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Highly Infectious, Less Pathogenic and Antibody Resistant Omicron Xbb.1, Xbb.1.5 and Xbb.1.5.1-Xbb.1.5.39 Subvariant Coronaviruses Do Not Produce Orf8 Protein Due To 8th Codon Gga=Tga Termination Codon Mutation

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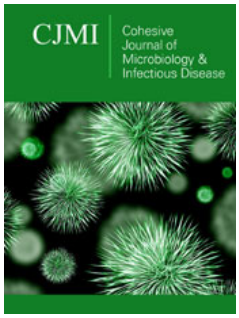

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Summary

RNA viruses are very mutation prone. We recently reported SARS-CoV-2 ORF8 gene CAA=TAA and AAA=TAA Termination Codon Mutations in B.1.1.7 variants with no production of viable ORF8 protein. We described here another GGA=TGA termination codon in the 8th codon of ORF8 gene located exclusively in XBB.1 (XBB.1.16 and XBB.1.22) and XBB.1.5 subvariants (XBB.1.5.1 to XBB.1.5.39) but not in XBB.2 variant or Alpha, Beta, Gamma, Delta and Omicron BA.1, BA.2, BA.4, BA.5, BF.7 and BQ.1 subvariants. However, G>T mutation at 27915 also created an alternate ATG codon but the protein product was short due to preceding TAG and TGA termination codons. The originally located following ATG codons were there but in alternate reading frames and ultimately no ORF8 protein was formed in XBB.1.5 subvariants which were spreading highly now over BA.2.75, BA.4.6, BA.5.2.1, BF.7 and BQ.1.1 subvariants. This is a vivid example of three termination codon mutations in the coronavirus ORF8 protein which was implicated as target for many human proteins regulating interferon production, chromosome instability, antibody production, patho genicity and virus clearance. The 30nt deletion in the 3'-UTR, including ^{24}LPP, and ^{145}Y deletions in Spike protein as well as N-protein ^{31}ERS and ORF1ab polyprotein ^{3675}SGF deletions made XBB.1.5 Omicron coronavirus weak and less pathogenic so that WHO declared coronaviruses as non-emergency pathogen.

Keywords: Termination codon mutants; ORF8 protein; COVID-19; Immunomodulation; Higher transmission; Lower pathogenicity

Introduction

The SARS-CoV-2 is the causative agent of the coronavirus disease 2019 with severe public health consequences and million deaths [1-9]. The novel SARS-CoV-2 shares nearly 96% similarity to the bat coronavirus isolate RaTG13, suggesting these animals are the likely natural reservoir of the virus [10,11]. Thus, different animal, birds and whale corona viruses were known since 2003 but human corona viruses were appeared in December 2019 at Wuhan province of China. So far million coronaviruses RNA were sequenced and divided into Alpha, Beta, Delta, Gamma etc. as well as Omicron variants with mutations, deletions and insertions [12-18]. There were many differences in pathogenic potential and immunogenicity among those VOCs. SARS-CoV-2 is a large positive-stranded RNA virus with round 30000 nucleotides genome (Figure1). It has structural proteins Membrane (M), Envelope (E), Nucleocapsid (N), Spike (S) coded from 3'-1/3 part of the virus independently, but RNA-dependent RNA polymerase was coded from nsp12 domain of ORF1ab polyprotein coded from 2/3 of the 5'-parts of the genome and such polyprotein was degraded into sixteen polypeptides (nsp1-nsp16). The nsp2 protein is RNA topoisomerase with new therapeutic target whereas Nsp3 and nsp5 are proteases that cleave polyprotein into sixteen polypeptides with diverse functions [18-23]. The nsp6, nsp7, nsp8, nsp9 and nsp10 were small accessory proteins involved in RNA polymerase replication complex. The nsp14 and nsp15 are ribonucleases

and nsp16 is 2'-O methyltransferase and nsp13 is RNA helicase and may have capping methyl transferase activity. The ORF3a, ORF6, ORF7a/7b, ORF8 and ORF10 small proteins also coded from 3' end of the genome and have roles in regulating cellular genes.

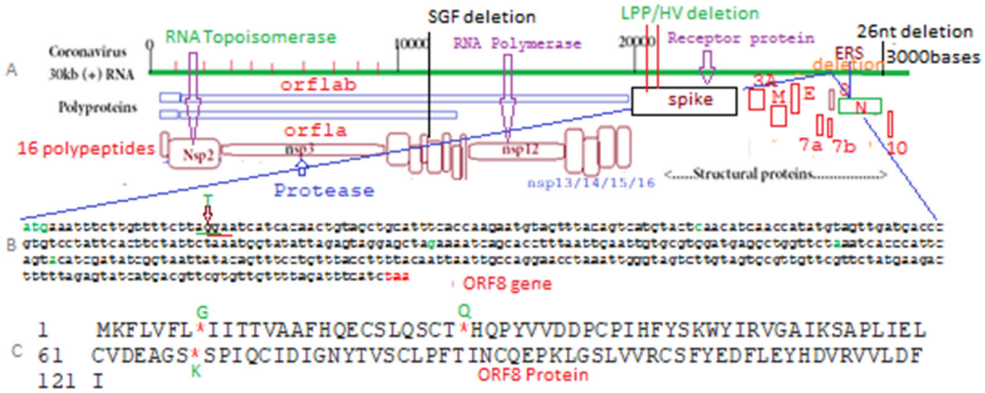

Figure 1: Structure of Omicron corona virus with polypeptides (A), nucleotide sequence of ORF8 gene (B) and ORF8 protein sequence with three termination codons detected (C).

In USA, Wuhan-D614G mutant first peak between March-August 2020, Alpha (B.1.1.7) 2nd peak with spike ^{69}HV deletion immune-escape mutant between January-June 2021 followed by 3rd peak of Delta (B.1.617.2, AY.X) with spike ^{157}FR deletion mutant between June to December 2021 [24]. Since last week of December 2022 4th peak of Omicron BA.1 variant (B.1.1.519) spread was evident followed by BA.2 variant spread since April 2022 with 29 mutations in the spike. From June-July 2022, Omicron BA.4/BA.5 variants were dominating worldwide followed BF.7, BQ.1, BQ.1.1, XBB.1 and XBB.1.5 subvariants recently (Figure 2).

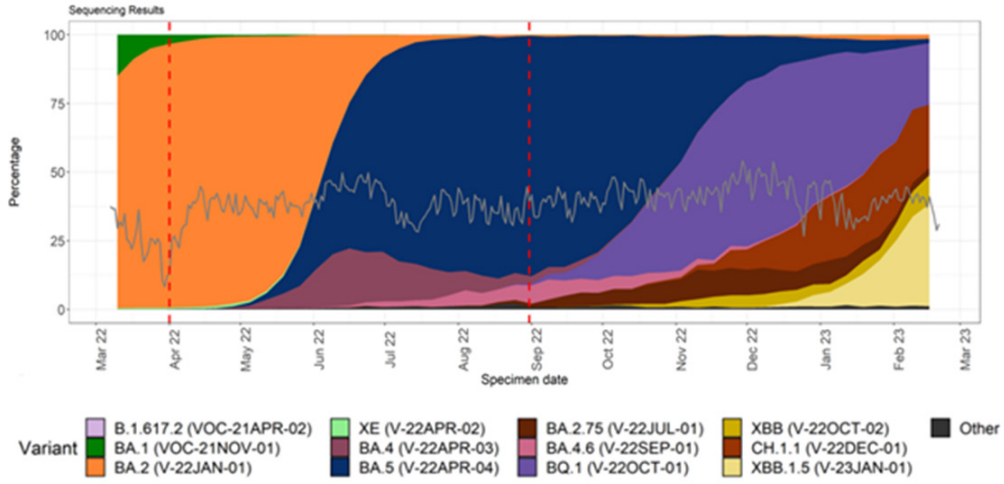

Figure 2: Distribution and sequencing data comparison for COVID-19 in the United Kingdom.

The Spike protein (1273AA) of COVID-19 had gone extensive mutations and deletions than large polyprotein ORF1ab (7096aa) particularly in Omicron lineages. The spike ^{24}LPP, ^{69}HV, ^{143}VYY, ^{157}FR, ^{212}L and ^{145}Y deletions were detected in different proportion whereas ^{215}EPE and ^{249}RWMD insertions were also reported in Omicron BA.1 variant and BQ.1 variant respectively [25-29]. Among the ORF1ab deletions, ^{141}KSF deletion in nsp1 domain was found only in omicron BA.4 subvariants and ^{3674}LSG deletion found in omicron BA.1 subvariant whereas ^{3675}SGF deletion in nsp6 domain was found in most Omicron (BA.1, BA.2, BA.4, BA.5) and Alpha (B.1.1.7) variants. Dominant point mutations D614G and N501Y were important for higher transmission whereas ~20 mutations in the RBD domain of Omicron were not found in deadly B.1.1.7, B.1.617.2 and AY.103 variants. The E484A, T478K, L452R and K417N/T mutations were very immune-modular and such mutant viruses were refractory to antibodies of patients.

More than a few dozen spike mutations were recently detected in XBB.1.5 and BQ.1.1.1 subvariants. However, Omicron variants were less pathogenic and usually did not require oxygen support and hospitalization unless co-morbidity [30]. Still pneumonia, chest pain, confusion, and headache with cough and cold, were different symptoms that affected over 650 million people worldwide. The gradual changes in different SARS-CoV-2 variants since 2019 was shown in Figure 3. Presently, infectivity of XBB.1.5 subvariant was dominating worldwide but very mild symptoms due to huge mutations in spike and deletion of 26nt 3'-UTR. However, the titer of such deleted coronaviruses was low and their spread may be due to better interaction with ACE-2 receptor and reinfections of patients [31-36].

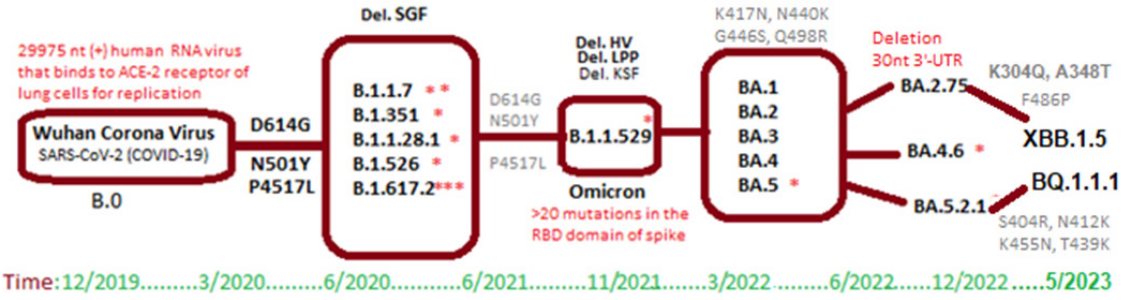

Figure 3: Conversion of Wuhan B.0 coronavirus into Omicron viruses like BQ.1.1.1 and XBB.1.5.3 types of sub subvariants.

Accumulating evidence suggested that small regulatory proteins (ORF3a, ORF7a, ORF8) of SARS-CoV-2 interacted highly with many cellular proteins. Preliminary 3-D graphics interactive studies indicated few dozen proteins like PVR, IRF3, ATF6, Beclin 1, FK506-binding protein 10, EDEM, vitronectin, OPJ94, Sec62163, VIP36, TRFT3 and PLAT etc interacted with ORF8 protein regulating protein folding, apoptosis and interferon production. Such process likely favours COVID-19 survival in host cells inhibiting immune control mechanisms [37]. Genetic analysis pointed a severe deletion in ORF8 (**Δ3**82) caused less severe corona infections likely due to low viral load with increased immune clearance.

However, in cell culture study with such deletion mutant contradicted the finding of lower viral load with no change of cellular transcriptional profile. The ORF8 protein also mediates immune evasion by downregulating MHC-I molecules like HLA-A2 [38]. The IgG domains similarity of ORF8 protein may be important to modulate host immune functions and chromatin structure. The C>T base change at 27972nt and another A>T base change at 28095nt created two termination codons (CAA=TAA and AAA=TAA) to produce 26AA and 67AA long ORF8 truncated proteins. Similar Blast-N search with mutated oligonucleotides detected many ORF8 mutants with distinct S24L, V32L, P38S, R52I, A65V, Y73C, L84S, K92E and V100L mutations with or without TAA termination mutations [39].

During our database search to characterize the XBB.1.5 lineages, we noticed no expression data for ORF8 protein in the genomic sequences. As we already known the two termination codons in ORF8 gene of Alpha variants, we searched the similar termination codon mutation in XBB-related variants which originated from BA.2.10 and BA.2.75 recombination and mutation [39-46]. We detected here a new termination codon mutation in ORF8 gene of XBB.1 lineage but not in XBB.2 lineage. Spike protein mutations and deletions had affected Omicron coronaviruses and for transformation of BA.2 to BA.2.75 required K147E, W152R, F157L, I210V, G257S, D339H, G446S, N460K and Q493R mutations in the spike protein and BA.2.75 was originated in 31.12.2021. Gene rearrangement and deletion in the 5'-UTR and 3'-UTR were also demonstrated in different SARS-CoV-2 variants. The article was deposited in Research Square preprint on 30th May 2023.

Methods

We searched PubMed to get an idea on published papers on ORF8 and searched SARS-CoV-2 NCBI database using BLAST-N and BLAST-X search methods. Multi-alignment of protein was done by Mult Alin software and multi-alignment of DNA by CLUSTAL-Omega software. 1st impression of ORF8 mutants was gained by Blast N searching of deletion boundary of 120nt sequence and analyzing the sequences with 90-100% similarities. Blast X search of ORF8 full length gene used to get mutant ORF8 proteins with or without termination codon. Then, the other ORF8 mutants were detected by Blast-N search of TAA mutant oligos as well as other oligos selected from point mutation boundaries [47,48]. The hairpin structure of ORF8 gene 222nt 5'-terminal sequence was done by Oligo Analyzer 3.1 software (Integrated DNA Technologies). The protein 3-D structure was determined by SWISS-Model software [49-61].

Result

In Table 1, we showed XBB.1.5. subvariants specific changes in the coronavirus proteins whereas K304Q and A411S two important mutations in the RBD of spike of XBB.1.5.3 sub-subvariant might be significant. Similarly, XBB.1.5.29 and XBB.1.5.30 had A348V and A348T mutations in the spike but roles of such mutations had to be tested yet. The never-the-less dominant common F486P spike mutation was implicated in antibody evasion and higher transmission in XBB.1.5 subvariants. We found six mutations (P2045S, T2137A, A3697V, T5941I, H5951Y, P6376S) in ORF1ab polyprotein of XBB.1.5.30 subvariant and four different mutations (S1188L, P2045L, P2110S, N6481K) in XBB.1.5.20 which also harboured A398V mutation in the N-protein (Table 1). Three spike mutations (P463S, E554K, P1162S) in XBB.1.5.8 might be significant and the penetration of such subvariant in the database was low. Similarly, four spike mutations (V83S, Y200C, V382L, T573I) in the XBB.1.22.1 subvariant was reported in Table 1. Interestingly, we detected total eight mutations in N-protein of XBB.1.5.sub-subvariants: R10Q in XBB.1.5.8, G25C in XBB.1.5.16, D81H in XBB.1.5.38, I131R in XBB.1.5.3, R195I in XBB.1.5.19, P279L in XBB.1.5.28, S327L in XBB.1.5.9 and A398V in XBB.1.5.20. Mutation rate was higher in recent coronavirus isolates (BQ.1.1.1 and XBB.1.5.1) and we also found four distinct mutations in ORF3A trans-activator protein: G49C in XBB.1.5.21, S92L in XBB.1.5.2, G172D in XBB.1.5.38 and H182Y in XBB.1.5.27 [61-68].

Table 1: Demonstration of major mutations in Omicron XBB.1.5.1-XBB.1.5.39 sub subvariants. XBB.1.5.26 (OQ783732) and XBB.1.5.31 (OQ758970) have no mutation in our search as stated here (ORF1ab, Spike, N, M, E, Orf3A) and also other small ORF proteins. No M protein was conserved but A63T mutation was found in all omicron variants and a D3G mutation in BA.1 variant and a I82T mutation in Iota variant were detected.

Acc. no.	Sub Subvariants	ORF1ab	Spike	N	E	Orf3A
OP699966	BA.2.75	S1221L	K444T			
OQ080316	XBB	G82D	V83A			
OQ244648	XBB.1	T2906I	G252V			
OQ681889	XBB.1.5	D3196G	F486P*			
OQ748396	XBB.1.5.1	K714R	T573I			
OQ748682	XBB.1.5.2	S3309P	K147I, T284I			S92L
OQ748578	XBB.1.5.3	S2048F	K304Q, A411S	I131R		
OQ759166	XBB.1.5.4		T883I			
OQ748550	XBB.1.5.5	T1822I, S2246l	T523A, K1181I			
OQ783432	XBB.1.5.6	S3158G				
OQ748657	XBB.1.5.7	V4649F				
OQ727842	XBB.1.5.8	L3116F	P463S, E554K, P1162S	R10Q		
OQ782510	XBB.1.5.9	R560C		S327L		
OQ748526	XBB.1.5.10	A6044V	F456L			
OQ748511	XBB.1.5.11	G401S				
OQ748664	XBB.1.5.12	S2285F	Q146K			
OQ748399	XBB.1.5.13		Q146K, V1104L			
OQ782367	XBB.1.5.14	R442C				
OQ748855	XBB.1.5.15	P5377S, S5674L	Q146K			
OQ782329	XBB.1.5.16	F6058L	E180V	G25C		
OQ782359	XBB.1.5.17				V62F	
OQ734082	XBB.1.5.18	T2300I, P4619L				
OQ782733	XBB.1.5.19	L3754F, A2128T, R4573C		R195I		
OQ748528	XBB.1.5.20	S1188L, P2045L, P2110S, N6481K,		A398V		
OQ748813	XBB.1.5.21	K322R				G49C
OQ783157	XBB.1.5.22	S167C	S247I			
OQ783778	XBB.1.5.23	N375S, P4220L, T5690A				
OQ802664	XBB.1.5.24	A2584V				
OQ783570	XBB.1.5.25	V665F, A1812D	K97T			
OQ748846	XBB.1.5.27	E1015G	K478R			H182Y
OQ748750	XBB.1.5.28		K478R	P279L		
OQ759251	XBB.1.5.29	L3808F, A6832V	A348V, G932S			
OQ748271	XBB.1.5.30	P2045S, T2137A, A3697V, T5941I, H5951Y, P6376S	A348T			
OQ748566	XBB.1.5.32	T2823I, Q3966R				
OQ783474	XBB.1.5.33	A138V, S5583L				
OQ748615	XBB.1.5.34	T1754I	T696S, C1253F			
OQ748647	XBB.1.5.35		S98F			
OQ782439	XBB.1.5.36	L293F	A475V, T547K			
OQ782618	XBB.1.5.37	E148G	K1045R			
OQ808416	XBB.1.5.38	T403I		D81H		G172D
OQ783588	XBB.1.5.39	S538L, M3684T, E4388G	Q52H, G1167V			

After the demarcation of XBB.1.5 sub-subvariants, we did multi-alignment analysis to pinpoint the genetic changes and if ORF8 termination codon mutation happened in all those sub-subvariants. For multi-alignment, we choose B.o, B.1.1.7, B.1.617.2, BF.7, BA.2.75 and BQ.1 as standard variant and subvariant whereas few XBB.1.5 sub-subvariants as experimental [69]. In truth, we

checked all XBB.1.5.1 to XBB.1.5.39 sub-subvariants for negative ORF8 expression in the database. Figure 4 stated that ORF8 gene mutation (GGA=TGA) in all XBB.1.5.1 sub-subvariants but not in XBB.2 as well as standard variants. We put standard CAA=TAA termination codon mutant (accession no. OP711844) and standard AAA=TAA termination codon mutant (accession no. OP683545) belonging to Alpha (B.1.1.7) variant for comparison and all three termination codon mutants did not produce viable ORF8 protein to interact and modulate host genes involved in interleukins expression and immune modulation [70-75].

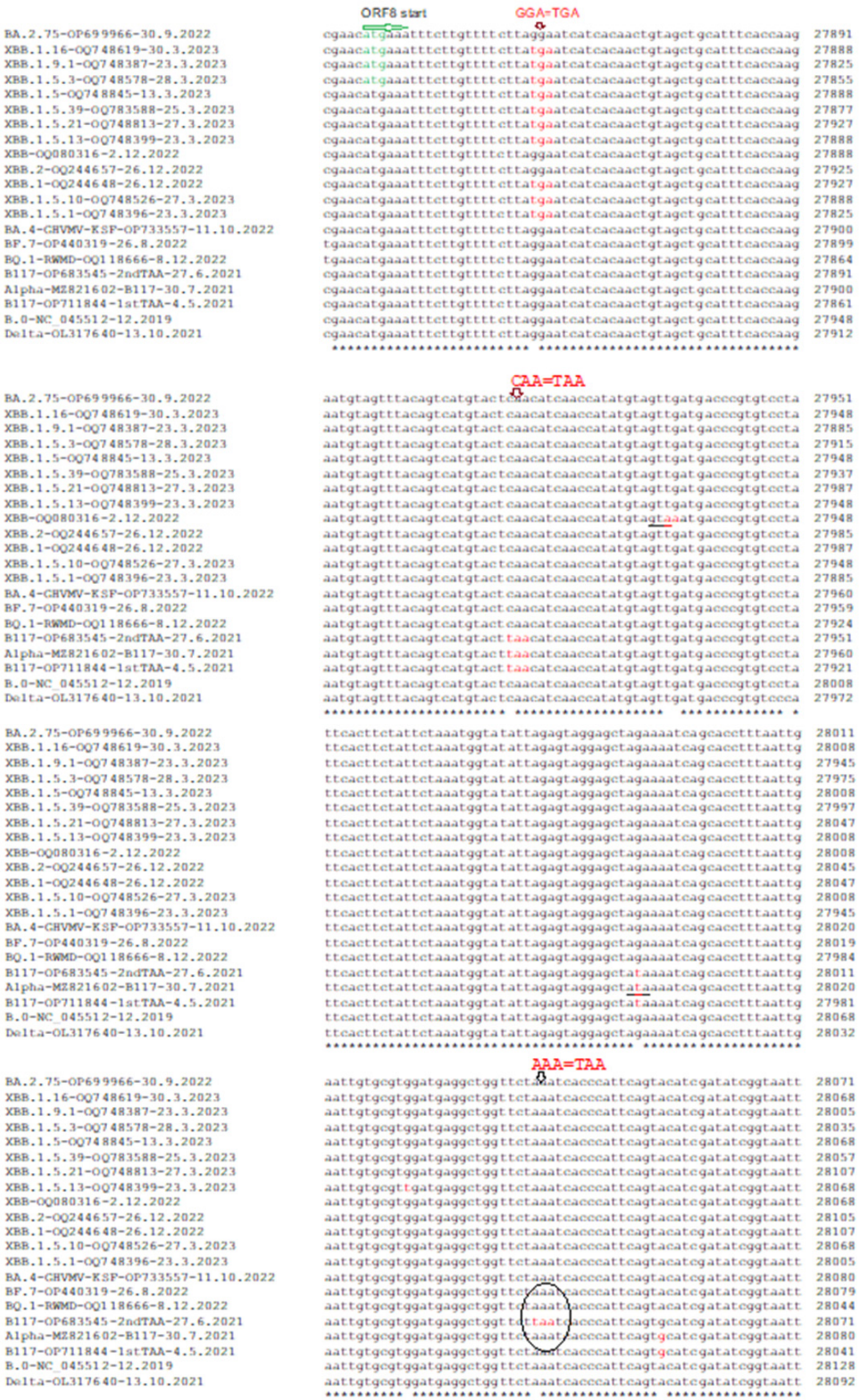

Figure 4: Localization of three termination codon mutants in ORF8 gene of SARS-CoV-2.

We checked the ^{3675}SGF dominant deletion in the nsp6 domain of ORF1ab polyprotein and except in Wuhan and Delta, all Omicron (BA.1/2/4/5, BF.7, BQ.1, XBB.1) and Alpha (B.1.1.7) lineages had such deletion (Figure 5). Similarly, we checked the nsp1 deletions

in XBB.1.5 and no such deletion was found (Figure 6). Astonishingly, we detected a B.1.1.7 alpha variant with GHVMV deletion which also had ORF8 termination codon mutation (accession no. OP711844). Next, we determined if there was any Spike deletion in XBB.1.5 subvariants. Spike ^{24}LPP deletion was found in BA.2 lineages including BA.2.75, XBB, XBB.1, XBB.1.5 (Figure 7) but no ^{69}HV deletion. On the contrary, both ^{24}LPP and ^{69}HV spike deletions were found in BQ.1, BF.7, BA.4/5 but not in Delta variant whereas only ^{69}HV deletion found in Alpha variant. We also demonstrated that BA.2.75, XBB, XBB.1 and XBB.1.5 subvariants had N501Y mutation (Figure 8) and D614G mutation both of which increased transmission to over 100% than Wuhan virus.

Figure 5: Demonstration of all omicrons including XBB, XBB.1, XBB.2 and XBB.1.5 subvariants as well as Alpha variant had SGF deletion in nsp6 protein but Delta and Wuhan.

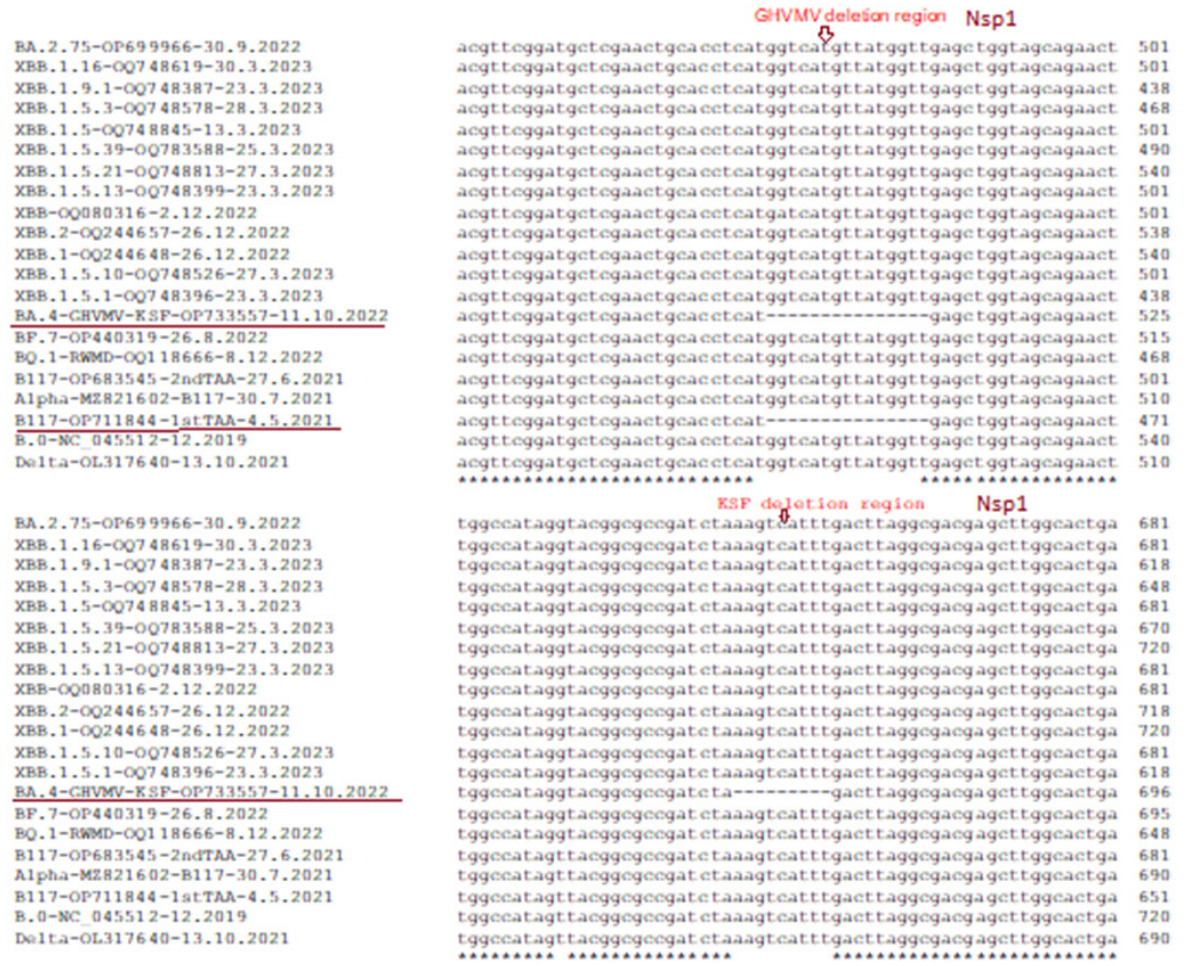

Figure 6: Demonstration of absence of GHVMV and KSF deletions in nsp1 protein of XBB.1.5 subvariants.

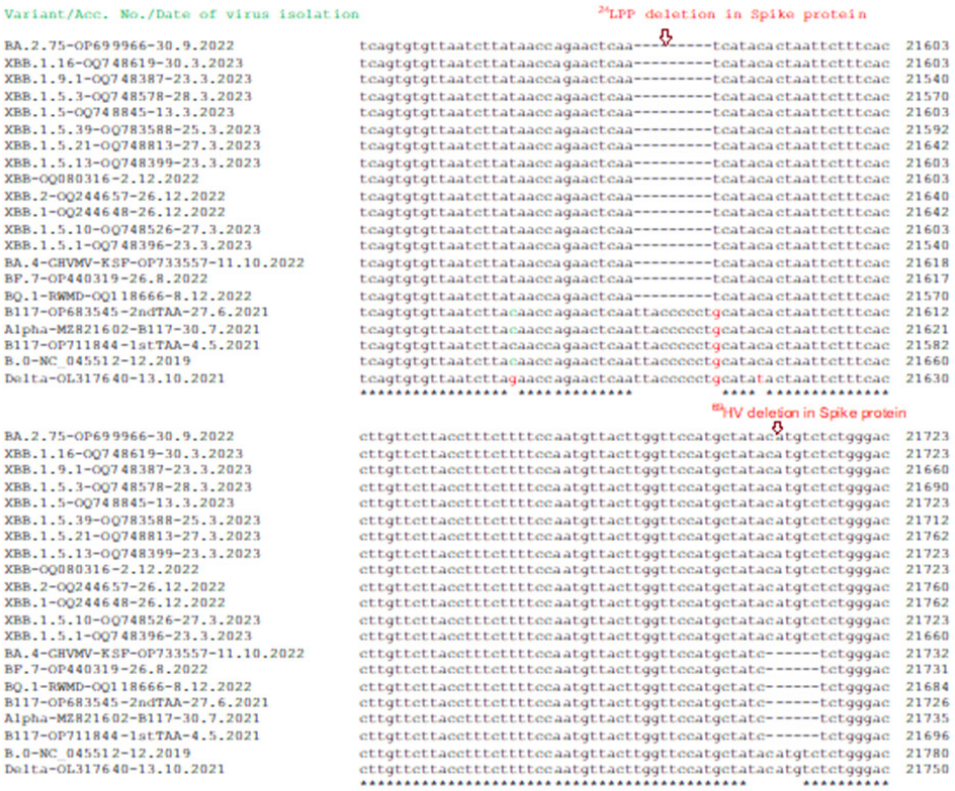

Figure 7: Demonstration of Spike deletion in XBB.1.5 subvariants. Spike [24]LPP deletion found in BA.2 lineages including BA.2.75, XBB, XBB.1, XBB.1.5 (A) but no spike [69]HV deletion (B). The [69]HV deletion was also found in Alpha, BQ.1, BF.7, BA.4 but not in Delta variant.

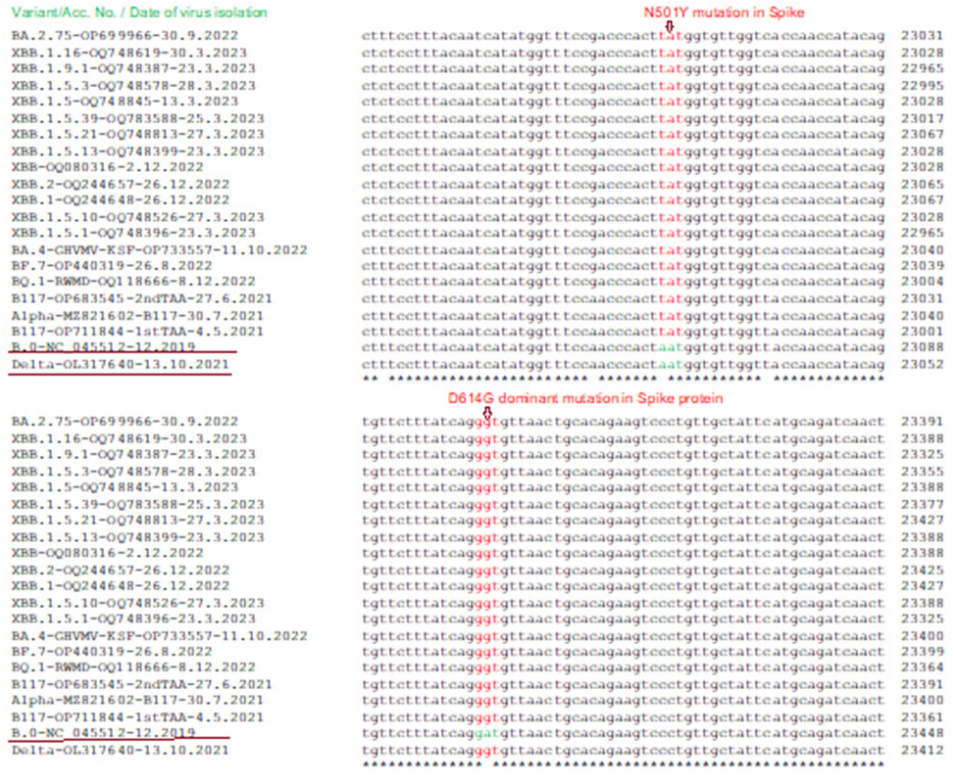

Figure 8: Multi-alignment demonstration that genomes of BA.2.75, XBB, XBB.1 and XBB.1.5 subvariants had N501Y mutation similar to B.1.1.7 (A) and D614G mutation similar to B.1.1.7, B.1.617.2 and others (B). Delta variant had D614G mutation but not N501Y mutation. Wuhan related early corona viruses (B.0, B.1, B.1.1) had no both D614G and N501Y mutations.

The deadly Delta varianN501YG mutation but no N501Y mutation whereas control 2019 Wuhan virus (B.0) had no D614G and N501Y both mutations. Previously, we showed that N-protein [31]ERS three amino acids deletion was prominent in Omicron variants starting from B.1.1.529 variants including BA.2, BA.2.9, BA.2.75 and BA.2.75.2 [76-78]. However, Wuhan, BA.1.1, B.1.1.1, BA.1.1.172, B.1.1.7, B.1.617.2 and other early coronavirus lineages had no such deletion. Figure 9 showed that BA.4, BA.5, BF.7, BQ.1, XBB.1 lineages also had such deletions which caused N-protein three amino acids shorter (216AAs). Most importantly, previously we reported 26nt deletion in 3'-UTR in many Omicron lineages (BA.2, BA.4, BA.5) which made coronavirus very replication defective. Interestingly, we did not find 26nt deletion in BA.1 early omicron lineages or old Wuhan, Alpha, Beta, Gamma, Delta coronavirus variants. In Figure 10, we showed that XBB.1.1 lineages retained such deletion including XBB.1, XBB.2, XBB.1.16, XBB.1.22.1, BF.7, BQ.1, BQ.1.1 and BQ.1.1.1 whereas spread of such variant likely increased than BA.2.75, BF.7 and BQ.1.1.1 subvariants.

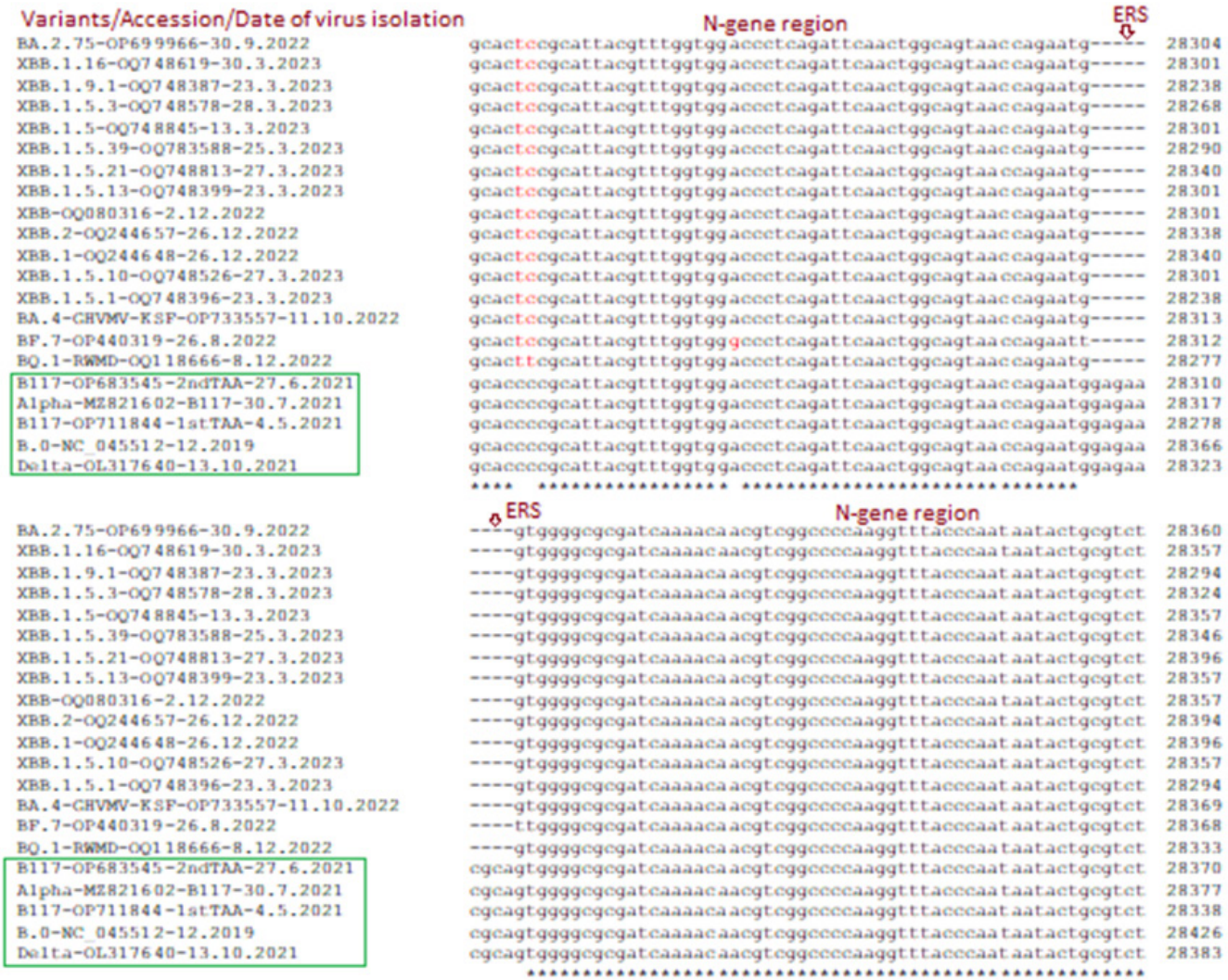

Figure 9: Multi-alignment demonstration of [31]ERS N-protein deletion in Omicron corona viruses but not in Alpha, Delta or Wuhan early coronaviruses. The other early VOCs like Beta, Gamma, Epsilon, Zeta also had no such deletion (data not shown).

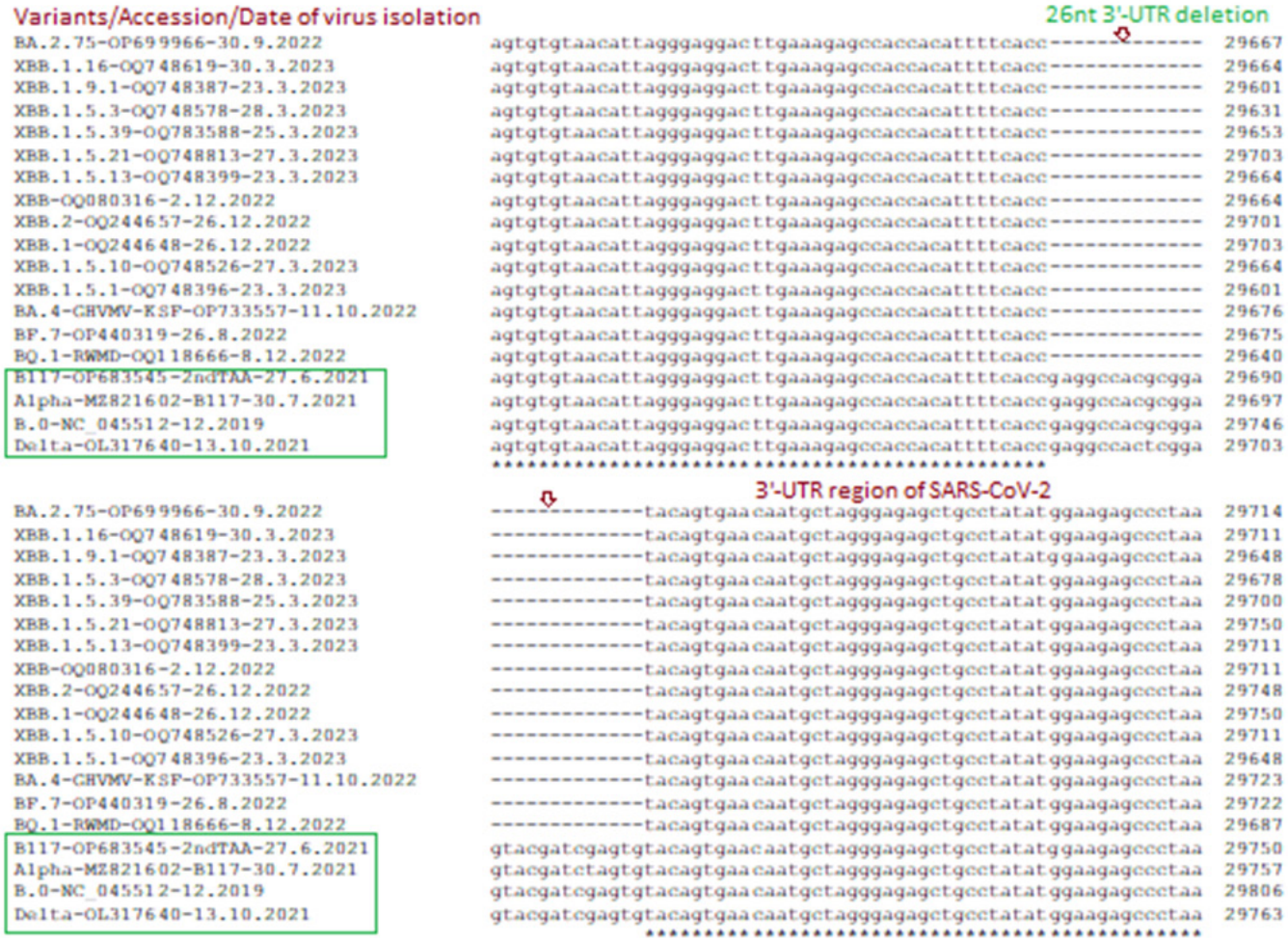

Figure 10: Multi-alignment demonstration of 26nt 3'-UTR deletion in most Omicron coronaviruses (BA.2.75, BA.4, BF.7, BQ.1, XBB.1.5.1) but such deletion was not reported in Alpha, Delta and Wuhan early coronaviruses.

We showed the XBB.1.5.1 to XBB.1.5.39 specific mutations in most COVID-19 proteins in Table 1. We showed the part of the multi-alignment of spike protein RBD domain in Figure 11. Figure 11 also demonstrated Omicron BA.2 specific mutations (green arrows), XBB.1.5 specific mutations (blue arrows), N501Y mutation (green circle) and XBB.1.5.1 sub subvariants mutations (grey circles). Further, A411S (XBB.1.3), P463S (XBB.1.5.8), F456l (XBB.1.5.10), A475V (XBB.1.5.36) including A520S (XBB.1.22) and S408W (XBB.1.22.1) sub subvariant specific mutations were demonstrated (Table 1). Those sub sub variant specific mutations will be utilized to make subvariant specific oligonucleotides for the detection of unknown COVID-19 variants as well as to demonstrate the database penetration of those sub subvariants by BLAST search.

Figure 11: Multi-alignment of spike protein RBD domain region to demonstrate Omicron BA.2 specific mutations (green arrows), XBB.1.5 specific mutations (blue arrows), N501Y mutation (green circle) and XBB.1.5.1 sub subvariants mutations (grey circles).

Discussion

Five VOCs of SARS-CoV-2 mainly caused million deaths worldwide and named as B.1.1.7 (U.K.), B.1.351 (South Africa), P.1 (Brazil), B.1.617.2 (India), and B.1.1.529 (Africa). The B.1.1.529 variant was named Omicron which diverged into different BA.1, BA.2, BA.3, BA.4 and BA.5 lineages worldwide during 2022 with mild infections. Viral transactivator proteins regulate cellular genes to modulate immunogenicity and pathogenicity. As for example, in HIV retrovirus mediated pathogenesis, TAT, NEF and REV small proteins modulate its own transcription as well as human cellular genes. Similarly, preliminary reports indicated that corona virus ORF8 protein (121 AAs) acts as histone mimics disrupting chromatic structure with many epigenetic changes and immune modulator functions. ORF8 protein could inhibit MHC-1 and IFN-beta functions due to some similarities to immunoglobulin domains and also modulate spike protein. A 382-nucleotide deletion (Δ382) in the ORF8 region of the corona virus genome causes weak virus load and weak pathogenicity (accession no.MT374101) [78-83]. Previously, we showed that C>T base change at 27972nt and another A>T base change at 28095nt created two termination codons (CAA=TAA and AAA=TAA) to produce 26AA and 67AA long ORF8 truncated proteins. Further, S24L, V32L, P38S, R52I, A65V, Y73C, L84S, K92E and V100L mutations in the ORF8 gene located with or without TAA termination mutations. Thus, ORF8 gene is very proven to mutation disrupting its function which controls immunogenicity and virus clearance.

Over time, the coronavirus has undergone mutations and deletions and different variants reported in different parts of the world with different time since December 2019. We found no GGA=TGA termination codon mutation in Alpha (B.1.1.7), Beta (B.1.351), Gamma (P.1/B.1.1.28.1), Delta (B.1.617.2), Kappa (B.1.617.1), Epsilon (B.1.427/B.1.429), Zeta (P.2); Eta (B.1.525), Iota (B.1.526) and B.1.1.298 (Mink Variant). Spike mutations are of major health concerns, as they reportedly exacerbate the infectious rate of the virus as in D614G and N50Y mutations. We found here major ORF8 truncated mutant in XBB.1 and XBB.1.5

lineages which were rapidly spreading now. Interestingly, RNA recombination generated few omicron lineages similar to XBB which was generated due to recombination between BA.1.10 and BA.2.75. Thus, XBB to XBB.1 and then XBB.1 to XBB.1.5 may be important but we characterized up to XBB.1.5.39 and in all we detected GGA=TGA termination codon mutation.

Thus, present outbreaks occurred with a corona virus with ORF8 gene product deficient. We also know that in most Omicron corona viruses, a 26nt deletion was prominent and we do not find any report that an insertion in that 3' UTR region has happened yet in Omicron lineages! Dominant mutation and deletion of small regulatory protein ORF7a limits viral suppression of the interferon response. We recently reported such deletions abolishing the production of both ORF7a and ORF7b proteins (see, accession no. OP711842). A WA1/BA.5 bivalent mRNA vaccine was inactive to kill BQ.1 and XBB.1 coronaviruses and titre against BQ and XBB subvariants were lower by 13-81fold and 66-155fold respectively as compared to Omicron BA.2 and BA.5 variants. Antiviral COVID-19 medications such as Paxlovid (Nirmatrelvir/Ritonsvir) and Remdesivir should still be effective against XBB.1.5 variant and both of these drugs will prevent virus replication as RdRp enzyme crucial function is not altered in XBB.1.5 [29,37].

The most sequences in SARS-CoV-2 database we analyzed were from USA (Howard, D. et al group) and we characterized some Bangladesh omicron data which confirmed that GGA=TGA termination codon was found in XBB.1 variant (accession no. OQ075411) but not in XBB variant (accession no. OQ075381) or XBB.2 variant (accession no. OQ075400). Interestingly, we also recently pinpointed round 400 ^{147}RWMD spike insertion mutants in US patients. We found three Northern Ireland (UK) based patients where such RWMD insertion was observed (accession numbers; OX527225, OX520545, OX486753). The 5'-UTR of SARS-CoV-2 forms five hairpin structures and nsp1 protein bound to first stem and loop structure helping transport of nsp1 to ribosome. Thus, 26nt 3'-UTR (29534-29870nt) S2M long stem-loop structure implicated in replication and other function in the coronavirus biology. The size of the genome has been changed from 29903nt to 29782nt. Thus, point mutation, deletion and insertion greatly affected coronavirus biology and WHO declared that recent isolates were no more so dangerous to cause death in COVID-19 infected individual [31,83].

HIV transactivator protein, TAT (71 AAs) binds to promoters of genes that are bound by the ETS1 transcription factor, the CBP histone acetyltransferase suggesting its role in regulating genes involved in T cell biology and immune response. Tat protein binds to TAR region where other cellular protein (puralpha) also docks. Further, Tat binds to breast cancer resistant protein implying its role in cancer pathogenesis. Nef protein (216 AAs) activates cellular Src and Tec tyrosine kinases after binding Nef-dimer to SH3-SH2 domain of tyrosine kinases activating transcription of HIV. The Rev protein (129 AAs) was suggested as HIV RNA transporter and such function was inhibited by nuclear factor 90, a dsRNA binding host protein whereas other host proteins XPO1 (CRM1) and RBM14 may be involved [9,69].

Strikingly, ORF8 protein 62-77 residues has Ig-like domain and binds CD16a (FcγRIIIA) with high affinity. Such interaction lowers the activity of CD16 at the surface of monocytes and NK cells reducing the capacity of PBMCs and monocytes to mediate antibody-dependent cellular cytotoxicity and humoral responses The crystal structure of SARS-CoV-2 ORF8 reveals a ~60-residue core polypeptides has potential similarity to ORF7a interface containing two dimerization interfaces and a covalent disulfide-linked dimer through an N-terminal sequence, while a separate noncovalent interface is formed by a carboxy-terminal SARS-CoV-2-specific sequence, $_{73}$YIDI$_{76}$. We found many deletions in the ORF7a protein also and thus both should be transactivator proteins absence of which markedly lower the pathogenicity. Unfortunately, although ORF7a and ORF8 NH2-terminal 40 AAs has 25% scattered similarities, no such minor similarity with TAT protein or other HIV transactivator proteins like Nef and Rev (data not shown). Never-the-less, the roles of ORF8 protein in COVID-19 replication and pathogenesis were emerging and absence of ORF8 protein due to termination codon mutation was now well established in XBB.1.5 and B.1.1.7 variants. Thus, translational suppression of ORF8 protein is a therapeutic method to control Corona virus pathogenicity [29,37].

Conclusion

Presently circulating XBB.1.5 sub subvariants (XBB.1.5.1-XBB.1.5.39, XBB.1.16, XBB.1.22, XBB.1.9.1, XBB.1.9.2 etc) have GGA=TGA termination codon mutation in the ORF8 gene and such ORF8-deficent coronaviruses are less pathogenic. The 26nt deletion in the 3'-UTR, ^{24}LPP and ^{140}Y deletions in spike and ^{31}ERS deletion in N-protein may be significant in such a process.

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