding to ACE2. While S477N, T478K, N501Y mutations have been found to enhance bonding with ACE2.[14] Similarly, combinations of mutations such as S477N, Q498R, and N501Y have been found to stabilize the RBD-ACE2 complex.[12] When the RBD-ACE2 binding affinity was explored using the noncompetitive ELISA approach, the Delta variant binding affinity was higher than the wild type and Omicron. It has also been found that the binding affinity of Omicron

is less than that of the wild type but has no statistically significant difference.[18] Furthermore, it is noteworthy that the Delta and Omicron variants were determined to have similar binding affinity when the RBD-ACE2 binding affinity was explored using the surface plasmon resonance approach.[19] The mutations Q493R, G496S, and Q498R in the Omicron variant RBD cause increased binding affinity by forming salt bridges and hydrogen bonds with human ACE2.[19] Similarly, when the RBD-ACE2 binding affinity was explored using the bio-layer interferometry approach, it was determined that the Omicron variant had a ~3-fold enhanced binding affinity with ACE2 over the Wuhan-Hu-1 and Delta RBDs.[20] It has been found that in cells without TMPRSS2, the Omicron variant enters the cells through the endosomal route, and in cells with TMPRSS2 through fusion, the S1/S2 cleavage mutations cause fusion and syncytium formation less than the Delta variant.[20,21] It is noteworthy that TMPRSS2 is more expressive in alveolar AT1 and AT2 pneumocytes and less expressive in the upper airway (trachea).[20] However, when mutations such as D614G, P681H, and H655Y at the S1/S2 cleavage site were examined individually, it was determined that they increased cleavage, fusion, and syncytium formation, thereby contributing to virus transmission.[22–26] Mutations in the RBD and S1/S2 cleavage site have been shown to exhibit one function as individuals but another as a combination of mutations.[19] This study reports that mutations in the RBD and S1/S2 cleavage sites are significantly altered. These mutation frequencies represent single mutations. Furthermore, these mutations can also be with combinations in some sequences that this analysis cannot determine. It needs to examine each sequence individually and examine their significance to see if these mutations are transformed into individuals or groups in each BA.1 sequence.

If any of these altered/changed/replaced amino acids become more likely to increases/decrease the binding capacity to ACE2 or S1/S2 cleavage site mutation increases the infection in TMPRSS2 expressing cells, it can be expected that this lineage is more likely to become a virus that causes severe infections. If BA.1 lineage spreads rapidly and becomes a virus that causes severe infections, it could shake the world's health care system. Therefore, sequences that show altered mutations within the receptor-binding domain and or S1/S2 cleavage site of the B.1.1.529 or BA.1 lineages may be used to diagnose or thoroughly examine the health status (mild, severe, hospitalized, etc), source of sample (nasal swab, throat, lung, etc), recovery, and vaccination status to predict the effect of the mutation in the disease outcome. Furthermore, viruses with these altered mutations should be examined experimentally to confirm the severity of the infection. It is hoped that this will help in controlling the spread of the disease at an early stage and preparing for vaccines, antiviral, and antibody treatment.

ACKNOWLEDGMENTS

Perumal A. Desingu is a DST-INSPIRE faculty is supported by research funding from the Department of Science and Technology (DST/INSPIRE/04/2016/001067), Government of India, and Core grant from the Science and Engineering Research Board (SERB) (CRG/2018/002192), Department of Science and Technology (DST), Government of India.

CONFLICTS OF INTEREST

The authors declare no conflicts of interest.

AUTHOR CONTRIBUTIONS

All the authors contributed significantly to this manuscript. Perumal A. Desingu analyzed and wrote the first draft, K. Nagarajan reviewed the manuscript. All the authors reviewed and approved the final submission.

DATA AVAILABILITY STATEMENT

Data sharing is not applicable to this article as no datasets were generated or analysed during the current study.

Perumal A. Desingu[1]
K. Nagarajan[2]

[1]*Department of Microbiology and Cell Biology, Indian Institute of Science, Bengaluru, India*
[2]*Department of Veterinary Pathology, Madras Veterinary College, Vepery, Tamil Nadu Veterinary and Animal Sciences University (TANUVAS), Chennai, India*

Correspondence
Perumal A. Desingu, Department of Microbiology and Cell Biology, Division of Biological Sciences, Indian Institute of Science, Bangalore 560012, India.
Email: perumald@iisc.ac.in, padesingu@gmail.com

ORCID

Perumal A. Desingu https://orcid.org/0000-0003-4395-4472
K. Nagarajan https://orcid.org/0000-0001-6420-0309

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