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Mutational cascade of SARS-CoV-2 leading to evolution and emergence of omicron variant

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VOI, variant of interest
VUM, variant under monitoring
NSP, non-structural protein
UTR, untranslated region
rdrp, RNA dependent RNA polymerase

ABSTRACT

Background: Emergence of new variant of SARS-CoV-2, namely omicron, has posed a global concern because of its high rate of transmissibility and mutations in its genome. Researchers worldwide are trying to understand the evolution and emergence of such variants to understand the mutational cascade events.

Methods: We have considered all omicron genomes (n = 302 genomes) available till 2nd December 2021 in the public repository of GISAID along with representatives of variants of concern (VOC), i.e., alpha, beta, gamma, delta, and omicron; variant of interest (VOI) mu and lambda; and variant under monitoring (VUM). Whole genome-based phylogeny and mutational analysis were performed to understand the evolution of SARS CoV-2 leading to emergence of omicron variant.

Results: Whole genome-based phylogeny depicted two phylogroups (PG-I and PG-II) forming variant specific clades except for gamma and VUM GH. Mutational analysis detected 18,261 mutations in the omicron variant, majority of which were non-synonymous mutations in spike (A67, T547K, D614G, H655Y, N679K, P681H, D796Y, N856K, Q954H), followed by RNA dependent RNA polymerase (rdrp) (A1892T, I189V, P314L, K38R, T492I, V57V), ORF6 (M19M) and nucleocapsid protein (RG203KR).

Conclusion: Delta and omicron have evolutionary diverged into distinct phylogroups and do not share a common ancestry. While, omicron shares common ancestry with VOI lambda and its evolution is mainly derived by the non-synonymous mutations.

1. Introduction

Throughout the globe resurgence of COVID-19 cases has been linked to the emergence of new variants of concern (<https://www.hopkinsmedicine.org/health/conditions-and-diseases/coronavirus/first-and-second-waves-of-coronavirus>) (Thakur et al., 2021). Currently, the world is witnessing a new variant namely, omicron which was first reported in South Africa on 24th November 2021 from the specimen collected on 9th November 2021 ([https://www.who.int/publications/m/item/enhancing-readiness-for-omicron-\(b.1.1.529\)-technical-brief-and-priority-actions-for-member-states](https://www.who.int/publications/m/item/enhancing-readiness-for-omicron-(b.1.1.529)-technical-brief-and-priority-actions-for-member-states)). On 26th November 2021, World Health Organisation (WHO) assigned omicron to the 'variant of concern' (VOC) category due to its ability to poses a higher risk of reinfection as compared to previously reported variants (<https://www.who.int/news/i>

tem/26-11-2021-classification-of-omicron-(b.1.1.529)-sars-cov-2-variant-of-concern; <https://www.who.int/news/item/28-11-2021-update-on-omicron>). According to the 1st December 2021 update, omicron is reported in at least 23 countries from five out of six WHO regions, with most cases in Africa and Europe (<https://www.cnb.com/2021/12/01/-who-says-omicron-has-been-found-in-23-countries-across-the-world.html>).

There is a lot of uncertainty surrounding the omicron variant. For its risk assessment, scientists and researchers are investigating the intensity of its spread, extent of its infection, effectiveness of detection methods, therapeutics, and vaccine efficacy (Knoll & Wonodi, 2021; Lipsitch & Dean, 2020; Pegu et al., 2021). The onset of omicron is reported with mild diseases suggests its low or mild severity than its previous counterparts like delta (Ewen Callaway, 2021; E. Callaway & Ledford, 2021).

Abbreviations: VOC, Variant of concern; VOI, Variant of interest; VUM, Variant under monitoring; NSP, Non-structural protein; UTR, Untranslated region; rdrp, RNA dependent RNA polymerase.

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It is known to have a very high mutation rate with more than 30 mutational changes in its spike protein (Ewen Callaway, 2021) ([https://www.who.int/publications/m/item/enhancing-readiness-for-omicron-\(b.1.1.529\)-technical-brief-and-priority-actions-for-member-states](https://www.who.int/publications/m/item/enhancing-readiness-for-omicron-(b.1.1.529)-technical-brief-and-priority-actions-for-member-states))

Globally, high risk of reinfection with omicron variant and its ability to evade vaccine-induced immunity resulting in the emergence of new variants of SARS-CoV-2 (Pulliam et al., 2021). Since COVID-19 inception, researchers have been trying to investigate its origin and evolution (Bansal, Kumar, & Patil, 2021; Singh & Soojin, 2021; Tang et al., 2020). We are currently witnessing a global molecular arms race between SARS-CoV-2 and its preventive therapeutics based on diverse regimes such as DNA, RNA, protein or inactivated whole-virion, etc. (Andradakis et al., 2020; Corey, Mascola, Fauci, & Collins, 2020; Sharma, Sultan, Ding, & Triggler, 2020). This global crisis can be addressed by a

very rapid immunization program worldwide. Moreover, the real-time monitoring of evolutionary cascade of SARS-CoV-2 leading to novel variants is utmost. Earlier investigation of several VOC and VOI suggests some of the crucial mutations for viral survival and high infectivity in humans (Boehm et al., 2021; Kumar & Bansal, 2021; Schmidt et al., 2021). However, mutations giving rise to omicron and intra-omicron genomic diversity are not yet analyzed at a population level.

In the present study, we aim to look for the mutational profile of under-monitoring variants reported till now to understand the emergence of a heavily mutated variant named omicron. Interestingly, whole genome-based phylogeny suggests two major phylogroups PG-I and PG-II. Further, mutational analysis depicted the key role of non-synonymous mutations in the evolution of novel variant. Such genome-wide mutational landscape is required for surveillance and vaccine development.

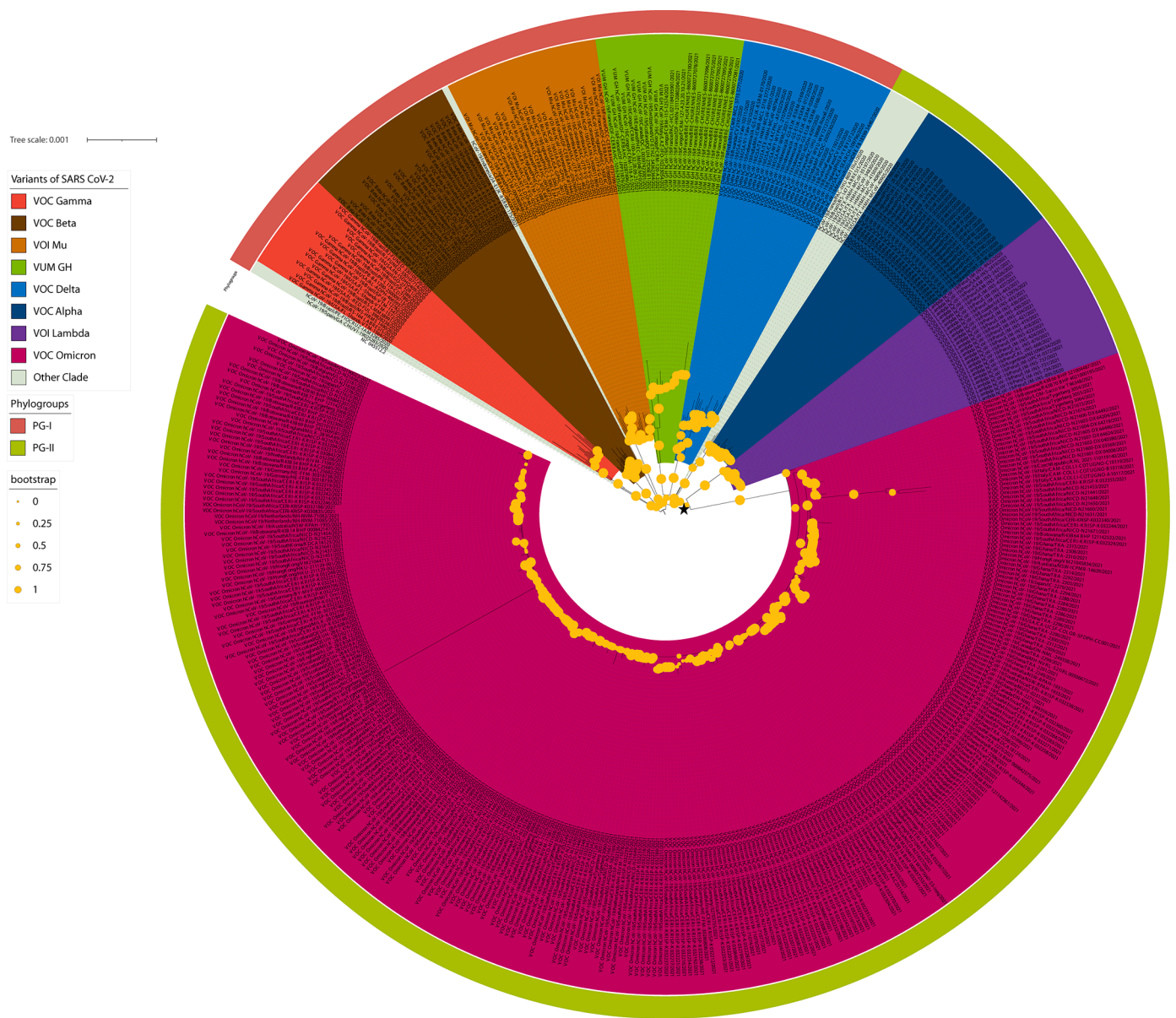


Fig. 1. Maximum likelihood whole genome-based phylogeny of SARS-CoV-2 VOCs, VOIs and VUMs. Here, phylogroups (PG-I and PG-II) and clades (alpha, beta, gamma, delta, omicron, mu etc.) are marked with respective colors as indicated. Bootstrap values are represented by the radius of circle at the nodes. Common ancestry of omicron and lambda is marked by black star.

Table 1
Metadata of the VOCs, VOIs and VUMs strains used in the present study.

Table with columns: Variant name, strain_analysis, vfaa, gbaat, gpcr, date, region, country, division, location, region, country, division, segment, length, host, age, sex, pangloss_ID, GISAID_ID, origination, submitting, author, url, date_uploaded. The table lists various SARS-CoV-2 variants such as Omicron, Delta, and Gamma, along with their specific strain identifiers and associated metadata.

(continued on next page)

Table 1 (continued)

Table with 10 columns: Accession, Country, Region, City, Date, Genotype, and various identifiers. The table lists numerous SARS-CoV-2 sequences from various countries including the USA, India, Mexico, and others, with associated dates and identifiers.

2. Results

2.1. Phylogenomics suggests common ancestry of omicron and lambda variants

Whole genome-based phylogeny (n = 478 genomes) representing VOC (alpha, beta, gamma, delta, and omicron), VOI (mu and lambda) and VUM depicts two major phylogroups PG-I and PG-II (Fig. 1 and Table 1). Here, the reference strain of SARS-CoV-2 (Wuhan-Hu-1, NC_045512.2) is taken as an outgroup. PG-I has VOC: gamma, beta, and delta; VOI: mu and VUM: GH. Whereas, PG-II includes VOC: alpha, omicron and VOI: lambda. Interestingly, two VOCs, delta and omicron, belong to different phylogroups. Phylogeny depicted that omicron shares a common ancestry with VOI: lambda represented by a black asterisk in Fig. 1. Interestingly, three isolates from Italy (EPI-ISL_6854346, EPI_ISL_6854347, and EPI_ISL_6854348) form a diversified sub-lineage among the omicron population. Additionally, EPI_ISL_6886594 from Germany is a diversified omicron strain.

2.2. Very high non-synonymous mutations give rise to omicron

Mutation is driving the evolution and emergence of new variants of COVID-19 worldwide (Islam et al., 2021; Kumar & Bansal, 2021; Thakur et al., 2021). Availability of genomic resources have enabled the research community in tracking mutational events and linking them to new variants (Mercatelli & Giorgi, 2020; Rambaut et al., 2020). Analysis and routine surveillance from South Africa suggested omicron ability to evade immunity from prior infection as compared to other VOCs (Pulliam et al., 2021). In the present study, we intend to understand the evolution and emergence of omicron by its mutational landscape at population level.

We have performed a mutational analysis with respect to the reference genome of SARS-CoV-2 (NC_045512.2) (Fig. 2). Total mutations detected in the dataset were 24,189, and omicron genomes constituted 18,261 mutations (supplementary table 1). For all the strains under study, we have calculated the total number of mutations detected (supplementary table 2). Average mutations per genome for the omicron variant were detected to be 60.5. For the limited genomes of VOCs, VOIs

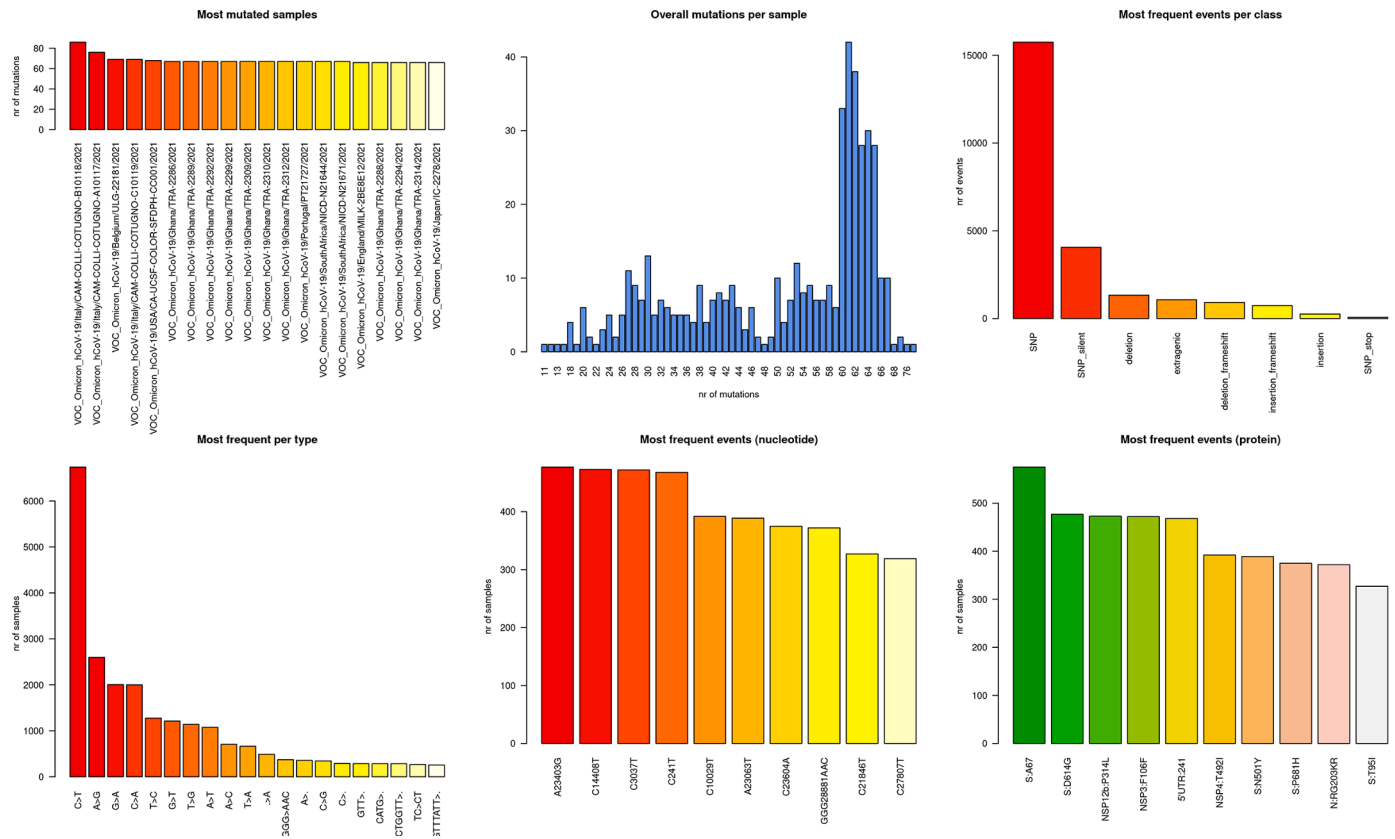


Fig. 2. Mutational analysis of omicron. Six panel image displays the most mutated samples, overall mutations per samples, most frequent events per class of mutation category, changes of nucleotide per type, nucleotide wise most frequent events and protein level most frequent events for the genomes used in the study.

and VUMs, average mutations for GH, delta, mu, gamma, alpha, lambda and beta were 48, 39, 38.5, 37.8, 30.7, 27.4 and 24.2 respectively. This clearly depicts high number of mutations in the omicron variant as compared to other variants of SARS-CoV-2. Except for omicron, average mutations for other variants were calculated on the basis of limited genomes, which might not represent the true mutational events for them. Since, omicron is the recently emerged variant, aim of present study was to understand its mutational landscape at population level.

Interestingly, >97% (n = 17,703 mutations) of the mutations in omicron were in the coding region, and remaining 558 were detected in the extragenic region of the genome. Amongst the coding gene mutations, 2965 were indels while 14,738 were SNPs constituting non-synonymous (n = 11,995 mutations) and synonymous mutations (n = 2743 mutations). Single nucleotide transitions are shown to be major mutational types amongst the SARS-CoV-2 genomes (Kumar & Bansal, 2021; Mercatelli & Giorgi, 2020).

Interestingly, mutational events are highly skewed towards the spike protein, which constitutes ~60% (n = 10,658) of the total mutations in the coding genomic region (n = 17,703) (Fig. 3). The majority of spike protein mutations encompass A67, T547K, D614G, H655Y, N679K, P681H, D796Y, N856K, Q954H, which are reported in all the omicron genomes analysed (Table 3). Count of mutations in the spike was followed by RNA dependent RNA polymerase (rdp) (n = 4142) constituting A1892T, I189V, P314L, K38R, T492I, V57V in all omicron genomes analyzed (Fig. 3 and Table 3). Remaining 2903 mutations were detected in rest of the coding genomic region (Table 2, 3, and supplementary table 1), where M19M in ORF6, and RG203KR in nucleocapsid

protein are amongst the most prevalent mutations in omicron (Fig. 3).

2.3. Low intra-sequence diversity amongst omicron variant

Intra-strain diversity among the omicron variant strains reported worldwide will be crucial in understanding the genome dynamics and rapid evolution of SARS-CoV-2. We performed the mutational analysis on the current dataset using omicron (OL677199) isolated from Canada on 23rd November 2021 as the reference genome (supplementary table 3). Most of the strains (n = 298), irrespective of their geographic origin, had less than ten mutations depicting low intra-strain diversity among omicron strains. We found omicron variants had >55 mutations when compared with other VOCs and VOIs. However, four of the isolates two from Europe (Italy) (EPI_ISL_6854347 (n = 23 mutations) and EPI_ISL_6854346 (n = 14 mutations) and two from South Africa (EPI_ISL_6699742 (n = 12 mutations) and EPI_ISL_6774091 (n = 11 mutations) were most diversified among the omicron genomes.

3. Methods

3.1. Identification and procurement of SARS-CoV-2 genome from the public repository

We have considered all the available genomes of omicron variant available in public domain until 6 pm Indian Standard Time (IST) on 2nd December 2021 from GISAID (n = 302 genomes). A total of 25 strains from each variant of concern, namely alpha (B.1.1.7), beta (B.1.351),

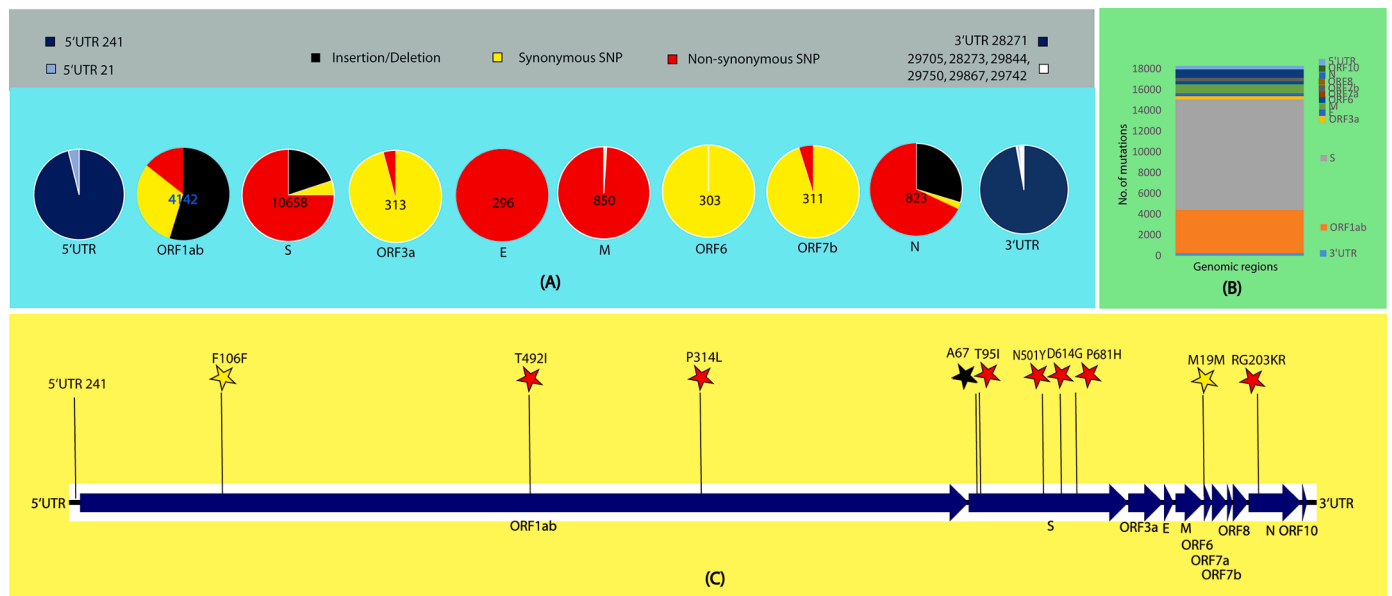


Fig. 3. Mutational analysis of omicron (A) Number of mutations in the coding region is in the centre of the pie-chart representing indels (black), synonymous (yellow) and non-synonymous (red) SNPs. Type and number of mutations in the exergenic region is represented by pie charts blue, light blue and white as represented in the color legends. (B) Bar graph representing number of mutations in the genomic region of SARS-CoV-2. (C) Some of the top mutations (pl. refer Table 3 for all top mutations in omicron) among the omicron variant are represented by stars of black: indels, yellow: synonymous and red: non-synonymous mutations.

Table 2

Genomic region wise mutational count of the omicron isolates by taking NC_045512.2 as a reference.

Genomic region	Mutational count	Annotation
5'UTR	309	5' Untranslated region
NSP1	5	RNA dependent RNA polymerase
NSP2	31	
NSP3	1572	
NSP4	325	
NSP5	317	
NSP6	595	
NSP7	0	
NSP8	2	
NSP9	9	
NSP10	301	
NSP11	0	
NSP12a	0	
NSP12b	632	
NSP13	14	
NSP14	319	
NSP15	6	
NSP16	14	
S	10,658	Spike
ORF3a	313	ORF3a protein
E	296	Envelope
M	850	Membrane
ORF6	303	ORF6 protein
ORF7a	2	ORF7a protein
ORF7b	311	ORF7b protein
ORF8	4	ORF8 protein
N	823	Nucleocapsid protein
ORF10	1	ORF10 protein
3'UTR	249	3' Untranslated region

gamma (P.1) and delta (B.1.617.2) and variant of interest, namely lambda (C.37) and mu (B.1.621). We have also considered 25 strains from variant under monitoring, namely GH (B.1.640). These all strains are from their respective earlier reports in the public domain. Pangolin COVID-19 lineage assigner webserver (<https://pangolin.cog-uk.io/>) was used to truly demarcate the strains of across variants. The investigation suggested that 9 out of 25 strains does not belong to gamma (P.1) and 1 out of 25 strains doesn't belong to VUM GH (B.1.640) and were wrongly

classified earlier. A detailed list of all the strains used in the study is provided in Table 1.

3.2. Phylogenetic analysis

A total of 477 high-quality genomes, including the major variants spread across the globe were taken into consideration. Multiple sequence alignment was performed for all the genomes using MAFFT v7.467 (Nakamura, Yamada, Tomii, & Katoh, 2018) followed by phylogenetic tree construction using fasttree v2.1.8 with double precision (Price, Dehal, & Arkin, 2010) with gamma time reversal method. Visualization of the obtained phylogenetic tree was performed using iTol v6 (Letunic & Bork, 2019). Different variants were marked in accordance with different colors as mentioned in the legends.

3.3. Mutational analysis

Mutational analysis of all the strains (n=477) in the study was performed with two different reference genomes. First with NC_045512.2 (Wuhan-Hu-1) strain (reference SARS CoV-2 strain) and another with first reported strain of omicron variant (OL677199.1) (<https://www.ncbi.nlm.nih.gov/nuccore/OL677199>) using nucmer v3.1 (Delcher, Phillippy, Carlton, & Salzberg, 2002). We have used a well-documented R script described earlier (Mercatelli & Giorgi, 2020). Here, we have used gff3 annotation and reference genome file to extract genomic co-ordinate of SARS-CoV-2 proteins. R library package seqinr (<https://cran.r-project.org/web/packages/seqinr/index.html>) and biostring package (<https://bioconductor.org/packages/release/bioc/html/Biostrings.html>) of bioconductor was implemented to obtain the list of all the mutational events. Mutational events were calculated with respect to two different references (Reference SARS CoV-2 strain: NC_045512.2) (https://www.ncbi.nlm.nih.gov/nuccore/NC_045512.2) and omicron (OL677199.1) (<https://www.ncbi.nlm.nih.gov/nuccore/OL677199>) separately. Further, the average mutations for a variant were calculated by adding up the mutations in each variant and dividing them by the total number of genomes of the variant used in the present study.

Table 3

Top mutations (>185 in count) in omicron variant as compared to the reference sequence NC_045512.2.

annotation	protein	variant	varclass	Count	Refpos	refvar	qvar	qpos	qlength
Spike	S	A67	deletion_frameshift	575	21,762	C	.	21,483	29,387
Predicted phosphoesterase, papain-like proteinase	NSP3	A1892T	SNP	302	8393	G	A	8124	29,387
Transmembrane protein	NSP6	I189V	SNP	302	11,537	A	G	11,259	29,387
RNA-dependent RNA polymerase, post-ribosomal frameshift	NSP12b	P314L	SNP	302	14,408	C	T	14,130	29,387
Spike	S	T547K	SNP	302	23,202	C	A	22,915	29,387
Spike	S	D614G	SNP	302	23,403	A	G	23,116	29,387
Spike	S	H655Y	SNP	302	23,525	C	T	23,238	29,387
ORF6 protein	ORF6	M19M	SNP_silent	302	27,259	A	C	26,972	29,387
Predicted phosphoesterase, papain-like proteinase	NSP3	K38R	SNP	301	2832	A	G	2566	29,387
Spike	S	N679K	SNP	301	23,599	T	G	23,312	29,387
Transmembrane protein	NSP4	T492I	SNP	301	10,029	C	T	9760	29,378
Nucleocapsid protein	N	RG203K*	SNP	301	28,881	GGG	AAT	28,806	29,693
Growth-factor-like protein	NSP10	V57V	SNP_silent	300	13,195	T	C	12,917	29,387
Spike	S	P681H	SNP	300	23,604	C	A	23,317	29,387
Spike	S	D796Y	SNP	300	23,948	G	T	23,661	29,387
Spike	S	N856K	SNP	300	24,130	C	A	23,843	29,387
Spike	S	Q954H	SNP	300	24,424	A	T	24,137	29,387
Nucleocapsid protein	N	RG203KR	SNP	300	28,881	GGG	AAC	28,594	29,387
RNA-dependent RNA polymerase, post-ribosomal frameshift	NSP12b	N591N	SNP_silent	298	15,240	C	T	14,962	29,387
Spike	S	T95I	SNP	298	21,846	C	T	21,562	29,387
Predicted phosphoesterase, papain-like proteinase	NSP3	F106F	SNP_silent	297	3037	C	T	2771	29,387
Spike	S	G339D	SNP	297	22,578	G	A	22,291	29,387
ORF3a protein	ORF3a	T64T	SNP_silent	297	25,584	C	T	25,297	29,387
NA	5'UTR	241	extragenic	297	241	C	T	187	29,693
3C-like proteinase	NSP5	P132H	SNP	296	10,449	C	A	10,180	29,387
3'-to-5' exonuclease	NSP14	I42V	SNP	296	18,163	A	G	17,885	29,387
Envelope	E	T9I	SNP	296	26,270	C	T	25,983	29,387
ORF7b protein	ORF7b	L17L	SNP_silent	296	27,807	C	T	27,520	29,387
Spike	S	N969K	SNP	294	24,469	T	A	24,182	29,387
Predicted phosphoesterase, papain-like proteinase	NSP3	A889A	SNP_silent	293	5386	T	G	5120	29,387
Spike	S	L981F	SNP	292	24,503	C	T	24,216	29,387
Spike	S	D1146D	SNP_silent	292	25,000	C	T	24,713	29,387
Membrane	M	A63T	SNP	289	26,709	G	A	26,422	29,387
Predicted phosphoesterase, papain-like proteinase	NSP3	S1265	deletion	288	6513	GTT	.	6246	29,387
Transmembrane protein	NSP6	L105	deletion	287	11,286	TGTCGGTT	.	11,016	29,387
Spike	S	I68	deletion_frameshift	287	21,767	CATG	.	21,486	29,387
Spike	S	E484A	SNP	284	23,013	A	C	22,726	29,387
Spike	S	S477N	SNP	283	22,992	G	A	22,705	29,387
Spike	S	T478K	SNP	283	22,995	C	A	22,708	29,387
Spike	S	Q493R	SNP	282	23,040	A	G	22,753	29,387
Spike	S	Q498R	SNP	281	23,055	A	G	22,768	29,387
Spike	S	N501Y	SNP	281	23,063	A	T	22,776	29,387
Spike	S	G496S	SNP	280	23,048	G	A	22,761	29,387
Spike	S	Y505H	SNP	277	23,075	T	C	22,788	29,387
Membrane	M	D3G	SNP	275	26,530	A	G	26,243	29,387
Membrane	M	Q19E	SNP	272	26,577	C	G	26,290	29,387
Spike	S	S371L	SNP	270	22,673	TC	CT	22,386	29,387
Spike	S	S373P	SNP	270	22,679	T	C	22,392	29,387
Spike	S	G142	deletion	260	21,987	GTGTTTATT	.	21,702	29,387
Spike	S	S375F	SNP	260	22,686	C	T	22,399	29,387
ORF7b protein	ORF7b	E3*	SNP_stop	253	27,762	G	T	27,687	29,752
Spike	S	I210	insertion_frameshift	243	22,193	.	T	21,901	29,387
Spike	S	R214	insertion_frameshift	243	22,203	.	A	21,916	29,387
Spike	S	R214R	SNP_silent	243	22,204	T	A	21,917	29,387
Nucleocapsid protein	N	E31	deletion	243	28,362	GAGAACGCA	.	28,074	29,378
Spike	S	L212*	SNP_stop	243	22,197	T	G	22,118	29,749
Spike	S	N211K	SNP	242	22,195	T	G	21,903	29,387
Spike	S	L212C	SNP	242	22,197	TA	GC	21,905	29,387
Spike	S	S214	insertion	242	22,201	.	AGC	21,910	29,387
Spike	S	V213	insertion_frameshift	242	22,202	.	A	21,914	29,387
NA	3'UTR	28,271	extragenic	242	28,271	A	T	27,984	29,378
Nucleocapsid protein	N	P13L	SNP	241	28,311	C	T	28,024	29,378
Spike	S	N764K	SNP	234	23,854	C	A	23,567	29,387
Spike	S	G446S	SNP	203	22,898	G	A	22,611	29,387
Spike	S	N440K	SNP	199	22,882	T	G	22,595	29,387
Spike	S	K417N	SNP	183	22,813	G	T	22,526	29,387

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Author contribution statement

Both the authors' KB and SK have contributed equally to the data curation, analysis, and writing of the manuscript.

CRediT authorship contribution statement

Kanika Bansal: Data curation, Formal analysis, Writing – original draft. **Sanjeet Kumar:** Data curation, Formal analysis, Writing – original draft.

Declaration of Competing Interest

The author declares no competing interest.

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Supplementary materials

Supplementary material associated with this article can be found, in the online version, at [doi:10.1016/j.virusres.2022.198765](https://doi.org/10.1016/j.virusres.2022.198765).

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