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Higher Omicron JN.1 and BA.2.86.1 Coronavirus Transmission Due to Unique ^{17}MPLF Spike Insertion Compensating ^{24}LPP, ^{69}HV, ^{145}Y, ^{211}N and ^{483}V Deletions in the Spike

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Abstract

Mutation, deletion and insertion in the spike likely select RBD in a favorable 3-D structure to interact with ACE-2 receptor of human cells for coronavirus entry. Our goal is to characterize the newly spreading JN.1 subvariant and related omicron coronaviruses. BLASTP search found a ^{17}MPLF four amino acid insertion in omicron BA.2.86 and BA.2.86.1 subvariants and its precedent JN.1 subvariant which had unique L452S (L455 in Wuhan) spike mutation. The JN.1 variant also contained ^{2375}SGF deletion in ORF1ab, ^{24}LPP, ^{69}HV, ^{145}Y, ^{211}N (^{208}N in BA.2) and ^{483}V (^{480}V in BA.2) deletions in the spike, ^{31}ERS deletion in N-protein and 26nt deletion in 3'-UTR (NC_045512.2). Many unique JN.1 spike mutations (242N=H249N, 261D=A268D, 352T=K360T, 400K=R407K, 442H=P449H, 449W=L456W, 474K=N485K, 480K=A488K and 566V=A574V) might be also important. The BLASTN search with insertion oligo found over 4984 JN.1 related sequences (10.1.2024) in the NCBI Database and were well distributed in America, Europe and Japan. Although, JN.1 acquired the 69HV deletion lately but did not generated from BA.4 or BA.5 lineages and it was solely generated from BA.2.86 variant. Swiss-Model detected a wing structure with basic amino acid in the middle of tripartite spike of JN.1 and important ACE-2 first interacting surface amino acids were changed. The small M protein of JN.1 had D3H, A63T and A104V mutations but Swiss Model showed no gross change in 3-D structure. Further, four JN.1 specific ORF1ab polyprotein mutations were detected: T170I mutation in nsp1 as well as D1600N, K1973R mutations in nsp3 protease and R3821K mutation in nsp6. Astonishingly, after a long journey of XBB.1.5.1 to XBB.1.5.100 subvariants spread, a sudden five amino acids deletion (^{176}EGKEG and ^{180}EGKQG in Wuhan) in the spike of XBB.1.5.103 subvariant was found. The ORF8 immune-regulatory protein expression was abolished in all XBB.1 subvariants including XBB.1.5.103 and XBB.1.16.23 as expected due to termination codon mutations (AAA=TAA, CAA=TAA, GGA=TGA). But such ORF8 gene mutation (GGA=TGA) was also found in ongoing dominated JD.1.1, FL.1.5.1, HV.1 and EG.5.1.1 subvariants, derived from XBB.1 lineage. The FL.1.5.1 variant also has ^{82}GHV deletion instead ^{82}GHVMV in the nsp1 protein as well as a 27nt deletion (27887 5'-aac gaa cat gaa att tct tgt ttt ctt-3') in the ORF7a gene. Partial or no expression of nsp1, ORF7a and ORF8 regulatory proteins cause coronavirus more immune deficient and less pathogenic. The spread of JN.1 has sent an alarm among health officials worldwide. It is worthwhile to see if BA.2.86.1 and JN.1 coronavirus goes nsp1 or OR7a deletion and ORF8 termination codon mutation with time lowering pathogenicity.

Keywords: JN.1 Omicron Coronavirus, ^{17}MPLF Spike Insertion, BA.2.86.1 Subvariant, XBB.1.5.103 Subvariant, ^{180}EGKQG Spike Deletion

1. Introduction

So far round 7000000 people have died between 2019-2024 due to deadly Delta, Alpha and to some extent Beta and Gamma lineages of SARS-CoV-2 [1, 2]. However, Mutation, Deletion and Insertion in omicron coronaviruses have reduced the viral tire and disease severity [3]. Still, NCBI Virus Database is depositing omicron COVID-19 sequences every day demonstrating that COVID-19 era has not been ended yet. Coronaviruses (family, Coronaviridae) are enveloped viruses with a largest positive sense, single-stranded RNA genome of 30kb. On genetic and antigenic criteria, CoVs have been organised into three groups: α-CoVs, β-CoVs, and γ-CoVs [4]. Coronaviruses (2003-2024) primarily infect birds, mammals and human, causing a variety of lethal respiratory diseases resembling the common cold, to lower respiratory tract infections such as bronchitis, pneumonia, and even severe acute respiratory syndrome (SARS) [5, 6]. The Covid-19 (SARS-CoV-2) preferably infects human lung cells. The virus enters cells through ACE2 receptor-mediated endocytosis. The receptor ACE2, was abundant in lungs AT2 alveolar epithelial cell as well as cells in the kidney, nose, heart and blood vessels [7, 8].

SARS-CoV-2 had structural proteins (S, M, N, E) at the 3'- end and two polyproteins, ORF1ab, ORF1a (7096aa and 4405aa)

were coded in same reading frame from 5' of 2/3 of the genome which were degraded into sixteen (nsp1-nsp16) non-structural proteins [9]. The ORF1ab generates sixteen functional peptides proteolytically: Nsp1(1-180aa), Nsp2(181-818aa), Nsp3(819-2763aa), Nsp4(2764-3263aa), Nsp5(3264-3569aa), Nsp6(3570-3859aa), Nsp7(3860-3942aa), Nsp8(3943-4140aa), Nsp9(4141-4253aa), Nsp10(4265—4392aa), Nsp11(4393-4400aa), Nsp12(4401-5324aa), Nsp13(5325-5925aa), Nsp14(5926-6462aa), Nsp15(6453-6798aa) and Nsp16(6799-7096aa) [10]. The functions of the most sixteen enzymes were reported: Trans-activator (nsp1), RNA topoisomerase (nsp2), two proteases (nsp3 and nsp5), RNA-dependent RNA polymerase (nsp12), RNA helicase and capping methyltransferase (nsp13), nucleases (nsp14 and nsp15) and methyl transferases (nsp16) and accessory proteins (nsp7, nsp8, nsp9 and nsp10) [11-18]. The ORF1ab protein was reported as 7096-7092 AAs in different variants. The spike protein (1273 AAs) is a trimeric class 1 transmembrane glycoprotein and it's RBD domain (335-515 aa) acts as receptor binding domain to bind ACE-2 receptor of host lung cells for virus entry [19]. The spike protein 1-13 AA acts as signal peptide and the S1 subunit (14-685 AAs) containing RBD domain and S2 subunit (686 to 1273 AAs) are important due to furine cleavage. Spike protein also contains fusion contact peptide (788-806 AA) as well as two hepta-peptide (HPPHCPC) repeats at 1163 and 1213 positions [20]. The ORF3a, ORF6, ORF7a, ORF7b, ORF8, ORF9 and ORF10 are small regulatory proteins also coded from 3' end of the SARS-CoV-2 genome and have roles in regulating cellular genes [21-26].

Spike protein in COVID-19 greatly varied in different lineages. Spike protein is 1273aa in Wuhan but it has changed in Alpha lineage to 1270 AA due to deletions of ^{69}HV and ^{145}Y positions; in Delta variant spike is 1271aa due to ^{156}FR deletion only. Spike protein of omicron BA.1 variant is 1270aa due to ^{69}HV, ^{143}VYY and ^{212}L deletions as well as ^{215}EPE three amino acid insertion [27-30]. The Spike protein of omicron BA.4 and BA.5 corona viruses is 1268 AA due to deletions of ^{24}LPP and ^{69}HV. Spike protein of omicron BA.2 has 1270 AAs due to ^{24}LPP deletion but no ^{69}HV and ^{143}VYY deletions or ^{215}EPE insertion [3, 10]. The ^{69}HV spike deletion found in B.1.1.7 first but also acquired in omicron BA.1, BA.4 and BA.5 variants but not in omicron BA.2 variant. Among the other structural proteins N-protein (419 AAs) binds to leader RNA of replicating corona virus and also regulates host-pathogen interactions. Three AAs deletion (^{31}ERS) was found in N-protein (416 AAs) of all omicron corona viruses (BA.1/2/4/5) and was very useful for diagnostics. Three amino acid deletions (^{3675}SGF) were found in ORF1ab protein (nsp6 protein region) of Alpha and Omicron BA.2 and BA.5 (ORF1ab=7093) but at the same region ^{3674}LSG deletion as well as extra ^{2083}S deletion were found in omicron BA.1 corona virus (ORF1ab=7092 AA). Interestingly, no ORF1ab deletions in notorious Delta variant (ORF1ab=7096 AAs). Whereas, a three amino acids ^{141}KSF deletion in the nsp1 protein was found in omicron BA.4 variant only (ORF1ab=7090 AAs) and such change was utilized to detect BA.4 omicron variant [31, 32]. Further, D614G mutation was detected in all variants since March, 2021 ongoing and such mutation increased 80% higher transmission [33]. The N501Y spike mutation was appeared first in Alpha (B.1.1.7) variant but also located in omicron variants BA.1, BA.4, and BA.5 followed by recent BF.1, BQ.1, XBB.1, JN.1, HV.1 and FL.1 lineages and sub subvariants [34]. The N501Y mutation increased transmission by more than 20-50% as well as immune escape. The P4715L ORF1ab mutation at RdRP was found in all variants since March, 2020 similar to D614G mutation. As N501Y and D614G both mutations appeared in omicron BA.1, BA.4 and BA.5, such viruses gained more spread than alpha, delta as well as BA.2 corona viruses but gave mild pathogenicity due to 25 more mutations in the spike and 26nt 3'-LTR deletion [20, 26]. The BA.2.12.1 lineage gained L452Q mutation facilitating immune-escape and high infectivity. However, BA.2.75.2 sub-lineage carrying additional R346T, F486S and D1199N mutations in the spike protein and gained more mutations and rearrangement to produce XBB variant. The BA.4.6 variant with R346T and N658S mutations was one of the dominating variants [26]. Delta variant contained L452R immune-escape mutation which also gained by omicron BA.1 as well as BA.4 and BA.5 variants. The BA.4 and BA.5 variants also gained important F486V mutation to produce immune escape similar to L452R mutation. A single mutation (E484K) present in SARS-CoV-2 Beta (B.1.351) and Gamma (P.1) lineages may alter the neutralising activity of convalescent (infected with previous COVID-19 strains) and post-vaccination polyclonal serum. Delta (B.1.617.2) variant has no N501Y mutation and spread of such viruses were diminished since 2021 due to vaccination. The P681R mutation in Delta variant was replaced by P681H in all omicron variants (BA.1/2/4/5). The Gamma (P.1 or B.1.1.28.1) variant had 3675LSG deletion in the ORF1ab protein (nsp6 region) and such information was used to make DelORF1ab3675-P1 oligonucleotide specific for P.1 variant [35].

Data analysis detected one amino acid deletion (^{140}Y=TAT; ^{145}Y in B.0) in spike in BA.4.6, BQ.1.5, BQ.1.8, BQ.1.14, BQ.1.1.5, XBB.1 as well as related BU.1, BW.1, CP.1 and CQ.1 subvariants. But Y140 deletion was not detected in BA.2.75, BF.7, XBD, BQ.1, BQ.1.1, BQ.1.2, BQ.1.10, BQ.1.12, BQ.1.16, BQ.1.19, BQ.1.22, BQ.1.1.1, BQ.1.1.4, BQ.1.1.12 and related BK.1, BN.1, BM.1.1.1, BU.1, CA.1, CH.1.1 subvariants [36]. Spike protein mutations were rampant in omicron coronaviruses. The 91% nucleotides changes in spike protein of BQ.1 variant was resulted in AA changes whereas only 52% nucleotides changes resulted in AAs changes in ORF1ab polyprotein [37]. The spike N460K and K444T mutations in BQ.1 may be important driving force for immune-escape similar to F486S and N480K mutations in BA.2.75 subvariant and related XBB.1 subvariant. Further, the R346T mutation as found in BA.4.6 and BF.7 was regained in the spike of BQ.1.1 and BA.2.75.2 or related recent lineages CH.1, BM.1 and CA.1 to enhance immune escape and infectivity (>80%). The L452R and F486V spike mutations were main drivers of Omicron BA.2 conversion to BA.4 and BA.5 in presence of ^{69}HV deletion and 30nt deletion in 3'-UTR. Whereas ^{24}LPP spike deletion and ^{3675}SGF ORF1ab protein deletion were found in all Omicron viruses including BQ.1, XBB.1, HV.1 and JN.1.

In USA, Wuhan D614G first peak occurred between March-

August, 2020, Alpha (B.1.1.7) 2nd peak between January-June, 2021 followed by 3rd peak of Delta (B.1.617.2, AY.X) between June to December, 2021 [27]. 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 in April, 2022. From June-July, 2022, omicron BA.4 and BA.5 variants are increasing worldwide [28]. From November 2022 we found the spread of BQ.1 subvariants and from March 2023, we find spread of XBB.1 lineage. In August 2023, we detected more than 448 249RWMD spike insertion sequences spread in the USA and Europe. But such lineage was not prominent at the end of 2023 [37]. Where as in December 2023 new sub-subvariants like XBB.1.16, XBB.1.5, EG.5.1, HV.1, FL.1.5, JN.1 and JD.1.1 were the major omicron coronaviruses sequences were deposited in the NCBI virus database [29, 30]. Similarly, we detected ORF7a protein deletions in BQ.1 variant and ORF8 termination codon mutations in XBB.1 lineage as well as ^{141}KSF, ^{82}GHV and ^{82}GHVMV deletions in the nsp1 protein of omicron BA.4 variant [22, 24, 26]. All these changes caused recent corona viruses weak and non-life threatening with very low viral titer as compared to Alpha and Delta variants [36, 37]. In this communication, we detected a continuous deletion and insertion in the spike of new corona virus variants like BA.2.86.1, JN.1, XBB.1.5.103 and HV.1 [38, 39]. We discussed the potential issues of such changes for its pathogenicity and worldwide transmission as also reported in many newspapers recently.

2. Materials and Methods

The BLAST search was done using web portal www.ncbi.nlm.nih.gov/blast and retrive of covid-19 and other corona viruses cDNA sequences were done using web portal www.ncbi.nlm.nih.gov/nucleotide or protein. NCBI Primer Design Software was used for primer selection and Oligoanalyzer 3.2 software was used to analyse primer dimmer and hairpin structure. MultAlin Software and CLUSTAL Omega Software were used to multiple align of protein sequences and NCBI BLAST seq-2 analysis portal (www.ncbi.nlm.nih.gov/blast) used to analyse homology between two sequences. NCBI PubMed portal (www.ncbi.nlm.nih.gov/pubmed) used to retrieve references and papers. NEB DNA cutter software was used to restriction map the DNA fragment. Swiss-Model software was used for 3-D structure prediction (http://swissmodel.expasy.org/interactive/Jpr JmW).

3. Result

Multi-alignment of spike proteins from highly spreading randomly selected six subvariants demonstrated a ^{17}MPLF four amino acids insertion at 16 position of spike in omicron JN.1 variant ahead of ^{24}LPP deletion. But such insertion was not detected in highly spreading HV.1, EG.5.1.1, JD.1.1, XBB.1.16 and XBB.1.5 new subvariants that were highly appeared in the NCBI Virus Database during December, 2023 (NIH, USA). Similarly, a ^{180}EGKQG deletion (^{176}EGKEG in Omicron; ^{176}VGKEG in XBB.1.6.23) in the 176 position of spike of XBB.1.5.103 subvariant (see, multi-alignment data, figure-1, lower portion) was also found. The genomes of such variants were multi-aligned and the data was presented in figure-2. We detected the ^{17}MPLF insertion (ATGCCGCTGTTT) in JN.1 and ^{176}EGKEG deletion (GAA GGA AAA GAG GGT) in XBB.1.5.103 in the genome wide search. Further, ^{24}LPP, ^{69}HV, ^{145}Y, ^{215}N and ^{480}V deletions in the spike of JN.1 variant was also found. Thus, few extra deletions in the spike were compensated by the insertion of ^{17}MPLF sequence in JN.1 and might be responsible for higher transmission.

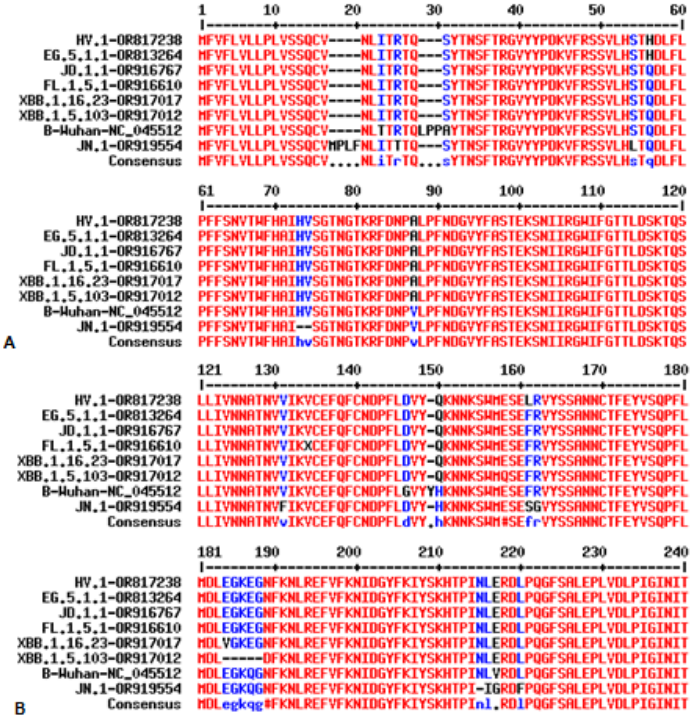

Figure 1: Demonstration of new spike protein insertion (A) and deletion (B) mutants of omicron JN.1 variant and omicron XBB.1.5.103 subvariant respectively. The data compared with standard Wuhan coronavirus (accession no. NC_045512.2)

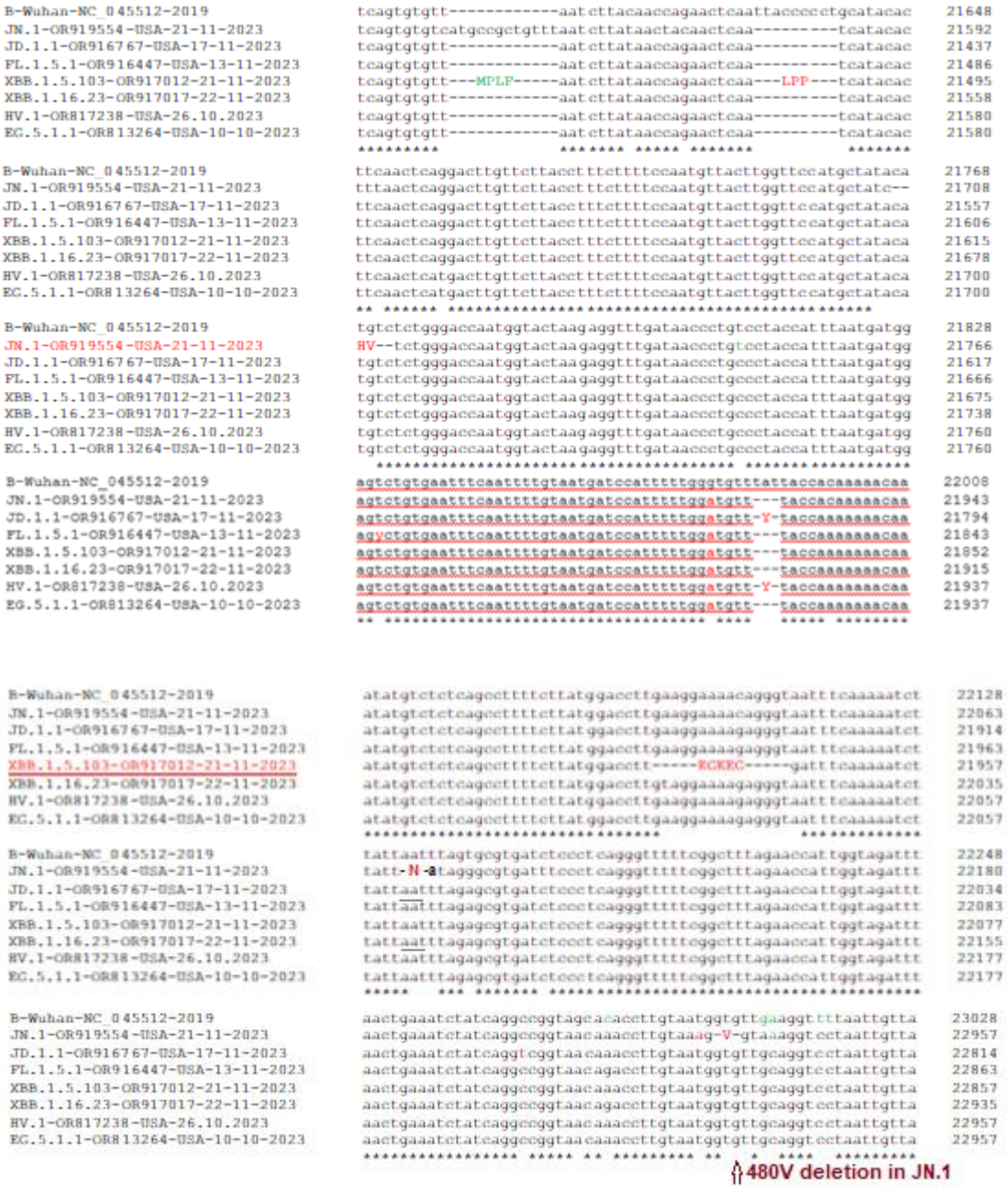

Figure 2: Multi-alignment of genomes of new corona virus variants with deletion and insertion in the spike as shown in figure-1. We confirmed the all deletions ([24]LPP, [69]HV, [145]Y, [211]N and [483]V) in the spike during genome wide search. The [17] MPLF insertion was found in the spike of omicron JN.1 subvariant only.

The previously reported the XBB.1.5 lineage GGA=TGA termination codon mutation of ORF8 gene was found in XBB.1.5.103 and XBB.1.16.23 as expected. But such mutation was also appeared in FL.1.5.1, HV.1, EG.5.1.1 and JD.1.1. Table-1 showed the main lineage (XBB.1) from where these new subvariant were derived. This data clearly demonstrated their common source lineage was XBB.1 and too many new names in the omicron sub-lineages spoiled the game. Thus, recent omicron coronavirus nomenclature was exacerbated without reason. As for example, FL.1.5.1 and EG.5 are essentially same and similarly JN.1 and BA.2.86.1 are very same with one spike mutation difference. We also found that FL.1.5.1 subvariant had a deletion in the ORF7b-ORF8 boundary that completely removed the ATG codon of ORF8 gene (figure-3). In figure-4, we showed the few new amino acid changes at the RBD of spike including N501Y mutation in those new variants of XBB.1 descendant.

New name	Source	Old name	Accession number
JN.1	BA.2.86	BA.2.86 + L455S Spike	OR919554
XBB.1.5.103	XBB.1.5	XBB.1.5 + E150Q, N181D, F452L+^{176}EGKEG Del Spike	OR917012
XBB.1.16.23	XBB.1.16	XBB.1.16 + P486S	OR917017
XBB.1.16	XBB.1	XBB.1 + Y145 Del + 15 spike mutations	OR871702
XBB.1.5	XBB.1	XBB.1 + F486P Spike	OQ816854
XBB.1	XBB	XBB + G252V Spike	OR741908
XBB	BJ.1+ BM.1.1.1	BJ.1 =BA.2.10.1 BM.1.1.1=BA.2.75.3.1.1.1	OQ080316 OQ166287
JD.1.1	XBB.1.5.102	XBB.1.5.102.1.1.1	OR916767
FL.1.5.1	XBB.1.9.1	XBB.1.9.1.1.5.1	OR916447
HV.1	EG.5 or XBB.1.9.2.5	XBB.1.9.2.5.1.6.1	OR817238
EG.5.1.1	XBB.1.9.2	XBB.1.9.2.5.1.1	OR813264
BA.1	B.1.1.529	B.1.1.529.1	OM542730
BN.1	BA.2.75	B.1.1.529.2.75.1	OQ114394
BF.7	BA.5.2	B.1.1.529.5.2.1.7	OP440319
BQ.1	BA.5.3	B.1.1.529.5.3.1.1.1.1.1	OQ392531

Table 1: Correct Nomenclature of New Omicron Variants with Recent Higher Transmission

Figure 3: Demonstration of ORF8 gene termination codon mutation at 8th codon in XBB.1.5.103 as well as XBB.1.1.6.23, JD.1.1, HV.1, EG5.1.1 as previously described in all XBB.1.5 variants (Chakraborty AK. Cohesive J Microbiol Infect Dis. 2023; 6(5): CJMI.000646).

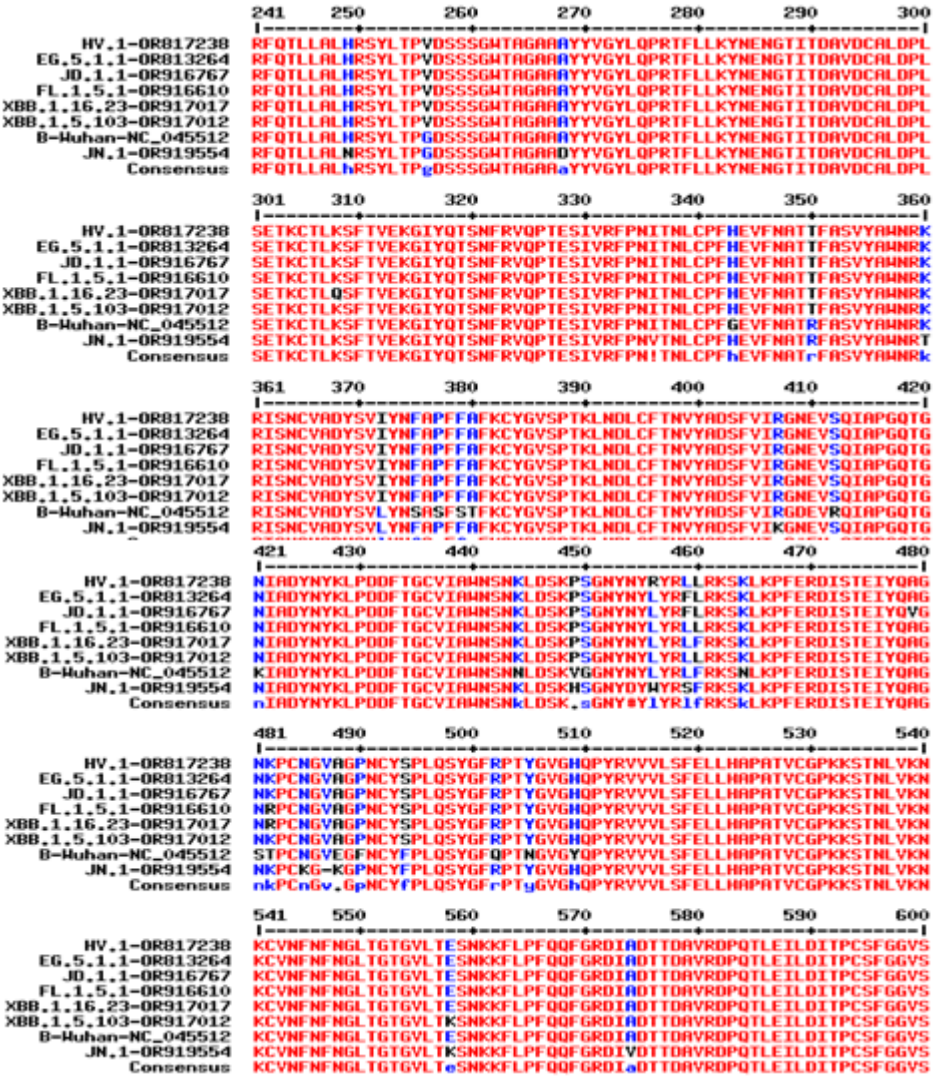

Figure 4: Demonstration of some unique new amino acid mutations (442H=P449H, 449W=L456W, 474K=N485K,480K= A488K etc) in the Receptor Binding Domain (RBD) of JN.1 variant.

Then, we want to see the database penetration of insertion and deletion spike mutants as discussed above. For that we made insertion-oligo and deletion-oligo at the insertion and deletion boundaries. The JN.1-MPLF-insertion-oligo is: 5'-gtc tct agt cag tgt gtc atg ccg ctg ttt aat ctt ata act aca act caa-3'. The XBB.1.5.103-EGKEG-Deletion oligo: 5'-cag cct ttt ctt atg gac ctt gat ttc aaa aat ctt agg gaa-3'. The BLASTN search detected about >3895 sequences for insertion-oligo for JN.1 variant on dated 1.1.2024 (figure-5) and >75 sequences for deletion oligo for deletion oligo for XBB.1.5.103 variant (figure-6). It was found that JN.1 sequences are more recent (November-December, 2023) than XBB.1.5.103 sequences still JN.1 sequences are progressing fast in the America and Europe (figure-7 and figure-8). However, last minute NCBI Virus Database search (10.1.2024) with JN.1-MPLF-oligo indicated more than 4984 JN.1 and BA.2.86.1 sequences which was 27% increase than we detected in 9 days ago. The Indian newspaper (The times of India, 20.12.2023, 31.12.2023, 9.1.2024) reported the spread (~500 infections and three deaths with co-morbilities) of such JN.1 coronavirus in India in the province of Goa, Tamil Nadu, Karnataka, Maharashtra and West Bengal. The British Newspaper indicated every twenty-four person, one JN.1 affected person could be found in England and Scotland (www.bloomberg.com 21st December, 2023). The USA was severely affected too as described by CDC (www.cdc.gov/respiratory-virus-updates 22th December, 2023).

Description	Scientific Name	Max Score	Total Score	Query Cover	E value	Per. Ident	Acc. Len	Accession
Severe acute respiratory syndrome coronavirus 2 isolate SARS-CoV-2/human/USA/2368605/2023 ORF1ab pol...	Severe acute res...	93.5	93.5	100%	2e-15	100.00%	29649	OR906019.1
Severe acute respiratory syndrome coronavirus 2 isolate SARS-CoV-2/human/USA/2368603/2023 ORF1ab pol...	Severe acute res...	93.5	93.5	100%	2e-15	100.00%	29649	OR906017.1
Severe acute respiratory syndrome coronavirus 2 isolate SARS-CoV-2/human/USA/2368366/2023 ORF1ab pol...	Severe acute res...	93.5	93.5	100%	2e-15	100.00%	29649	OR906008.1
Severe acute respiratory syndrome coronavirus 2 isolate SARS-CoV-2/human/USA/2367509/2023 ORF1ab pol...	Severe acute res...	93.5	93.5	100%	2e-15	100.00%	29649	OR905970.1
Severe acute respiratory syndrome coronavirus 2 isolate SARS-CoV-2/human/USA/2367130/2023 ORF1ab pol...	Severe acute res...	93.5	93.5	100%	2e-15	100.00%	29649	OR905965.1
Severe acute respiratory syndrome coronavirus 2 isolate SARS-CoV-2/human/USA/2366815/2023 ORF1ab pol...	Severe acute res...	93.5	93.5	100%	2e-15	100.00%	29649	OR905961.1
Severe acute respiratory syndrome coronavirus 2 isolate SARS-CoV-2/human/USA/2366814/2023 ORF1ab pol...	Severe acute res...	93.5	93.5	100%	2e-15	100.00%	29649	OR905960.1
Severe acute respiratory syndrome coronavirus 2 genome assembly, chromosome: 1	Severe acute res...	93.5	93.5	100%	2e-15	100.00%	29850	OY855842.1
Severe acute respiratory syndrome coronavirus 2 genome assembly, chromosome: 1	Severe acute res...	93.5	93.5	100%	2e-15	100.00%	29850	OY855833.1
Severe acute respiratory syndrome coronavirus 2 genome assembly, chromosome: 1	Severe acute res...	93.5	93.5	100%	2e-15	100.00%	29850	OY855825.1
Severe acute respiratory syndrome coronavirus 2 genome assembly, chromosome: 1	Severe acute res...	93.5	93.5	100%	2e-15	100.00%	29850	OY855814.1
Severe acute respiratory syndrome coronavirus 2 genome assembly, chromosome: 1	Severe acute res...	93.5	93.5	100%	2e-15	100.00%	29826	OY855617.1
Severe acute respiratory syndrome coronavirus 2 genome assembly, chromosome: 1	Severe acute res...	93.5	93.5	100%	2e-15	100.00%	29873	OY855616.1

Figure 5: BlastN search to demonstrate the huge spread (>500) of spike MPLF insertion omicron Coronavirus JN.1 variant sequences. JN.1-MPLF-insertion-oligo: 5'-gtc tct agt cag tgt gtc atg ccg ctg ttt aat ctt ata act aca act caa-3'.

Description	Scientific Name	Max Score	Total Score	Query Cover	E value	Per. Ident	Acc. Len	Accession
Severe acute respiratory syndrome coronavirus 2 isolate SARS-CoV-2/human/USA/ND-USAFSAM-S21586/2023...	Severe acute res...	78.7	78.7	100%	3e-11	100.00%	29739	OR886861.1
Severe acute respiratory syndrome coronavirus 2 genome assembly, chromosome: 1	Severe acute res...	78.7	78.7	100%	3e-11	100.00%	29858	OY790445.1
Severe acute respiratory syndrome coronavirus 2 genome assembly, chromosome: 1	Severe acute res...	78.7	78.7	100%	3e-11	100.00%	29858	OY788425.1
Severe acute respiratory syndrome coronavirus 2 isolate SARS-CoV-2/human/USA/GA-GPHL-1018/2023 ORF1...	Severe acute res...	78.7	78.7	100%	3e-11	100.00%	29741	OR838630.1
Severe acute respiratory syndrome coronavirus 2 genome assembly, chromosome: 1	Severe acute res...	78.7	78.7	100%	3e-11	100.00%	29858	OY783551.1
Severe acute respiratory syndrome coronavirus 2 genome assembly, chromosome: 1	Severe acute res...	78.7	78.7	100%	3e-11	100.00%	29858	OY780265.1
Severe acute respiratory syndrome coronavirus 2 genome assembly, chromosome: 1	Severe acute res...	78.7	78.7	100%	3e-11	100.00%	29858	OY779010.1
Severe acute respiratory syndrome coronavirus 2 genome assembly, chromosome: 1	Severe acute res...	78.7	78.7	100%	3e-11	100.00%	29823	OY777884.1
Severe acute respiratory syndrome coronavirus 2 isolate SARS-CoV-2/human/USA/ND-USAFSAM-S21543/2023...	Severe acute res...	78.7	78.7	100%	3e-11	100.00%	29740	OR825084.1
Severe acute respiratory syndrome coronavirus 2 isolate SARS-CoV-2/human/USA/ND-USAFSAM-S21542/2023...	Severe acute res...	78.7	78.7	100%	3e-11	100.00%	29739	OR825083.1
Severe acute respiratory syndrome coronavirus 2 isolate SARS-CoV-2/human/USA/CT-DPH-1177255001/2023...	Severe acute res...	78.7	78.7	100%	3e-11	100.00%	29633	OR791623.1
Severe acute respiratory syndrome coronavirus 2 isolate SARS-CoV-2/human/USA/VT-23-008705-WGS-02/2023...	Severe acute res...	78.7	78.7	100%	3e-11	100.00%	29691	OR783752.1
Severe acute respiratory syndrome coronavirus 2 isolate SARS-CoV-2/human/USA/NY-PRL-231020_81A01/202...	Severe acute res...	78.7	78.7	100%	3e-11	100.00%	29738	OR710511.1
Severe acute respiratory syndrome coronavirus 2 isolate SARS-CoV-2/human/USA/CA-CDPH-500125630/2023...	Severe acute res...	78.7	78.7	100%	3e-11	100.00%	29756	OR703517.1
Severe acute respiratory syndrome coronavirus 2 isolate SARS-CoV-2/human/USA/CA-CDPH-500125112/2023...	Severe acute res...	78.7	78.7	100%	3e-11	100.00%	29756	OR703431.1
Severe acute respiratory syndrome coronavirus 2 isolate SARS-CoV-2/human/USA/NJ-PHEL-V23016091/2023...	Severe acute res...	78.7	78.7	100%	3e-11	100.00%	29634	OR689681.1
Severe acute respiratory syndrome coronavirus 2 isolate SARS-CoV-2/human/USA/WA-PHL-034887/2023 ORF1...	Severe acute res...	78.7	78.7	100%	3e-11	100.00%	29676	OR680213.1

Figure 6: Blast search to demonstrate penetration of ^{184}EGKQG spike deletion variant of omicron coronavirus XBB.1.5.103 sequences in the database (>75). XBB.1.5.103-EGKQG-oligo: 5'-cag cct ttt ctt atg gac ctt gat ttc aaa aat ctt agg gaa-3'.

```
Variant/Accession/Date/Country          S gene region                                                  Position
B-Wuhan-NC_045512-2019                  tcagtgtgtt----MPLF----aatcttacaaccagaactcaattacccccctgcatacac    21648
OY854261-20-11-2023-Denmark             tcagtgtgtcatgccgctgtttaatcttataactacaactcaa---------tcatacac    21642
OY855842-20-11-2023-Denmark             tcagtgtgtcatgccgctgtttaatcttataactacaactcaa---------tcatacac    21642
OY855585-20-11-2023-Denmark             tcagtgtgtcatgccgctgtttaatcttataactacaactcaa---------tcatacac    21642
OR906019-28-11-2023-USA                 tcagtgtgtcatgccgctgtttaatcttataactacaactcaa---LPP---tcatacac    21588
OR882520-8-11-2023-USA                  tcagtgtgtcatgccgctgtttaatcttataactacaactcaa---------tcatacac    21595
OR862048-3-11-2023-USA                  tcagtgtgtcatgccgctgtttaatcttataactacaactcaa---------tcatacac    21540
OR864490-9-11-2023-USA                  tcagtgtgtcatgccgctgtttaatcttataactacaactcaa---------tcatacac    21570
JN.1-OR919554-USA-21-11-2023            tcagtgtgtcatgccgctgtttaatcttataactacaactcaa---------tcatacac    21592
JD.1.1-OR916767-USA-17-11-2023          tcagtgtgtt------------aatcttataaccagaactcaa---------tcatacac    21437
FL.1.5.1-OR916447-USA-13-11-2023        tcagtgtgtt------------aatcttataaccagaactcaa---------tcatacac    21486
XBB.1.5.103-OR917012-21-11-2023         tcagtgtgtt----MPLF----aatcttataaccagaactcaa---LPP---tcatacac    21495
XBB.1.16.23-OR917017-22-11-2023         tcagtgtgtt------------aatcttataaccagaactcaa---------tcatacac    21558
HV.1-OR817238-USA-26.10.2023            tcagtgtgtt------------aatcttataaccagaactcaa---------tcatacac    21580
EG.5.1.1-OR813264-USA-10-10-2023        tcagtgtgtt------------aatcttataaccagaactcaa---------tcatacac    21580
                                        *********              ******* *** * ********         *******
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Figure 7: Genome-wide search of more spike 17MPLF insertion mutants and multi-alignment. It was found that Denmark and USA had high hit for mutant coronavirus. The 24LPP deletion was rampant in all omicron variants.

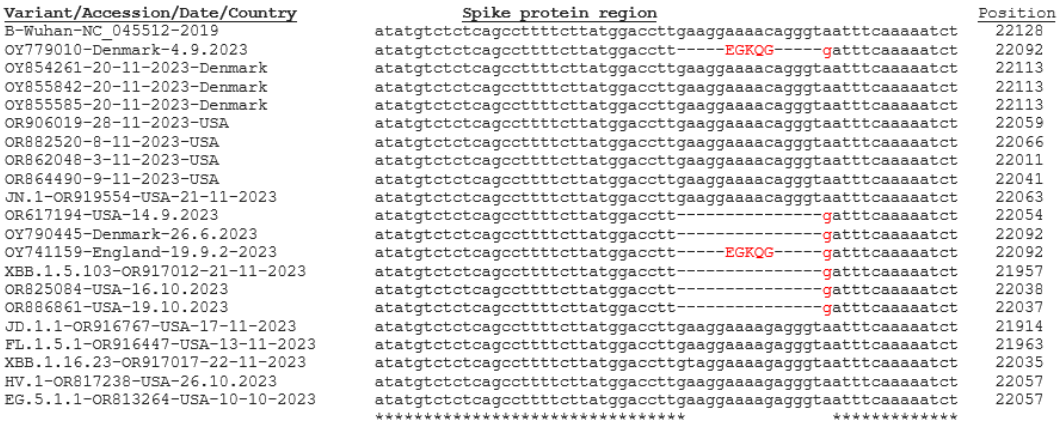


Figure 8: Genome-wide search for spike EGKEG deletion more mutants. It was also distributed in Europe and America. After WHO declared recent spread of coronavirus was mild. So, poor countries including India had stopped further WGS of newly infected coronaviruses.

Then, we wanted to see the spread of [17]MPLF insertion (ATGCCGCTGTTT) among the different BA.2 lineages. It was found that [17]MPLF insertion first appeared in BA.2.86 and BA.2.86.1 subvariants but not in other BA.2 lineages like BA.2.12.1, BA.2.48, BA.2.75 and BA.2.75.10 (figure-9). Further, the spike 69HV deletion was not found in all BA.2 variants (BA.2, BA.2.12, BA.2.48 and BA.2.75) except recently was found in BA.2.86 or BA.2.86.1 lineage as well as new JN.1, JN.2 and JN.3 lineages (figure-9). The mutated JN.1 genome sequences were authentic and such sequences were done by different famous groups like Howard et al, Freeman et al and Laurin et al (USA) whereas we found JN.1 spread in the 35 US States. Such sequences were also found in Japan (accession nos. BS007751 and BS007749). The partial sequence data was not included in this analysis. In figure-10, we demonstrated the unique spike L455S mutation (L452S in JN.1) suggested in immune escape and higher transmission and such mutation was not detected in BA.2.86 variant.

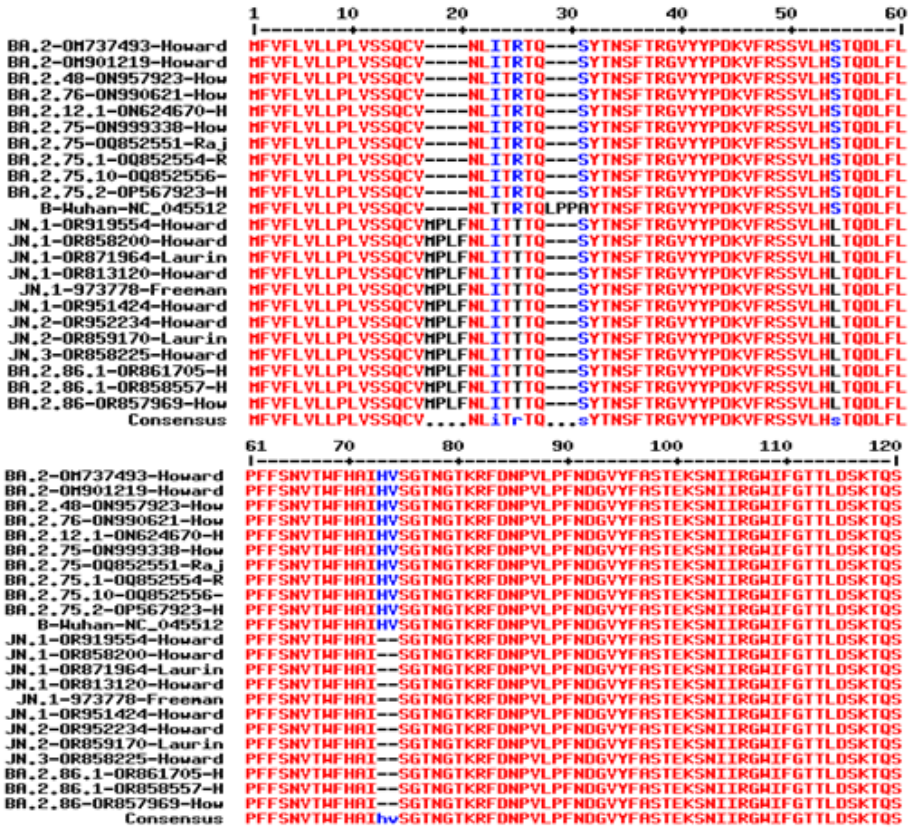


Figure 9: Demonstration of most spike deletions of JN.1 variant was occurred in BA.2.86 and BA.2.86.1 subvariants. The important [69]HV deletion was first occurred in B.1.1.7 variant in 2020 and the was found in BA.1, BA.4 and BA.5 omicron variant in 2021. The [69]HV deletion was not found in all BA.2 variants (BA.2, BA.2.12, BA.2.48 and BA.2.75) except recently was found in BA.2.86 and BA.2.86.1 lineages as well as JN.1, JN.2 and JN.3 lineages. The JN.1 genomes were sequenced by different author and groups: Howard et al, Freeman et al and Laurin et al and Partial sequence data was not included.

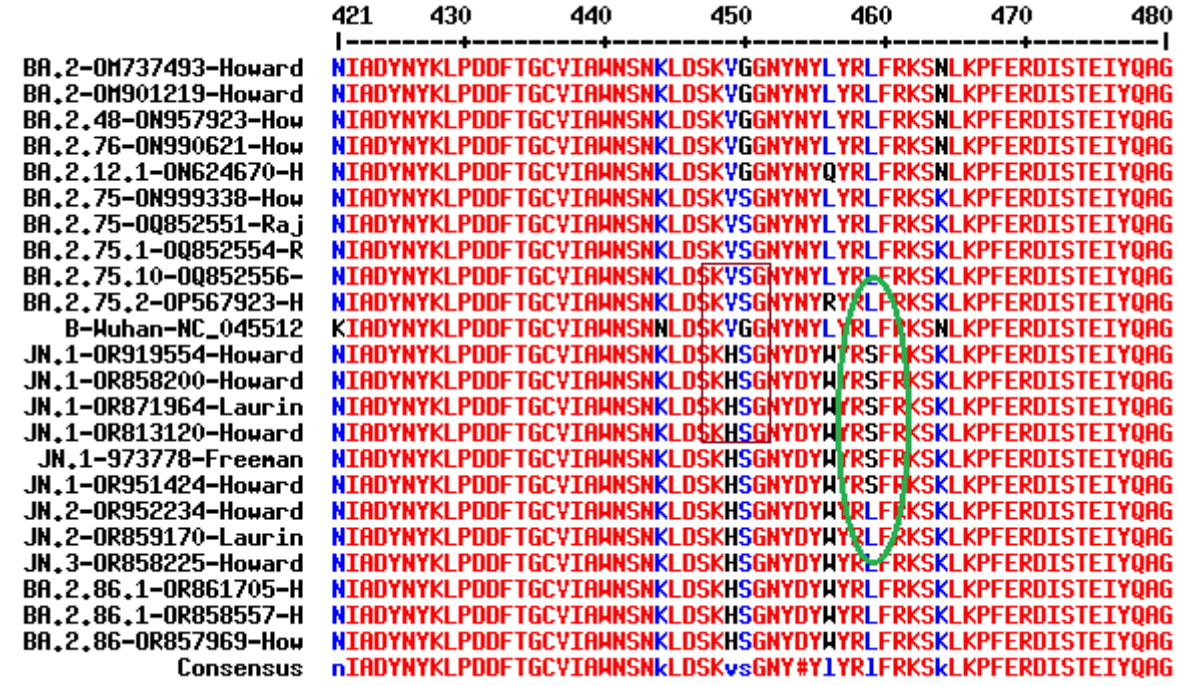


Figure 10: Demonstration that the main difference between the spike of BA.2.86 and JN.1 was L455S (S452 in JN.1) mutation. The important spike V445H mutation in JN.1 and BA.2.86 was also shown.

We also demonstrated that only XBB.1.5.103 variant has [176]EGKEG spike deletion but not in other XBB.1.5.1-XBB.1.5.100 subvariants (figure-11). Much of the such data was reported in a book recently published: Chakraborty AK. 2023. Life and Death of COVID-19: Molecular Genetics of SARS-CoV-2. ISBN: 978-1-68576-454-8. Iterative International Publishers, Karnataka, India. First edition, Pp. 119-121. However, part of such data was also published in an Open Access journal: Chakraborty AK. Highly infectious, less pathogenic and antibody resistant omicron XBB.1.5.1-XBB.1.5.39 subvariant coronaviruses do not produce ORF8 protein due to 8th codon GGA=TGA termination codon mutation. Cohesive J Microbiol Infect Dis. 6(5): CJMI.000648.2023. Doi:10.31031/CJMI.2023.06.000648. We also reported such data in preprint server: Research Square (Preprint), 30th May, 2023. We knew if both 69HV and 24LPP double deletions in the spike, then the variant would be BA.4 or BA.5 omicron variants. But data presented in figure-12 showed that omicron BA.4 and BA.5 spike sequences were very different than JN.1 variant which was selected from BA.2.86 lineage and BA.2.86.1 was also spreading highly.

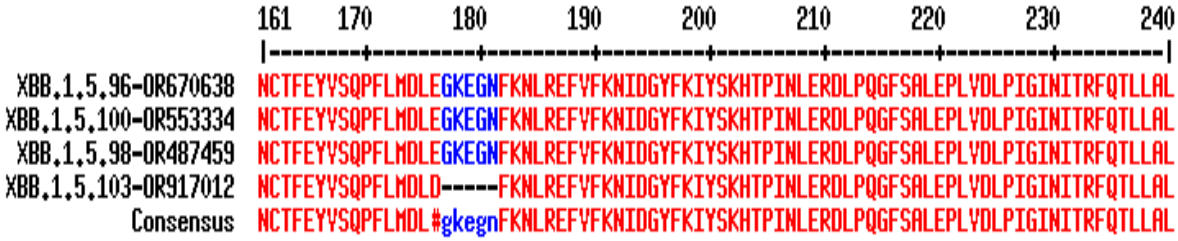


Figure 11: Demonstration that only XBB.1.5.103 variant has EGKEG deletion but not in XBB.1.5.1-XBB.1.5.100 (Chakraborty AK. 2023. Life and Death of COVID-19: Molecular Genetics of SARS-CoV-2. Book, ISBN: 978-1-68576-454-8. Iterative International Publishers, Karnataka, India. First edition, Pp. 119-121 and Chakraborty AK. Highly infectious, less pathogenic and antibody resistant omicron XBB.1.5.1-XBB.1.5.39 subvariant coronaviruses do not produce ORF8 protein due to 8th codon GGA=TGA termination codon mutation. Cohesive J Microbiol Infect Dis. 6(5): CJMI.000648.2023. Doi:10.31031/CJMI.2023.06.000648) and also deposited in Research Square (Preprint) on 30th May, 2023.

Figure 12: Demonstration that even JN.1 has [24]LPP and [69]HV deletions but could not be assigned to as BA.4 or BA.5 subvariant (more than 18 AAs variation in the spike). The part of the alignment was shown. Thus, multi-alignment clearly indicated OR855646, OR855645 and OR821989 were pre-JN.1 or pre-BA.2.86 subvariant (only variation in the [17]MPLF spike insertion).

In figure-13, figure-14 and figure-15, we demonstrated the Swiss-Model structures of JN.1 spike comparing with known 3-D spike structures (7CN8.1.A; 7CJL.1.A; 7CNB.1.A; 8WTJ.1.B). A potential spike trimers 3-D fold was detected as previously reported but basic amino acid protruding wings were also found in the middle (figure-14). The Swiss-Model predicted **Class score** 1.64 for Wuhan spike whereas for JN.1 spike 0.61 suggesting comparable 3D structures well suited for Wuhan spike but in JN.1 spike had much variation which was further clarified by the **MolProbity score** for Wuhan spike 1.54 vs 1.39 for JN.1 spike. The **Ramachandran plot** favored was 94.01% vs 95.08% indicating JN.1 spike protein had more compact 3-D fold than Wuhan spike.

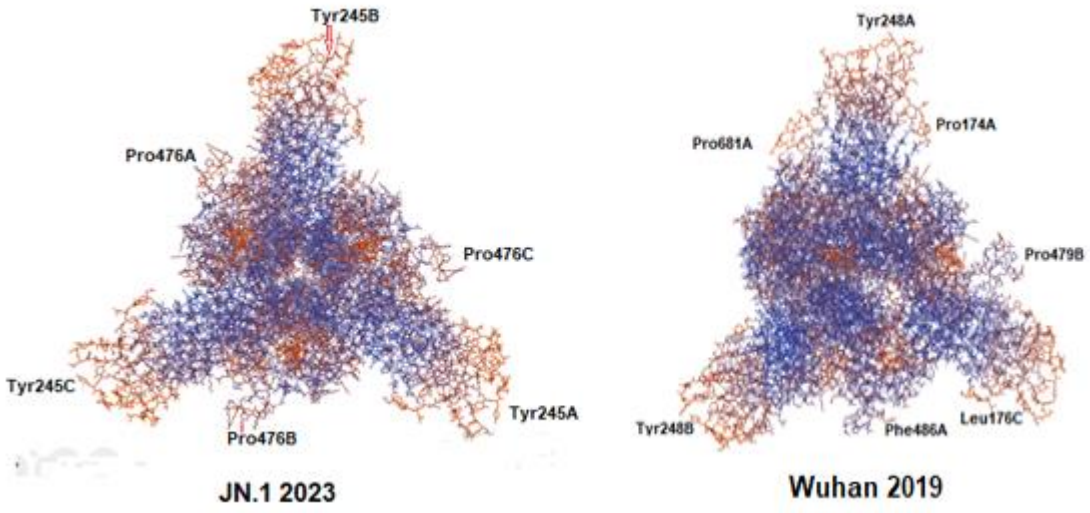


Figure 13: Comparison of SWISS-Model structures of Spike protein from JN.1 (2023) and Wuhan (2019) coronavirus (front view).

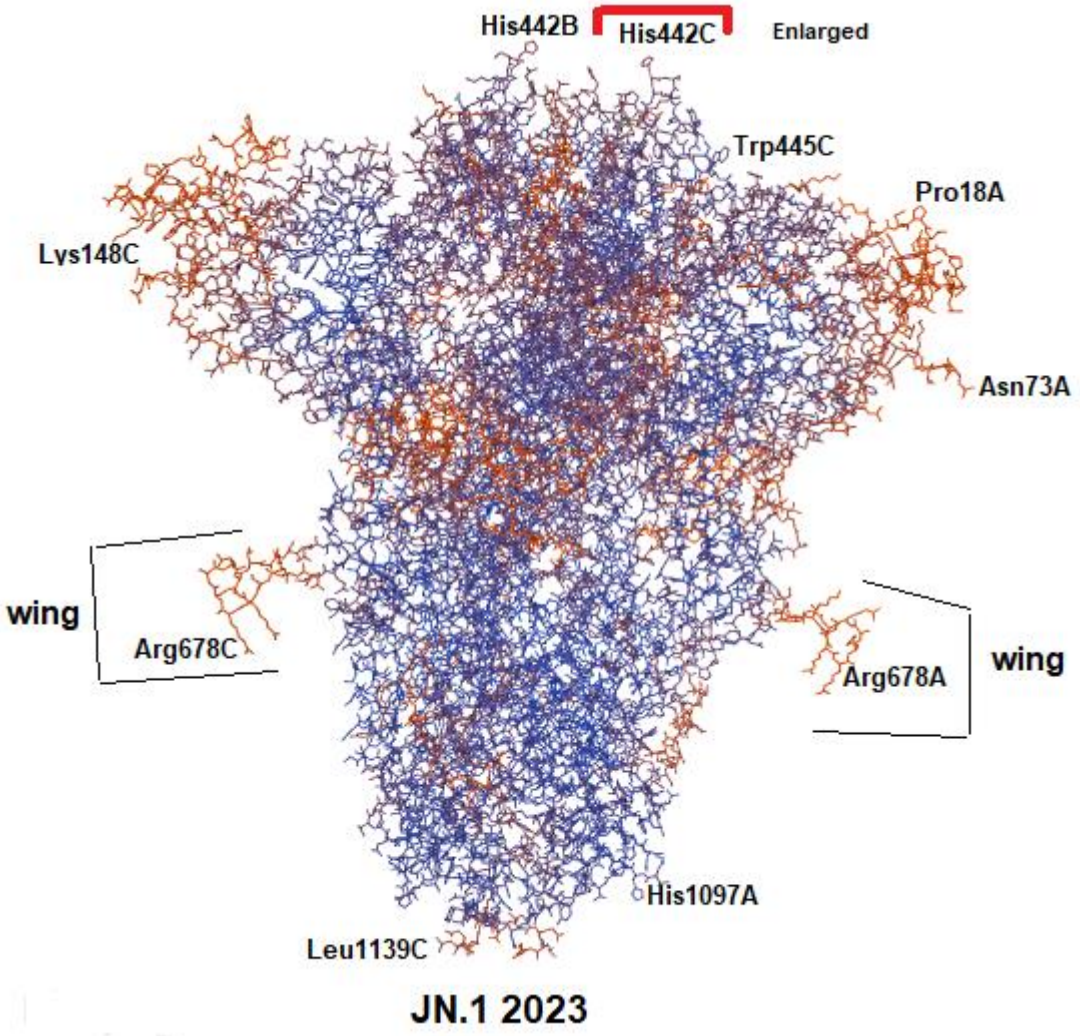
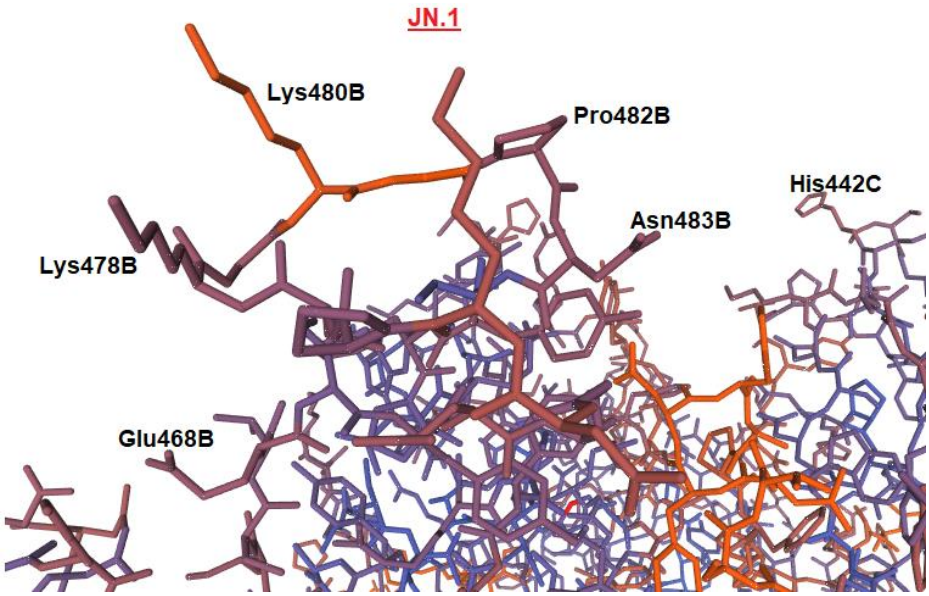


Figure 14: Longitudinal Swiss-Model view of spike protein of JN.1 omicron coronavirus showing important surface amino acids and postulated wings with basic amino acids. We postulated that mutations and deletions in the JN.1 spike caused more compact well-suited spike tripartite 3-D structure to interact with ACE-2 receptor.



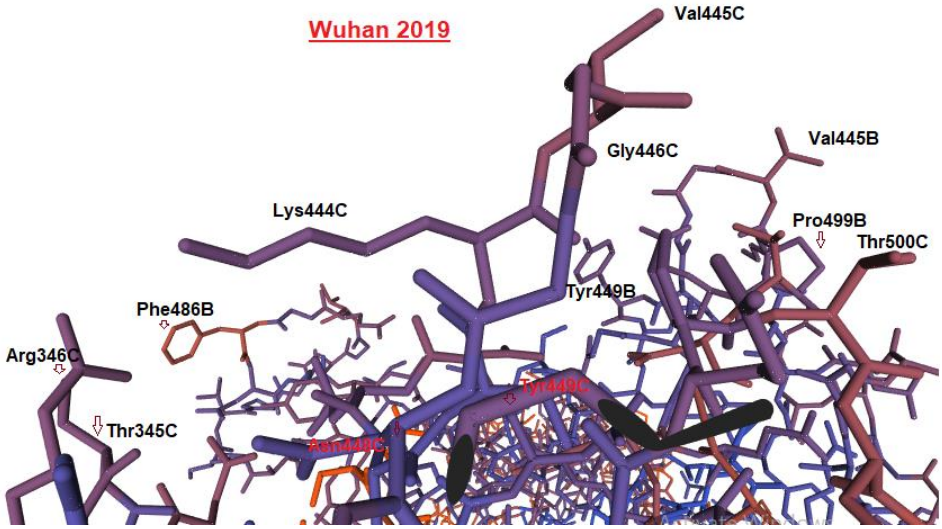


Figure 15: The view of enlarged top point of the tripartite Spike protein amino acids that involved in ACE-2 receptor interactions was shown. We found differences among the interacting amino acids between JN.1 and Wuhan variants. The amino acids from three same subunits were involved in ACE-2 and spike RBD interactions.

We also demonstrated the overwhelming ^{3675}SGF deletion in ORF1ab, ^{31}ERS deletion in N-protein and 26nt deletion in 3'-UTR respectively in the suppl-Figures (figure-16, figure-17 and figure-18) among the omicron coronaviruses including JN.1 variant. The heterogeneity among the different lineages was evident. Few omicron strains had L452S mutation (L455S in Wuhan) for JN.1 but no ^{17}MPLF spike insertion was found (accession numbers: OR674602, OR865731 and OR861833). Similarly, Protein ids. WPK95661, WPK95649 and WPF60514 had no ^{17}MPLF insertion but ^{24}LPP, ^{69}HV, ^{145}Y, ^{211}N, ^{483}V (NC_045512.2 Wuhan position) deletions and important L452S mutations were detected and could be assigned as pre-JN.1 and pre-BA.2.86 variants.

```
B-Wuhan-NC_045512-2019                tagtttgtctggttttaagctaaaagactgtgttatgtatgcatcagctgtagtgttact      11340
OY779010-Denmark-4.9.2023             tagtttg---------aagctaaaagactgtgttatgtatgcatcagctgtagtgttact      11331
OY854261-20-11-2023-Denmark           tagtttg---------aagctaaaagactgtgttatgtatgcatcagctgtagtgttact      11331
OY855842-20-11-2023-Denmark           tagtttg---------aagctaaaagactgtgttatgtatgcatcagctgtagtgttact      11331
OY855585-20-11-2023-Denmark           tagtttg---------aagctaaaagactgtgttatgtatgcatcagctgtagtgttact      11331
OR906019-28-11-2023-USA               tagtttg---------aagctaaaagactgtgttatgtatgcatcagctgtagtgttact      11277
OR882520-8-11-2023-USA                tagtttg---------aagctaaaagactgtgttatgtatgcatcagctgtagtgttact      11284
OR862048-3-11-2023-USA                tagtttg---------aagctaaaagactgtgttatgtatgcatcagctgtagtgttact      11229
OR864490-9-11-2023-USA                tagtttg---------aagctaaaagactgtgttatgtatgcatcagctgtagtgttact      11259
JN.1-OR919554-USA-21-11-2023          tagtttg---------aagctaaaagactgtgttatgtatgcatcagctgtagtgttact      11281
OR617194-USA-14.9.2023                tagtttg---------aagctaaaagactgtgttatgtatgcatcagctgtagtgttact      11293
OY790445-Denmark-26.6.2023            tagtttg---------aagctaaaagactgtgttatgtatgcatcagctgtagtgttact      11331
OY741159-England-19.9.2-2023          tagtttg---------aagctaaaagactgtgttatgtatgcatcagctgtagtgttact      11331
XBB.1.5.103-OR917012-21-11-2023       tagtttg---------aagctaaaagactgtgttatgtatgcatcagctgtagtgttact      11196
OR825084-USA-16.10.2023               tagtttg---------aagctaaaagactgtgttatgtatgcatcagctgtagtgttact      11277
OR886861-USA-19.10.2023               tagtttg---------aagctaaaagactgtgttatgtatgcatcagctgtagtgttact      11276
JD.1.1-OR916767-USA-17-11-2023        tagtttg---------aagctaaaagactgtgttatgtatgcatcagctgtagtgttact      11138
FL.1.5.1-OR916447-USA-13-11-2023      tagtttg---------aagctaaaagactgtgttatgtatgcatcagctgtagtgttact      11187
XBB.1.16.23-OR917017-22-11-2023       tagtttg---------aagctaaaagactgtgttatgtatgcatcagctgtagtgttact      11259
HV.1-OR817238-USA-26.10.2023          tagtttg---------aagctaaaagactgtgttatgtatgcatcagctgtagtgttact      11281
EG.5.1.1-OR813264-USA-10-10-2023      tagtttg---------aagctaaaagactgtgttatgtatgcatcagctgtagtgttact      11281
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Figure 16: Genome-wide search and multi-alignment to demonstrate the ^{3675}SGF deletion in the nsp6 protein of ORF1ab polyprotein of most omicron corona viruses. Part of the alignment was shown.

Figure 17: Genome-wide search and multi-alignment to demonstrate the [31]ERS deletion in the nucleocapsid protein (N gene) in all omicron coronaviruses.

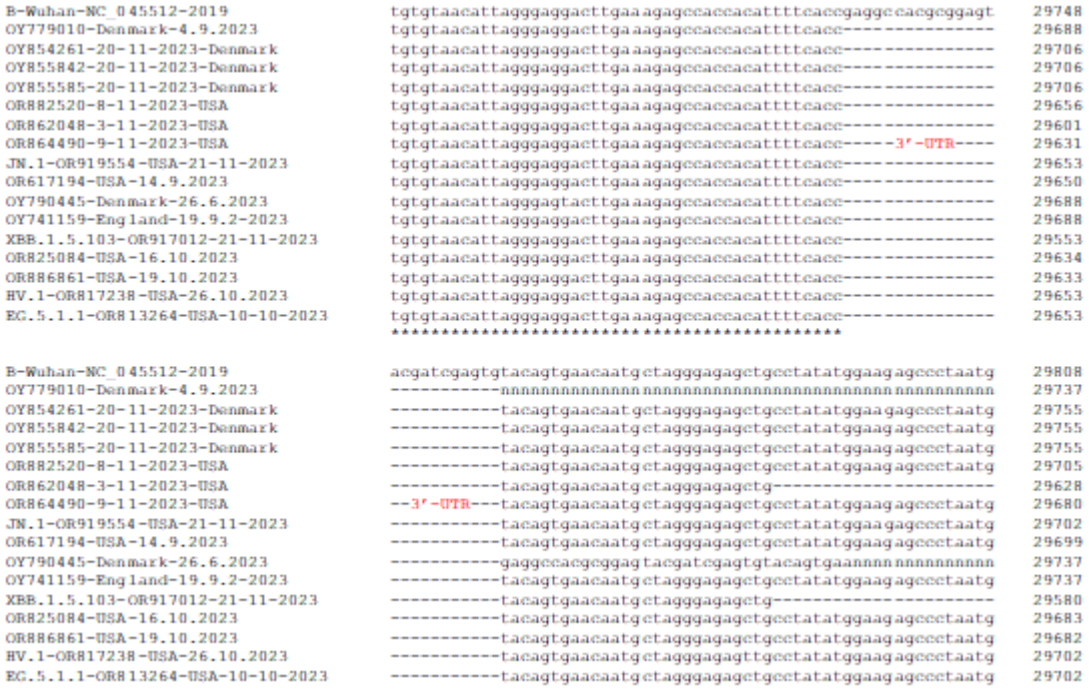

Figure 18: Genome-wide search and multi-alignment to demonstrate the 26nt 3'-UTR deletion at nucleotide 29733 position in most recent omicron coronaviruses.

Multi-alignment detected four JN.1 specific new mutations in the ORF1ab polyprotein (figure-19): T170I mutation in the nsp1 and D1600N plus K1973R two mutations in the nsp3 protease whereas R3821K mutation in the nsp6 protein. Similarly, three mutations (D3H, A63T and A104V) in very conserved membrane protein in both BA.2.86 and JN.1 variants were detected whereas A63T mutation was appeared first in BA.2.75 variant (figure-20). Genome wide search predicted that not all JN.1 variants were selected for A104V mutation yet (figure-21). The Swiss Model analysis showed that no gross changes in the 3-D structure of M protein as compared to published SARS-CoV-2 M protein 3-D models (PDB ID: 8CTK.1.A; 7VGGR.1.E; 7Y96.1.A) (figure-22). The News Papers in India are claiming that there are worldwide increase in JN.1 outbreaks. However, sequence data has not deposited yet from India or China or Brazil. Surely, [17]MPLF insertion in spike including important L452S mutation favored the spread of JN.1 variant. The orf3A trans-activator protein mutation was not found in JN.1 or BA.2.86.1 subvariants whereasT223I mutation was selected in BA.2 and was found in JN.1, BQ.1, BF.1 as well as BA.4 and BA.5 variants. Interestingly orf3A mutation in Alpha variant (T151I), Beta variant (Q57H and S171L), Gamma variant (W131C and S253P) and Delta variant (S26L) were suggested to increase virulence (data not shown). Data submitted by Opentrons P et al (USA) showed a cluster of BA.2.86.1 spread (PP087519) whereas we found many silent mutations (25421 C>T in ORF3a and 26894 C>A in M-protein) in such coronavirus sequences as shown in figure-23. In figure-24, we disclosed the new point mutations in the JN.1 coronaviruses just have selected in one JN.1 subvariant but not in other JN.1 subvariants and ongoing mutations will dictate the future spread. Thus, analysis of such mutations is important task to monitor coronavirus spread in different countries.

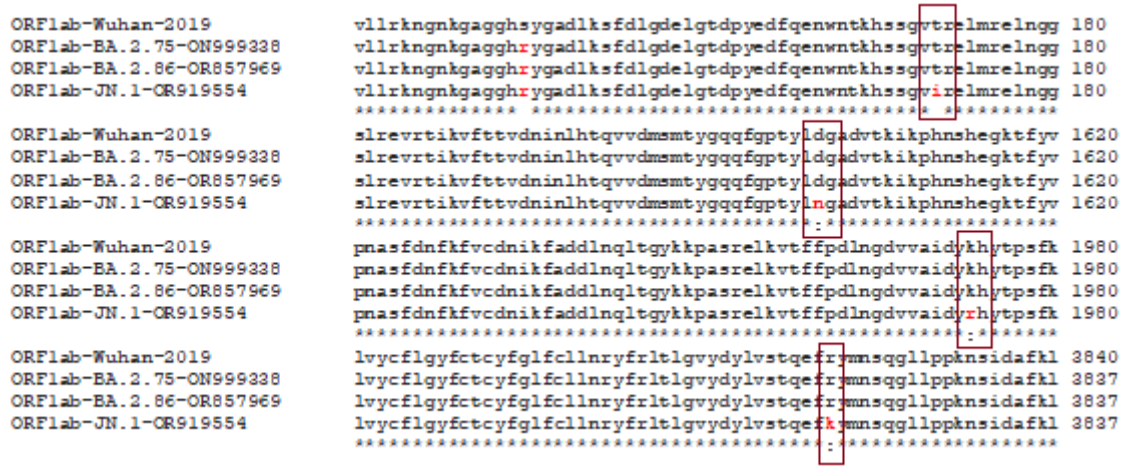

Figure 19: The JN.1 coronavirus variant specific ORF1ab polyprotein mutations. The mutations T170I, D1600N, K1973R and R3821K were detected (NC_045512/YP_009724389 based position numbering).

Figure 20: Important mutations (D3H, A63T, A104V) in the Membrane protein of JN.1 and BA.2.86 variants.

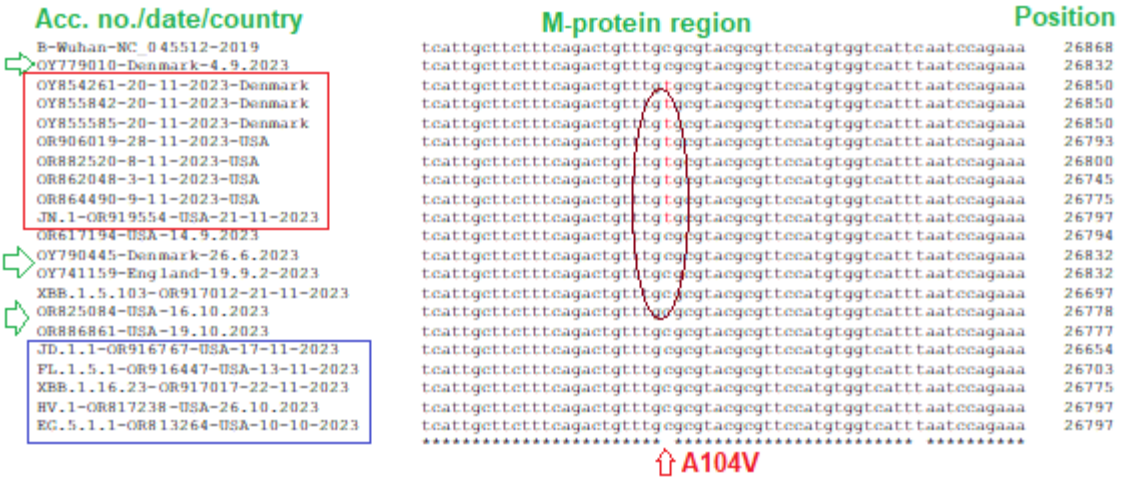

Figure 21: Genome wide search for JN.1 M-protein A104V mutation (red box). It appeared few JN.1 sequences have not introduced A104V mutation yet (green arrows). Interestingly, XBB.1.5.103, PD.1.1, XBB.1.16.23, HV.1, FL.1.5.1 and EG.5.1.1 had no such mutation (blue box)

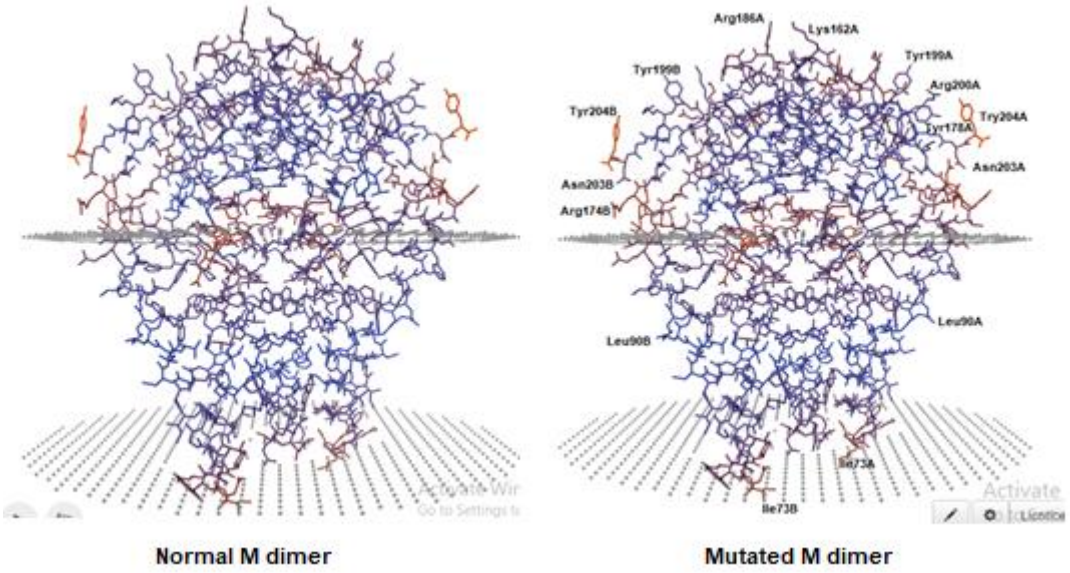

Figure 22: Swiss Model Dimeric 3-D structures of normal M protein of COVID-19 (NC_045512.2) as well as mutated M protein found in JN.1 and BA.2.86 subvariants.

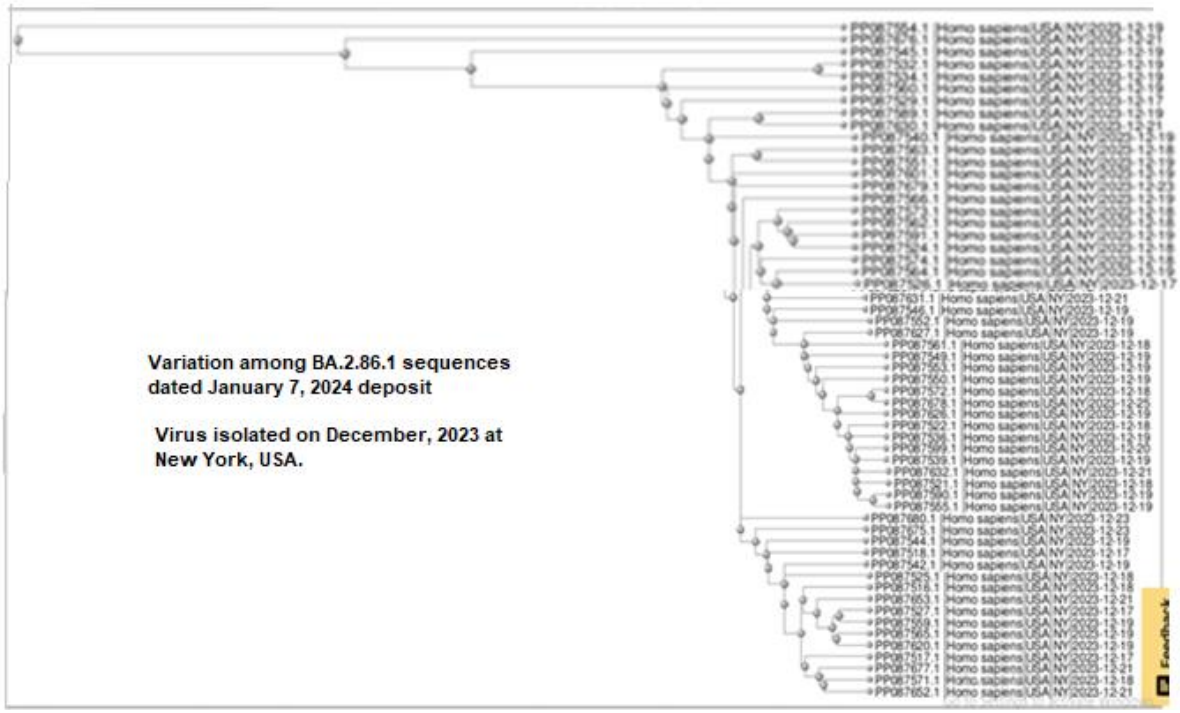

Figure 23: Phylogenetic differences among newly BA.2.86.1 subvariant sequences which were very similar to JN.1 subvariant (data deposited on 7th January, 2024). Important mutations were found 26894C>T with no amino acid change in M-protein (Leucine) and ORF3a 25421 C>T mutation also.

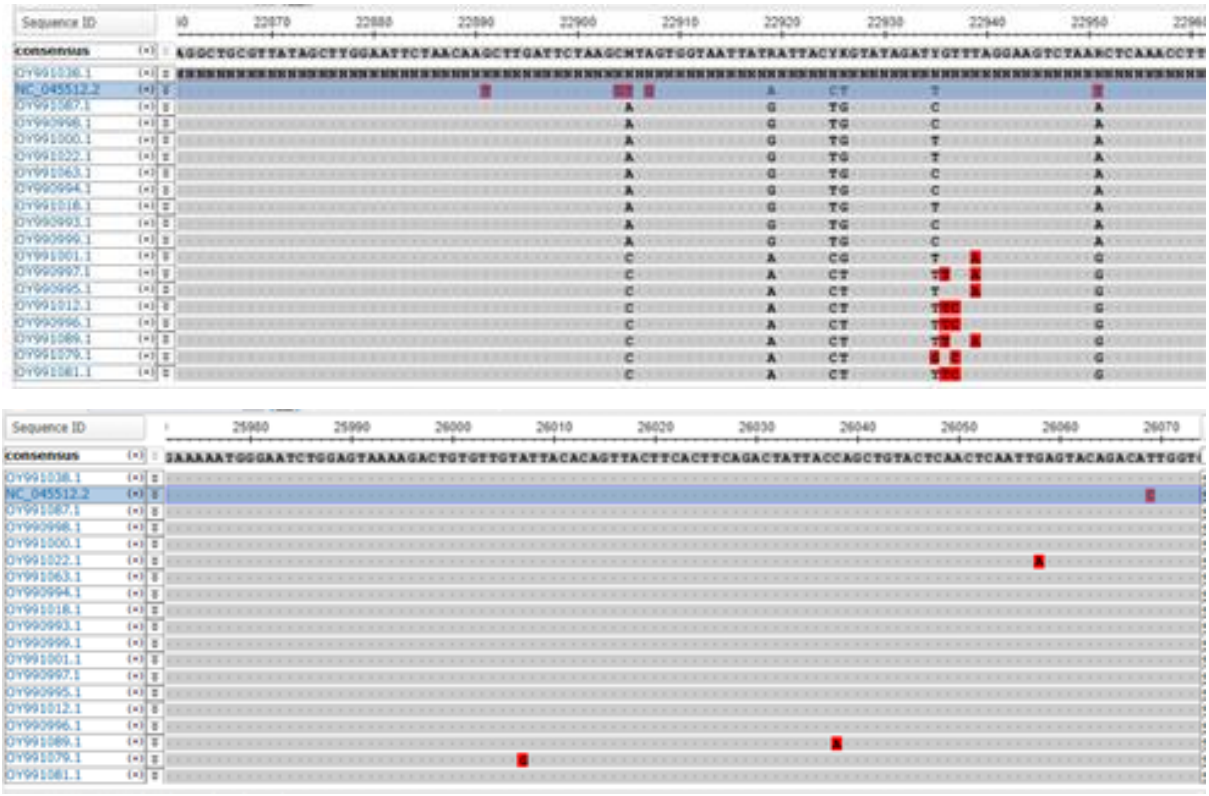

Figure 24: Appearance of new mutations in the JN.1 coronavirus sequences implying hundred subvariants of coronaviruses are still originated every day.

We always check the potential penetration of the specific variant in the database and try to understand their spread in different countries. BLASTP search with mutated nsp1-peptide sequence (vll rkn gnk gag ghr yga dlk sfd lgd elg tdp yed fqe nwn tkh ssg vir elm rel ngg-180) yielded 492 JN.1 sequences (9.1.2024) in the NCBI database. But BLAST search with mutant M-peptide (tlt cfv laa vyr inw itg gia iam acl vgl mwl syf ias frl fvr trs mws fnp etn ill-120) yielded only 98 sequences out of 500 sequences searched indicating most sequences were monopartite deposited from Europe (no protein data uploaded) or all JN.1 or B.2.86 M-protein might not be selected A104V mutation (figure-21). To resolve the issue, we BLASTP searched A63T mutant peptide sequence of M-protein (fly iik lif lwl lwp vtl tcf vla avy rin wit ggi aia mac-86) producing over 5000 sequences (above out search limit due to partial sequences of PCR diagnostics and incomplete sequences with errors). To catch the residual portion of A104V mutants in the NCBI monopartite JN.1 sequences, we BLASTN searched with mutant oligo surrounding A104V mutation (5'-tca ttg ctt ctt tca gac tgt ttg tgc gta cgc gtt cca tgt ggt cat tta atc cag aaa-3') producing more than 1000 sequences. However, with spike mutant oligo BLASTN search (22548-tcc taa tgt tac aaa ctt gtg ccc ttt tca tga agt t) produced over 4984 sequences as before. Such analysis might be useful to conclude that monopartite JN.1 related sequences (starting accession number with "AY") were more in the database signalling more and more JN.1 and BA.2.86.1 subvariants spread in the Europe than USA (at least more JN.1 sequences were deposited from Europe). Major JN.1 sequences obtained from European countries like England (OY990490), Denmark (OY984429), Switzerland (OY991000), Scotland (OY989956) and Ireland (OY989348). Astonishingly, we found a dated 31.1.2023 coronavirus sequence with only ^{24}LPP spike deletion that also had 22555 A>G mutation as above but no ^{69}HV deletion nor ^{17}MPLF spike insertions. Thus, mutation, deletion and insertion among the different coronaviruses selected under pressure and nomenclature of those very similar sequences remains controversial.

4. Discussion

I was studying the coronavirus NCBI portal and its sequence database since 2020 and had published twenty papers in new open-access journals. Sequence homology search showed that nsp2 was an RNA topoisomerase, nsp13 as capping methyl transferase. and nsp16 as 2'-O-Methyl 2251Uridine RlmE rRNA methyltransferase. The mutations, deletions and insertions in new coronavirus variants occurred frequently and various names were introduced for such mutants. The omicron subvariants BF.7 and BQ.1 sequences were greatly diminished recently with the spread of omicron XBB.1 lineages. During database search, subvariants HV.1, JN.1 EG.1 and JD.1 have deposited predominantly (December, 2023). In January, 2024, we found clonal expansion of JN.1 worldwide and thus we studied molecular detail of this coronavirus subvariant to find reason of spread.

The JN.1 coronavirus lineage was originated from BA.2.86 which was also known as Pirola variant and first detected in Denmark [38, 39]. The JN.1 subvariant was first reported in

Luxembourg in August and CDC suggested that its L455S (NC_045512.2 position) mutation might be caused the immune evasion properties. Considering the potential changes in BA.2.86, I would prefer the name JN.1 for BA.2.86 and present JN.1 should be then JN.1.1 and surely BA.2 vs BA.2.75 might understandable but BA.2 vs BA.2.86 would not match at all due to more distinct mutations and deletions in the spike.

We disclosed the ^{17}MPLF four amino acids spike insertion in JN.1 that might be involved in the higher transmission properties because of compensation eight deletions (^{24}LPP, ^{69}HV, ^{145}Y, ^{211}N and ^{483}V) in the spike. Data indicated the spread of JN.1 variant was maximum (3.5% of coronavirus sequences in the database) in the Denmark and USA during September, 2023. The JN.1 spread in South India has first detected in December, 2023 but scientists have cautioned such subvariant to be the major variant with 20-30% share of all omicron infections worldwide in 2024. Interestingly, JN.1 showed lower affinity for ACE-2 receptor with higher immune evasion as compared to BA.2.86.1 subvariant [38]. In August, 2023, BA.2.86 was found to be variant of concern (VOC) with many new mutations (WHO) and JN.1 was the precedent of BA.2.86 with one amino acid change in spike. We first time documented the insertion and important spike mutations in JN.1 subvariant as compared to BA.2.86 subvariant and others BA.2 subvariant (BA.2.3, BA.2.9, BA.2.48, BA.2.75). The report suggested EG.5.1, FL.1, XBB.1.5, XBB.1.16, XBB.1.9, XBB.1.42, XBB.2.86, XBB.2.3 were the major coronavirus variants spreading in December, 2023 and the BQ.1, BN.1, CH.1 and BQ.1.1 lineages were gradually diminished since January-March, 2023. The JN.1 variant was spreading highly worldwide and the virus did not replicate in Vero E6 cells and poor fusogenic [39]. The spread of ^{17}MPLF insertion (ATG CCG CTG TTT) in JN.1 spike is important (figure-1) which also has found in BA.2.86 and BA.2.86.1 subvariants but not in lower BA.2 lineages like BA.2.12.1, BA.2.48, BA.2.75 and BA.2.75.10 (figure-9). The database penetration was found for JN.1 and BA.2.86 variants (figure-5 and figure-7) over 500 sequences and maximum in September to December. However, spread of XBB.1.5.103 spike ^{176}EGKEG deletion mutants were found from June-September, 2023 and only ~75 sequences were detected so far (figure-6 and figure-8). Database suggests that JN.1 is rapidly spreading in the Europe as well as USA. The BA.2.86 variant is deadly as it has acquired the important mutations in the spike including ^{69}HV spike deletion and the only main difference between JN.1 and BA.2.86 is spike L452S mutation (L455 in Wuhan) (figure-10). It appears that JN1 ^{17}MPLF spike insertion is also a driving force for higher transmission compensating eight amino acids deletions. The JN.1 is BA.2.86 precedent and it has no similarity to omicron BA.4 and BA.5 lineage coronaviruses (figure-12) but it has unique ^{69}HV spike deletion which has not seen among other BA.2 lineages including BA.2.75 variant [40].

SWISS-Model predicted a more compact spike (figure-14) although the surface amino acids for interaction with ACE-2 receptor were changed (figure-15). The Swiss-Model structure of JN.1 spike predicted a more compact 3-D tripartite structure to interact with ACE-2 receptor [41-44]. Possibly middle wings with basic amino acid interacted with viral RNA for quick complete inclusion of virus into lungs cells and thus might be involved for rapid spread among the people whose immunity to COVID-19 vaccines was lost considerably. Spike protein of JN.1 has more mutations and deletions and thus will be less protective to COVAXIN and COVISHILD vaccines [45-51]. The JN.1 coronavirus thus, was more refractile to antibody of previously coronavirus-infected (Omicron BA.1/BA.2/BA.4/BA.5) individual [47, 49].

Data indicated that in the RBD of spike, amino acids Glu484, Phe486, Gln474, Lys417, Tyr453 and Asn501 might be involved to interact with the ACE-2 receptor [49-53]. But our data with JN.1 spike model (figure-15) suggested amino acids His442, Pro482, Lys480, Lys478 might be important in this aspect. As compare with Wuhan coronavirus spike, data indicated Arg346, Phe486, Lys444, Gly446, Val445 and tyr449 might be first interacted. Truly, 3-D crystal structure of JN.1 spike must be known to give a more conclusive data on RBD-ACE-2 interaction, transmission and pathogenicity.

The SARS-CoV-2 variants Delta, Kappa and Lambda with the double mutations T478K/L452R, E484Q/L452R, and F490S/L452Q, respectively, in their receptor binding domains (RBDs) of the spike protein were resistant to antibodies against Wuhan coronavirus but such mutations might not be sufficient to reduce the interaction between RBD and ACE-2 receptor [50, 51]. However, S373L (L368), S375P (P370), S377F (F372), K419N (N414), N442K (K437), G448S (S443), S479N (N474), E486A (A481), Q495R (R490), G498S (S493), Q500R (R495), and Y507H (H502) mutations in the RBD spike of omicron viruses changed such interacted amino acids as reflected in our JN.1 spike modelling data [3, 28]. More interestingly, 15 amino acids were changed further in the JN.1 spike making more confusion in our modelling data as compared to previously published data [49].

Data indicated more JN.1 sequences were deposited from the Europe. Does this imply that more JN.1 spread in Europe than USA? However, our finding of conserved M-protein mutations in BA.2.86 and JN.1 subvariants speculated if M-protein 3-D structure played a role for its higher transmission. Molecular dynamics simulations showed a high degree of structural rigidity in a simple lipid bilayer and supported a role for M homodimers in scaffolding viral assembly. Further, M displayed an important electropositive cytosolic surface that might be important for interactions with N, S, and viral RNA [54]. The D3H, A63T, A104V mutations in the M protein should change the charges in its surface. The M protein domains AA 1-19, AAs 20-40, AAs 51-71 and AAs 80-100 and AAs 101-222 were designated as NTD, TMI, TMII, TMIII and CTD respectively. The common interacting residues of the M-protein with S and N proteins were suggested as C-terminal Phe103, Arg107, Met109, Trp110, Arg131, and Glu135 [55-57]. However, at least we found that Ala104 of M-protein had interacting role with Ser186 of N protein changing M-N interaction in JN.1 and BA.2.86 variants [55].

Such studies are important to develop new drugs against COVID-19. The remdesivir, favipiravir, ribavirin and sofosbuvir drugs used to inhibit viral RNA-dependent RNA polymerase (RdRp), as well as drugs presumably acting on IL6 blockers, tocilizumab and doxycycline, acting on viral entry such as arbidol and hydroxychloroquine, protease inhibitors lopinavir-ritonavir and darunavir, JAK-1/2 inhibitors baricitinib and ruxolitinib are under different stages of clinical trials but remdesivir plus nirmatrelvir as Paxlovid was now used against COVID-19 worldwide [58-60]. The RBD spike-ACE-2 binding blocker (violacein) will be good candidate drug against RNA viruses [61]. The synthetic peptide like P9R (HOOC-NGA ICW GPC PTA FRQ IGN CGR FRV RCC RIR-NH2) and Peptide-3 (HOOC-DKF NHE AED LFY QSS LAS WNY NT-NH2) suggested to inhibit RBD-ACE-2 interactions to stop COVID-19 replication [62]. Recently, TEMP106B protein was suggested as a new receptor of COVID-19 entry into different human cells and TMEM106B-specific monoclonal antibodies blocked SARS-CoV-2 infection [63]. However, spike substitution E484D increased TMEM106B binding, thereby enhancing TMEM106B-mediated COVID-19 entry providing an alternate route of RBD mutation and COVID-19 spread as likely had happened in JN.1 variant. Thus, more and more drugs will be screened to stop omicron coronavirus spread.

5. Conclusion

Time to time, I am elegantly disclosing the nature of mutations, deletions and insertions in the genome of different coronavirus variants favoring higher transmission. My goal is to track genetic changes among coronavirus variants with time. The new JN.1 ^{17}MPLF spike insertion variant with L452S mutation is spreading highly worldwide. Similarly, XBB.1.5.103 176EGKEG deletion in the spike with popular ORF8 gene GGA=TGA termination codon mutation in XBB.1.103, XBB.1.16.23 as well as highly transmissible HV.1, EG.5.1.1 and FL.1.5.1 is important VIC to consider for further study. Surely, inactivation of nsp1, ORF7a and ORF8 small immuno-regulatory proteins lower the pathogenic potential of coronaviruses and such deletions were not detected in JN.1. It was therefore suggested that predominance of 69HV deletion and RBD mutations in JN.1 might be due to favorable escape of coronaviruses from immune-drugs. The JN.1 RBD-ACE-2 and RBD-TMEM106B binding inhibitors may be important drug to curve recent omicron JN.1 coronavirus spread.

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