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Omicron SARS-CoV-2 Variants in an *In Silico* Genomic Comparison Study with the Original Wuhan Strain and WHO-Recognized Variants of Concern

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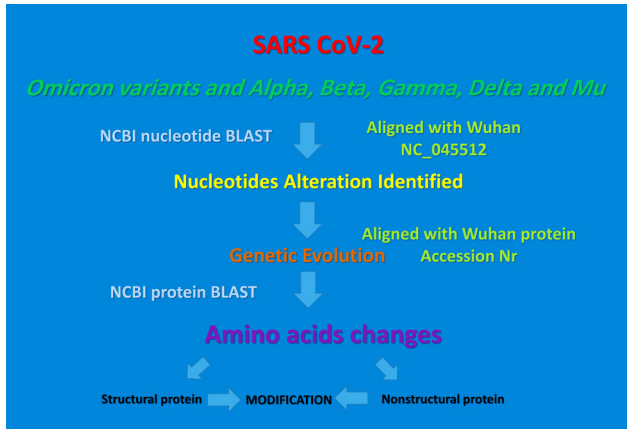
Abstract

This study aimed to determine the genetic alterations in the Omicron variants compared to other variants of concern (VOCs) to trace the evolutionary genetics of the SARS-CoV-2 variants responsible for the multiple COVID-19 waves globally. The present study is an *in silico* analysis determining the evolution of selected 11 VOCs compared to the original Wuhan strain. The variants included six Omicrons and one variant of Alpha, Beta, Delta, Gamma, and Mu. The pairwise alignment with the local alignment search tool of NCBI Nucleotide-BLAST and NCBI Protein-BLAST were used to determine the nucleotide base changes and corresponding amino acid changes in proteins, respectively. The genomic analysis revealed 210 nucleotide changes; most of these changes (127/210, 60.5%) were non-synonymous mutations that occurred mainly in the S gene (52/127, 40.1%). The remaining 10.5% (22/210) and 1.9% (4/210) of the mutations were frameshift deletions and frameshift insertions, respectively. The frameshift insertion (Ins22194T T22195G) led to frameshift deletion (Δ211N). Only four mutations (C241T, C3037T, C14408T, and A23403G) were shared among all the VOCs. The nucleotide changes among Omicron variants resulted in 61 amino acid changes, while the nucleotide changes in other VOCs showed 11 amino acid changes. The present study showed that most mutations (38/61, 62.3%) among Omicron variants occurred in the S gene; and 34.2% of them (13/38) occurred in the receptor-binding domain. The present study confirmed that most of mutations developed by Omicron variants occurred in the vaccine target gene (S gene).



K e y w o r d s: *in silico*, Omicron, VOCs, Nucleotide-BLAST, Protein-BLAST

Introduction

The COVID-19 pandemic, which started at the end of 2019, continues at the dawn of 2022, with an unprecedented vigor against humanity that was unheard of in recent times (Sofi et al. 2020). The global crisis demonstrated the loss of millions of human lives, and billions underwent a dreadful experience of economic, social, and psychological distress (Rudrapal et al. 2020). The healthcare systems across the world are exhausted and racing against time to save millions of SARS-CoV-2 -infected people. During this long pandemic, different

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parts of the world saw multiple waves of the disease that were driven by dominant variants originating through mutations in the original SARS-CoV-2 viral genome. Consequently, Alpha, Beta, Gamma, Kappa, Delta, Mu, IHU, and Omicron have originated as novel variants with the varying potential of virulence, transmissibility, and disease severity (Choi and Smith 2021; Karim and Karim 2021). The SARS-CoV-2, like other coronaviruses, possesses an enveloped genome of positive-sense single-stranded ~ 30 kb RNA with six open reading frames (Cao et al. 2021). The genome's two-thirds of length comprises 265 nucleotides 5'UTR, followed by 21,290 nucleotides long ORF1ab that encode poly-protein for 16 non-structural proteins. The other one-third at the 3' end has 229 nucleotide 3'UTR and several genes that encode surface, envelope, membrane, and nucleocapsid structural proteins such as 3,822 nucleotide-S, 228 nucleotide-E, 669 nucleotide-M, and 908 nucleotide-N, respectively. Six accessory proteins are encoded by 828 nucleotide ORF3a, 186 nucleotide ORF6, 366 nucleotide ORF7a, 132 nucleotide ORF7b, 193 nucleotide ORF8, and 117 nucleotide ORF10 genes. It is known that the viral genome is a hotspot of mutations with one of the highest mutation rates among all organisms.

Moreover, compared to DNA viruses, RNA viruses are more prone to mutations due to the low fidelity of RNA polymerase, resulting in higher rates of erroneous miss-incorporation of bases during replication. Some of these mutations might provide the endurance to the replicating virus that grants higher potential of transmission and pathogenicity, causing the emergence of a dominant variant associated with higher rates of infection, mortality, and evasiveness to the existing natural or vaccines elicited immunity. The Delta variant of SARS-CoV-2 (B.1.617.2) which was detected in India, exhibited characteristics that outweighed the previously existing Alpha and Kappa lineages, making it a dominant variant that was responsible for the calamitous second wave in India with devastating human sufferings and loss of lives (Adiga and Nayak 2021). Experimental evidence suggests that the Delta variant as compared to wild-type Wuhan-1, was less sensitive to neutralizing antibodies from the serum of recovered individuals (six-fold loss of efficacy), and vaccine-induced antibodies (eight-fold loss of efficacy), demonstrating a potential risk of infection due to a compromised vaccine efficacy even in the vaccinated population (Mlcochova et al. 2021).

Consequently, the Delta variant was observed to be associated with a longer period of infections, higher viral load, and higher rates of re-infections, emerging as a globally dominant variant driving 2nd, 3rd, and 4th waves of COVID-19 infections in many countries worldwide. Recently on November 25, 2021, another variant of concern named Omicron (B.1.1.529) was reported in South Africa, leaving the world apprehensive about the next course of COVID-19 disease and the efficacy of vaccines (Callaway 2021). Since then, the Omicron infection has been reported in fully vaccinated individuals, and the probability of higher transmission rates has also been suggested (Gu et al. 2021). The emergence of new variants with higher virulence downplays the containment strategies and increases the risk of greater harm to human lives. Studies characterizing the genetic diversity of such variants are therefore considered significant to tracing the course of the pandemic. In this study, we have characterized the genetic alterations in the variants of concern (VOC) including the Omicron variant which exhibits a continuous evolution of the heterogeneity in the viral genome (structural and non-structural genes) in comparison to the original Wuhan SARS-CoV-2 genome and other VOCs such as Alpha, Beta, Delta, Gamma, and Mu.

The novelty of our work can be assessed from the results wherein we have described the genetic variations in the whole genome of the Omicron SARS-CoV-2 variants as compared to other studies which focused only on the spike protein of the virus. It is also the first study wherein inter-variant genomic variations have been compared, tracing the genetic variations from the original Wuhan strain to variants of concern that emerged during different waves of COVID-19 globally.

Experimental

Materials and Methods

Our laboratory has been engaged in studies related to the genetic variations in SARS-CoV-2 over the last two years. This study is an *in silico* analysis of the evolutionary heterogeneity of selected variants of concern compared to the original Wuhan reference. The present study included six Omicron variants retrieved from GISAID database that were Omicron (EPI_ISL_6640916) from Botswana, Omicron (EPI_ISL_6647956) from South Africa, Omicron (EPI_ISL_6647957) from South Africa, Omicron (EPI_ISL_6647961) from South Africa, Omicron (EPI_ISL_7740798) from South Africa and Omicron (EPI_ISL_8182845) from South Africa plus five VOCs isolated from Japan and retrieved from GISAID database that were Alpha (EPI_ISL_6756515), Beta (EPI_ISL_5416540), Gamma (EPI_ISL_6228367), Delta (EPI_ISL_6832166) and Mu (EPI_ISL_4470504).

The sequences of SARS-CoV-2 of the original Wuhan variant were retrieved from NCBI COVID-19 Resource Repository (https://www.ncbi.nlm.nih.gov/genbank/sars-cov-2-seqs). The selected variants included complete genomic sequences aligned with the first

characterized isolate, the Wuhan strain from China (NC_045512.2) (Lu et al. 2020). The pairwise alignment with the local alignment search tool Nucleotide-BLAST (NCBI) was used to determine the nucleotide base changes and gene variation against Wuhan standard reference. Moreover, the NCBI Protein-BLAST was used to report the corresponding amino acid changes in the protein.

Evolutionary relationships: The Neighbor-Joining method was used to build a phylogenetic tree for understanding the evolutionary relationship of the evolving variants of SARS-CoV-2 using Molecular Evolutionary Genetics Analysis Software (MEGA 4), Philadelphia, USA (Saitou and Nei 1987). As reported in the results, the clustered taxa clad in the bootstrap test included 500 replicates, which are represented as a percentage of replicate trees shown close to the branches (Felsenstein 1985). The evolutionary distances were determined by the Maximum Composite Likelihood method (Tamura et al. 2004) showing the number of base substitutions/site units. This analysis involved a set of SARS-CoV-2 variants of concerns, including Beta, Gamma, Delta, Mu, and Omicron, along with the original Wuhan sequence. The FASTA sequence for each of the specimen variants retrieved from the database were selected, aligned, and phylogenetic association was obtained using the tools incorporated within the software. The analysis disregarded all the enigmatic positions with a pairwise deletion option for each sequence pair. A total of 29,903 positions representing the approximate length of the whole viral genome were present in the final dataset.

Results

The multi-alignment analysis showed that Omicron variants have the lowest homology compared to the original strain; they exhibited homology ranging between 99.74 to 99.3% except for Omicron (EPI_ISL_8182845), which showed 99.84%, while the other VOCs showed homology ranging between 99.82 to 99.85%. The genomic analysis of the VOCs of SARS-CoV-2 (Alpha, Beta, Delta, Gamma, Mu, and Omicron variants) revealed 210 nucleotide changes. Most of these changes (127/210, 60.5%) were non-synonymous mutations that occurred mainly in the S gene (52/127, 40.1%) followed by ORF1a/b (43/127, 33.9%) and the N gene (11/127, 8.9%). The remaining 10.5% (22/210) and 1.9% (4/210) of the mutations were frameshift deletions and frameshift insertions, respectively (Table I).

The comparative analysis showed that only four mutations were common among all the VOCs; one silent mutation (C241T) on the 5'UTR region, one synonymous mutation in ORF1a/b (C3037T), one non-synonymous mutation in ORF1a/b (C14408T), which changed the amino acid (P4715L), and one non-synonymous mutation in the S gene (A23403G), which changed the amino acid (D614G).

The nucleotide changes among Omicron variants resulted in 61 amino acid changes, while the nucleotide changes in other VOCs of SARS-CoV-2 (Alpha, Beta, Delta, Gamma, and Mu) showed 11 amino acid changes (Table II, III, and IV). The present study exhibited that the majority of mutations (38/61, 62.3%) among Omicron variants occurred in the S gene, 34.2% (13/38) out of that occurred in the receptor-binding domain RBD (the RBD has involved 541–319 residues of the S1 subunit), as follows: G339D, S371L, S373P, **N440K**, **G446S**, **K417N**, **S477N**, **T478K**, **E484A**, **Q493R**, **G496S**, **Q498R**, **N501Y**. The mutations indicated with the bold font representing 69.2% (9/13) of the mutations in BDR were located in the receptor-binding motif (508aa–438aa).

The mutations among Omicron variants were categorized into three groups; the unique common mutations group which involved 50.8% (31/61) of the mutations (Table II); the unique non-common mutations

Table I
Distribution and type of mutations among variants of concern of SARS-CoV-2.

Type of mutation	3'UTR	ORF10	N gene	ORF8	ORF7b	ORF7a	ORF6	M gene	E gene	ORF3	S gene	ORF1 a/b	5'UTR	Total
Non-coding	2	–	–	–	–	–	–	–	–	–	–	–	3	5
Non-synonymous	–	–	11	6	1	3	–	4	2	5	52	43	–	127
Synonymous	–	–	5	1	1	–	1	–	1	2	4	36	–	51
Frame shift/insertion	–	–	1	–	–	–	–	–	–	–	3	–	–	4
Frame shift/deletion	–	–	4	–	–	–	–	–	–	–	15	3	–	22
Nonsense	–	–	–	1	–	–	–	–	–	–	–	–	–	1
	2		21	8	2	3	1	4	3	7	74	82	3	210

Table II
The unique common mutations among Omicron variants.

Nucleotide Mutation	Protein mutation	Type of mutation	Gene
A2832G	K856R	Nonsynonymous	ORF1a/b
3T5386G	–	Synonymous	ORF1a/b
G8393A	A2710T	Nonsynonymous	ORF1a/b
C10449A	–	Synonymous	ORF1a/b
A11537G	I3758V	Nonsynonymous	ORF1a/b
C15240T	–	Synonymous	ORF1a/b
A18163G	I5968V	Nonsynonymous	ORF1a/b
Δ21762 ΔC Δ21764 ΔA	A67V	Frameshift (deletion)	S gene
Δ21767–21769 ΔCAT	Δ69H	Frameshift (deletion)	S gene
Δ21770 ΔG	Δ70V	Frameshift (deletion)	S gene
Δ21987–21988 ΔGT	G142D	Frameshift (deletion)	S gene
Δ21989–21991 ΔGTT	Δ143V	Frameshift (deletion)	S gene
Δ21992–21994 ΔTAT	Δ144Y	Frameshift (deletion)	S gene
Δ21995 ΔG	Δ145Y	Frameshift (deletion)	S gene
G22578A	G339D	Nonsynonymous	S gene
C23202A	T547K	Nonsynonymous	S gene
C23525T	H655Y	Nonsynonymous	S gene
T23599G	N679K	Nonsynonymous	S gene
C23854A	N764K	Nonsynonymous	S gene
G23948T	D796Y	Nonsynonymous	S gene
C24130A	N856K	Nonsynonymous	S gene
A24424T	Q954H	Nonsynonymous	S gene
T24469A	N969K	Nonsynonymous	S gene
C25000T	–	Synonymous	S gene
C25584T	–	Synonymous	ORF3a
C26270T	T9I	Nonsynonymous	E gene
A26530G	D3G	Nonsynonymous	M gene
G26709A	A63T	Nonsynonymous	M gene
C28311T	P13L	Nonsynonymous	N gene
Δ28363–28364 ΔGA	–	Frameshift (deletion)	N gene
Δ28365–28367 ΔGAA	Δ31E	Frameshift (deletion)	N gene
Δ28368–28370 ΔCGC	Δ32R	Frameshift (deletion)	N gene
Δ28371 ΔA	Δ33S	Frameshift (deletion)	N gene

group, which involved 31.1% (19/61) of the mutations (Table III), and the shared mutations group which involved 18.0% (11/61) of the mutations (Table IV). The unique common mutations characterized and differentiated Omicron variants from the other VOCs. These mutations were present only in Omicron variants, unique non-common mutations were also associated with Omicron variants but were absent in all variants, while the shared mutations existed in Omicron as well the other VOCs. Most of the unique common mutations 54.8% (17/31) were non-synonymous mutations, followed by 29% (9/31) frameshift deletions, which were observed in the S gene 55.6% (5/9) and in the N gene 44.4% (4/9) (Table II and Fig. 1).

Similarly, many unique non-common mutations (12/19, 63.2%) were non-synonymous. Some of them (3/19, 15.8%) were frameshift deletions and (1/19, 5.3%) frameshift insertions (Table III and Fig. 1). Interestingly the frameshift insertion (Ins22194, T22195G) led to frameshift deletion (Δ211N) (Table III and Fig. 1).

All Omicron variants showed frameshift deletions in the S and N genes and ORF1a/b except (EPI_ISL_6647956) variant, which did not show deletion in the ORF1a/b. All Omicron variants showed frameshift insertion in the S gene except (EPI_ISL_8182845) variant. The Alpha, Beta, and Gamma variants showed frameshift deletions in ORF1a/b, while the Mu variant showed frameshift insertion in the S gene.

Table III
The unique non-common mutations among Omicron variants.

Nucleotide mutation	Protein mutation	Type of mutation	Gene	Comment
C24503T	L981F	Nonsynonymous	S gene	Not detected in (EPI-ISL-6640916) variant
Δ11283–1129 ΔGTTTGTCTG	Δ3674–3676 ΔLSG	Frameshift (deletion)	ORF1a/b	Not detected in variant (EPI_ISL_6647956)
C27807T		Synonymous	ORF7b	Not detected in (EPI_ISL_8182845) variant
T13195C		synonymous	ORF1a/b	Not detected in one (EPI_ISL_8182845) variant
Ins22194T T22195G	Δ211N	Frameshift (deletion)	S gene	
T22197G A22198C	L212I	Nonsynonymous	S gene	
INS22202–22203 AG INS22203–22204 CA T22204A INS22205 A	INS214–216 EPE	Frameshift (insertion)	S gene	
T22673C C22674T	S371L	Nonsynonymous	S gene	
T22882G	N440K	Nonsynonymous	S gene	
G22898A	G446S	Nonsynonymous	S gene	
T22679C	S373P	Nonsynonymous	S gene	
G22813T	K417N	Nonsynonymous	S gene	Not detected in one (EPI_ISL_8182845) variant
Δ6513–6515 ΔGTT	Δ2083 S L2084I	Frameshift (deletion)	ORF1a/b	Not detected in two variants (EPI-ISL-6640916 and EPI_ISL_6647956)
A27259C		Synonymous	M gen	Not detected in two variants (EPI_ISL_6647956 and (EPI_ISL_8182845)
G22992A	S477N	Nonsynonymous	S gene	Not detected in three variants (EPI_ISL_6647956, EPI_ISL_6647957 and EPI_ISL_8182845)
A23013C	E484A	Nonsynonymous	S gene	
A23040G	Q493R	Nonsynonymous	S gene	
G23048A	G496S	Nonsynonymous	S gene	
A23055G	Q498R	Nonsynonymous	S gene	

Table IV
Mutations shared between Omicron and other VOCs.

Nucleotide mutation	Protein mutation	Type of mutation	Gene	Comment
Δ21767-21769 ΔCAT	Δ69H	Frameshift (deletion)	S gene	Shared with Alpha variant (EPI_ISL_6756515)
Δ21770 ΔG	Δ70V	Frameshift (deletion)	S gene	
C23525T	H655Y	Nonsynonymous	S gene	Shared with Gamma variant (EPI_ISL_6228367)
C10029T	T3255I	Nonsynonymous	ORF1a/b	Shared with Delta (EPI_ISL_6832166) and Mu (EPI_ISL_4470504) variants
C21846T	T95I	Nonsynonymous	S gene	
C23604A	P681H	Nonsynonymous	S gene	Shared with Alpha (EPI_ISL_6756515) and Mu (EPI_ISL_4470504) variants
G28881T	R203K	Nonsynonymous	N gene	Shared with Alpha (EPI_ISL_6756515) and Gamma (EPI_ISL_6228367) variants
G28882A		Synonymous	N gene	
G28883C	G204R	Nonsynonymous	N gene	
C22995A	T478K	Nonsynonymous	S gene	Shared with Delta (EPI_ISL_6832166) variant
A23063T	N501Y	Nonsynonymous	S gene	Shared with Alpha (EPI_ISL_6756515), Beta (EPI_ISL_5416540), Gamma (EPI_ISL_6228367), Mu (EPI_ISL_4470504), variants

Furthermore, 11 mutations were shared between Omicron variants and other VOCs (Alpha, Beta, Delta, Gamma, and Mu); 63.6% (7/11) of these mutations occurred on the S gene, 18.2% (2/11) were frameshift deletion (Δ21767-21769 ΔCAT (Δ69H) and Δ21770 ΔG (Δ70V)) that were shared with Alpha variant and three mutations in the N gene; G28881T (R203K), G28882A, G28883C (G204R) shared with Alpha (EPI_ISL_6756515) and Gamma (EPI_ISL_6228367) variants, one mutation occurred in ORF1a/b C10029T

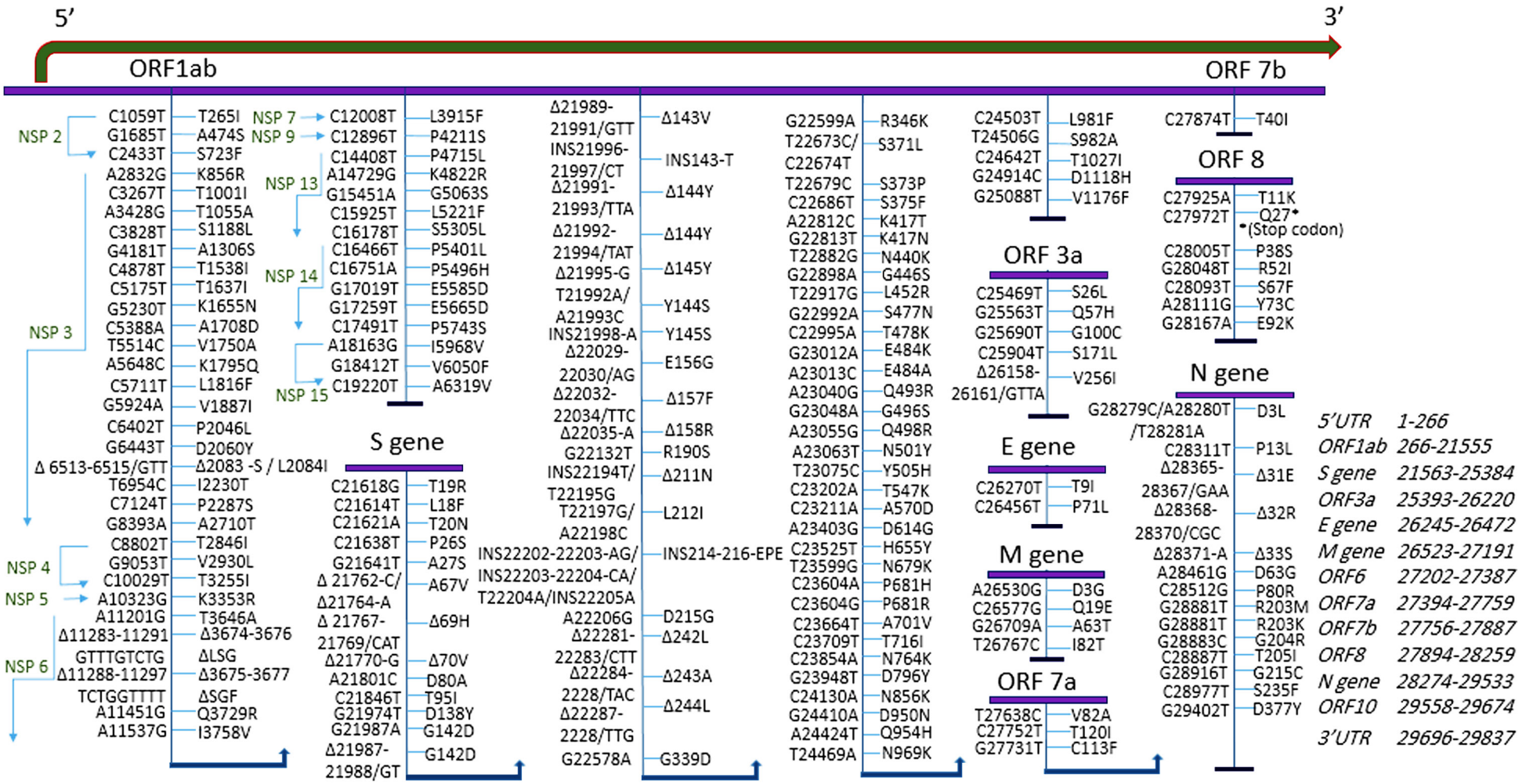

Fig. 1. The genomic landscape representing nucleotides changes for variants of concerns of SARS-CoV-2 and corresponding amino acid changes.

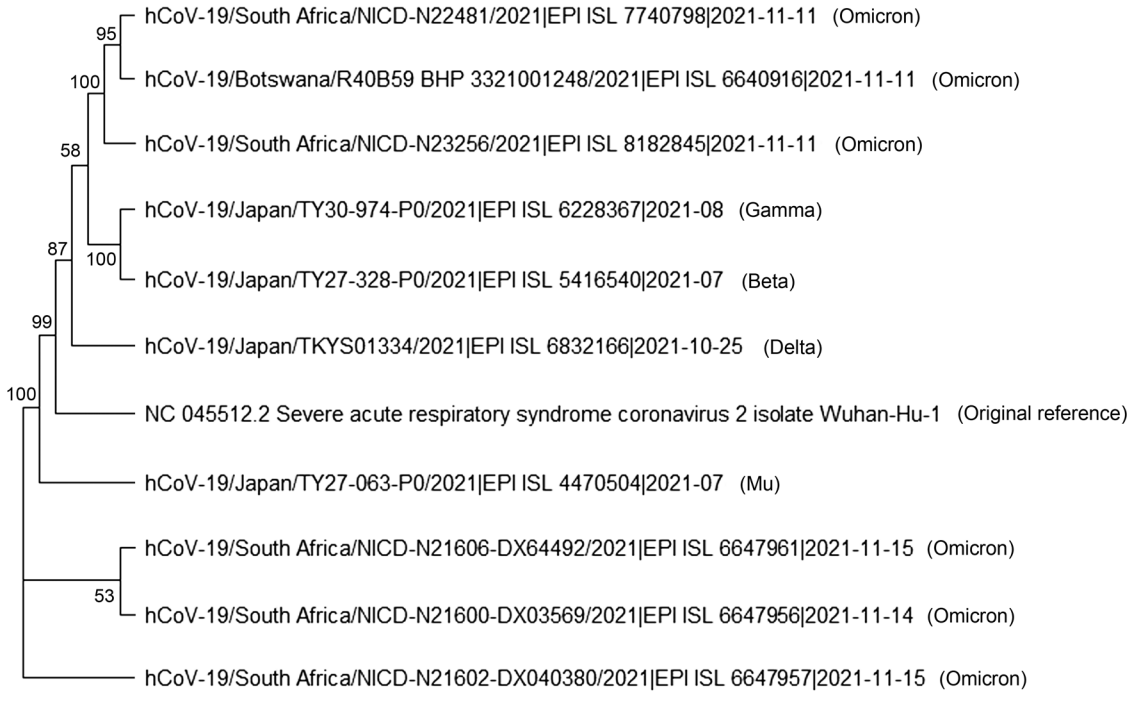

Fig. 2. Phylogenetic affiliation for variants of concerns of SARS-CoV-2.

(T3255I) shared with Delta (EPI_ISL_6832166) and Mu (EPI_ISL_4470504) variants. The mutation A23063T (N501Y) in the S gene was shared with Alpha (EPI_ISL_6756515), Beta (EPI_ISL_5416540), Gamma (EPI_ISL_6228367), Mu (EPI_ISL_4470504), and Omicron variants (EPI_ISL_8182845, EPI_ISL_6647961, EPI_ISL_7740798, EPI-ISL-6640916) (Table IV and Fig. 1).

A phylogenetic relationship between Omicron variants, Wuhan reference, and certain variants of concerns revealed two distinct clads of Omicron: one at the top and one at the bottom of the evolutionary tree (Fig. 2).

Discussion

Omicron variants showed too many mutations compared to the previously evolved variants of concern (Kannan et al. 2021), which is why it has been described as a "worrying type". These mutations may alter the virus's conformation and affect the capacity for immune evasion, disease severity, and transmissibility (Zhang et al. 2021). However, the information about the Omicron variants is limited (Kannan et al. 2022).

The present study displayed that most of the mutations (60.5%) among the VOCs were non-synonymous mutations that occurred mainly in the S gene. This finding was contradictory to our earlier reports at the beginning of this pandemic, in which we found that most mutations occurred in ORF1a/b (Ahmed-Abakur and Alnour 2020). Several other authors stated that ORF1a/b was occupied with more than 60% of mutations (Khailany et al. 2020; Shishir et al. 2021). The recent reports approved that the principal mechanism of SARS-CoV-2 evolution is natural selection, so the heavy mutations in the S gene could be attributed to the breakthrough of the SARS-CoV-2 vaccine. Wang et al. 2021 proposed that vaccine breakthroughs will become a major mechanism of SARS-CoV-2 evolution as most of the population is either infected or vaccinated.

Our study showed 61 amino acid mutations among Omicron variants; 31 of 61 (50.8%) were unique common mutations, 19 of 61 (31.1%) were unique non-common mutations, and 11 of 61 (18%) were shared mutations. These results reflected a high occurrence of mutations among the Omicron variants under the study compared to the most published reports that indicated 47 to 58 mutations in Omicron variants (Jia et al. 2022; Poudel et al. 2022; Tan et al. 2022; Thakur and Ratho 2022). Kannan et al. (2022) studied the unique features of the Omicron variant and reported 46 mutations, 23 of which were unique to the Omicron variant. Jia et al. (2022) and Kannan et al. (2022) mentioned that some Omicron signature mutations might not be present in some variants, similar to our finding concerning the unique non-common mutations.

The present study showed that only four common mutations were present in all the VOCs; one silent mutation (C241T) in the 5'UTR region, one synonymous mutation (C3037T) in ORF1, one non-synonymous mutation in ORF1a/b (C14408T) which changed the amino acid (P4715L), and one non-synonymous mutation in the S gene (A23403G) changing the amino acid

(D614G). These findings showed the importance and role of these four mutations. We propose that they have become part of each circulating variant of SARS-CoV-2. Although the mutations (C241T) and (C3037T) do not affect protein structure or function, they may support the virus to cloak itself within the host or affect transmission. The (C14408T) might influence the replication rate; it altered the amino acid in RNA-dependent RNA polymerase (Ahmed-Abakur et al. 2022). The (D614G) mutation occurred in the S2 domain, which is important for the fusion of the spike protein with the host cell membrane, thus may increase the infectivity and spread of the virus (Callaway 2021; Quarleri et al. 2022). Corresponding to this finding, Korber et al. (2020) reported that SARS-CoV-2 carrying the mutation D614G has become the dominant form. However, many reports showed the co-existence of C241T, C3037T, C14408T, and A23403G (Ahmed-Abakur et al. 2022; Kandeel et al. 2022; Kumar et al. 2022).

Our study showed 14 frameshift deletions (nine in the unique common mutations group, three in the unique non-common mutations group, and two in the shared mutations group) and two frameshift insertions. A higher number of deletions and insertions were reported by Tan et al. (2022), who characterized the first two cases of the Omicron variant in China. They showed 39 deletions and nine insertions. The CDC (2021) stated that the Omicron variant (B.1.1.529) has one tiny insertion and three small deletions in the spike glycoprotein Δ69-70 Δ143-145, Δ211 (28). The previously mentioned frameshift affected five amino acids that were Δ69H, Δ70V, Δ143V, Δ144Y, and Δ145Y. In addition to these deletions, our study pointed out nine frameshift deletions more: three in the S gene (A67V, G142D, and Δ211N), four in the N gene (Δ31E, Δ32R, Δ33S, and one synonymous), and two in ORF1a/b (Δ3674-3676 ΔLSG and Δ2083S L2084I). Thakur and Ratho (2022) reported that the deletions at positions H69- and V70- result in failure of the S-gene target. Recently, some studies proposed that the SARS-CoV-2 spike protein insertion sequences may be derived from either host or other coronaviruses. These findings might have subsequently affected the viral entry and failure of antibodies to deactivate this variant (Kannan et al. 2022).

The present study exhibited heavy mutations in the S gene compared to the previous studies; several authors figured out 30–32 mutations in the S gene of Omicron variants, one minor insertion, and three deletions (Karim and Karim 2021; Zhang et al. 2021; Gao et al. 2022; Gowrisankar et al. 2022; Kumar et al 2022;). However, our study showed that 34.2% (13/38) of the mutations in the S gene occurred in the receptor-binding domain (RBD). This site (RBD) is important for the entry of SARS-CoV-2, represents the binding site to the host receptor (ACE2), and is the main target of antibodies and therapeutics agents (Hu et al. 2022). Therefore, mutations at this site mainly affected the transmission, efficiency of the available vaccine, and treatment. In alignment with our findings, Zhang et al. (2021) mentioned that Q498R, Q493K, G496S, S477N, G446S, N440K, S375F, S373P, S371, and G339D were the new mutations in the RBD of Omicron variants. They concluded that the mutation on the receptor-binding motif leads to conformational changes that may potentiate the ability of immune evasion. Also, Lupala et al. 2022 found that most of the mutations on RBD were located at the RBD-ACE2 interface. Subsequently, these mutations alter the electrostatic charges at the interface which affects the binding of neutralizing antibodies and medications targeting the interface (Zhang et al. 2021; Mohapatra et al. 2022; Saxena et al. 2022). The modification at the RBD-ACE2 interface increases the binding through increasing buried solvent accessible surface area and enhancing the hydrogen bonding interaction (Lupala et al. 2022). Similarly, Andreata-Santos et al. (2022) stated that the mutations G142D and P681H corresponded to vital regions targeted by neutralizing antibodies. Kannan et al. (2021) reported that N501Y, Q493R, E484A, and T478K were the vital mutations in the RBM. The mutation T478K was also found in Delta variants, and it was linked to infections of vaccinated people (Zhang et al. 2021). Likewise, the mutation in 484 was observed in Gamma and Beta variants and associated with the reinfection with the Gamma variant (Kannan et al. 2021), where the glutamic acid is replaced by lysine (E484K). Interestingly, in the Omicron variant, it is replaced by alanine (E484A). The mutation E484A might modify the interaction between human angiotensin-converting hACE2 and RBD. The mutation N501Y was detected earlier in the Gamma, Beta, and Alpha variants and was recognized as having a strong affinity to hACE2 (CDC 2021).

However, Karim and Karim (2021) reported that most Omicron mutations' effects are unknown, leading to uncertainty about how these mutation combinations could affect the response to natural and acquired immunity. Poudel et al. (2022) mentioned that only twelve mutations were studied in the past, and it is early to realize the new mutations and how they affect the virus behavior.

The mutations in the ORF1a/b gene in the present study were almost matched with the results by Thakur and Ratho (2022), who reported that the mutations in ORF1a/b compromise the cell's capability to destroy viral components and therefore assist in the evasion of innate immunity. The mutations in the E gene in our study were in alignment with Saxena et al. (2022) and Kannan et al. (2021). Opposite to our results for the M gene, numerous reports showed two non-synony-

mous mutations (D3G and Q19E) and one synonymous mutation (A63T) (Kannan et al. 2021; Saxena et al. 2022; Thakur and Ratho 2022). The changings in the N gene in our study matched with Kannan et al. (2021), Saxena et al. (2022), and Thakur and Ratho (2022), concerning the non-synonymous mutations (P13L, N: R203K, and N: G204R). Moreover, Saxena et al. (2022) showed one deletion (Δ31-33). In addition to the previous mutations, we pointed out four frameshift deletions (Δ28363-28364 ΔGA, Δ31E, Δ32R, Δ33S), and one synonymous G28882A.

Our study showed that 11 mutations were shared between Omicron and other VOCs; N501Y was the most common mutation; it appeared in Omicron, Alpha, Beta, Gamma, and Mu. T3255I and T95I were shared with Delta, Mu, and Omicron. The deletions Δ69H and Δ70V were observed in Alpha and Omicron variants. T478K appeared in Omicron and Delta. R203K, G204R, and the synonymous mutation G28882A were found in Alpha, Gamma, and Omicron variants. However, numerous other mutations have been reported by other authors, such as Lys38Arg, SΔ1265, Leu1266Ile, Ala1892Thr, Thr492Ile, Phe132His, Δ105-107, Ile189Val, Pro323Leu NSP14-Ile42Val (Saxena et al. 2022). P3395H, S3675-, G3676- (Thakur and Ratho 2022). T492I, P314L, P323L (Kannan et al. 2021). L141F, R346K, V367F 5 L455, P499, A475 and F486 (Yi et al.2020).

The phylogenetic tree in our study showed two distinct clads of Omicron; one at the top and one at the bottom. Such a pattern is as arduous to explain as the Omicron origin and its higher number of mutations within a limited time frame compared to the original SARS-CoV-2 and its other variants. Nonetheless, the three sub-lineages of Omicron have been reported to be sufficiently distinct (Mahase 2022). It might be presumed that the Omicron has originated from an early lineage of wild-type SARS-CoV-2 and subsequently, its sub-lineages evolved independently in an unmonitored environment which was not traced in the reservoir population somewhere in the world until it became dominant with the steady weakening of other VOCs due to herd, vaccine-induced or hybrid immunity (Mallapaty 2022). Moreover, a study by Wang and Cheng (2022) analyzed the sequence of Omicron variants in South Africa and reported two subclades based on the sequence of spike genes.

Indeed, the impact of this variant had been reported to be high as incidence of Omicron infections increased exponentially in various affected regions such as South Africa, United Kingdom, and USA, which in a short-span overtook the delta variant, thus implying that the variant was highly transmissible (Sharma et al. 2022). Interestingly, the variant emerged when the vaccine breakthrough was already achieved (Rauf et al. 2022). As mentioned, spike proteins which are the target of vaccine immunity were found to be heavily mutated in the variant. Various comparative studies emerged suggesting new approaches to target viral-host molecular interactions and evolve preventive strategies (Isidoro et al 2022; Vardhan and Sahoo 2022a; 2022b).

Conclusions

The present study confirmed that the majority of mutations developed by Omicron variants occurred in the vaccine target gene (S gene) and most of the mutations in the receptor-binding domain occurred in the receptor-binding motif. Thus, we propose that the vaccine breakthrough has the potential to affect the genetic evolution of SARS-CoV-2.

Future perspectives

The inevitable stress caused by the COVID-19 disease globally, with the loss of human lives, post-COVID-19 mental and physical health issues, and disturbing social cohesion and economic failure has been an unprecedented incidence in recent times. Henceforth in a short time, enormous experimental research and meta-analyses have brought forward a bulk of studies that facilitated the determination of viral genomic landscape, sources and mode of viral transmission, pathogenesis, preventive strategies, and acquired immunity through vaccines. Our study is a significant piece of work that has the potential to be used as a reference in the future for the evolutionary genetics of SARS-CoV-2 and relate to any further risk of emerging variants and abeyant COVID-19 waves.

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Author contributions
The conception and design of the study was done by all authors, the second and third authors retrieved the data from NCBI. The first, second, and third authors analyzed the data while the fourth author arranged the results. The third and fourth authors wrote the manuscript whereas the rest of the authors revised it.

Conflict of interest
The authors do not report any financial or personal connections with other persons or organizations, which might negatively affect the contents of this publication and/or claim authorship rights to this publication.

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