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The ^{82}GHVMV and ^{141}KSF Deletions in the Nsp1 Protein of ORF1ab Polyprotein Favour the Creation of Immune-Weak SARS-CoV-2

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Abstract

The Wuhan corona virus was mutated and deleted at different positions of the genome specifically in deadly Alpha and Delta variants whereas spike protein was mostly affected in Omicron variants. The nsp1 protein (180 AAs) is the first protein of ORF1ab polyprotein which was degraded in host into sixteen (nsp1-nsp16) polypeptides with diverse functions. The most popular deletion was ^{3675}SGF in the nsp6 domain which was first appeared in early 2021 in B.1.1.7, B.1.351 and B.1.1.28.1 variants but now carried into most Omicron variants. We investigated here the deletion in the nsp1 protein which interacted with many cellular proteins preventing viral clearance. A ^{141}KSF three amino acids deletion in nsp1 was persistent in all Omicron BA.4 variant while another ^{82}GHVMV five amino acids deletion was detected at the upstream of ^{141}KSF in some recent isolates. BLAST-N search with ^{82}GHVMV oligo gave no ^{141}KSF deletion mutant but selection with GHVMV-KSF oligo gave all ^{82}GHVMV plus ^{141}KSF deletion mutants including ^{3675}SGF (ORF1ab), ^{31}ERS (N-protein), ^{24}LPP (Spike) as well as 26nt 3'-UTR deletions. Sequences surrounding ^{82}GHVMV and ^{141}KSF deletions formed hairpin structures that were changed in deletion mutants and 3-D structure of mutant nsp1 was also changed. Previously, we showed the frequent deletions in ORF7a and ORF7b as well as termination codon mutations in ORF8 genes. In summary, we postulated that such changes might be favoured host from severe effects of those viral moderator proteins sustaining viral growth in same cells. On the contrary, absence of those small transacting proteins favoured the clearance of SARS-CoV-2 by host immune system generating mild infections.

Keywords: Nsp1 protein; Genome deletion; Deletion boundary oligos; ORF1ab protein; ORF7a/b deletions; SARS-CoV-2

Introduction

Corona virus infections claimed >600000 lives in two years recently and its genetic structure was known extensively due to worldwide sequencing efforts [1]. The SARS-CoV-2 is a large positive-stranded RNA virus with~30000 nucleotides genome and it was to MERS, SARS-CoV, CoV 229E etc. related human corona viruses that were known for long time [2-4]. 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 from 2/3 of the 5'-part of the genome [5]. The ORF1ab polyprotein was degraded into sixteen polypeptides (nsp1-nsp16) (Figure-1). The ORF1ab generated sixteen peptides are: 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). The nsp2 protein is RNA topoisomerase whereas Nsp3 and nsp5 are proteases and nsp12 is RNA-dependent RNA polymerase [6-9]. The nsp6, nsp7, nsp8, nsp9 and nsp10 were small accessory proteins involved in RNA polymerase replication complex [10-12]. The nsp14 and nsp15 are nucleases to degrade RNA and

nsp16 is 2'-O Uridine methyltransferase and as well as nsp13 is RNA helicase with capping methyl transferase similarity [13-15]. Nsp11 is a small peptide and function was not known. The ORF3a, ORF6, ORF7a, ORF7b, ORF8, ORF9 and ORF10 small proteins also coded from 3' end of the SARS-CoV-2 genome and have roles in regulating cellular genes [16-20]. Many drugs were discovered against proteases and RNA polymerases but vaccines (specifically recombinant spike vaccine) were only important remedy that halted the corona virus spread [21,22]. The most frequent mutation that occurred in most corona virus isolates was 3037C>T which is a synonymous change that usually accompanied 3 other mutations that include 241C>T, 14408C>T (P323L in RdRp) and 23403A>G (D614G in S-protein). The omicron corona virus (B.1.1.529) spike mutations were: A67V (V67), T95I (I93), N211I (I206), L212V (V207), V215P (P210), R216E (E211), G341D (D336), S373L (L368), S375P (P370), S377F (F372), K419N (N414), N442K (K437), G448S (S443), S479N (N474), E486A (A481), Q495R (R490), G498S (S493), Q500R (R495), Y507H (H502), T549K (K544), H657Y (Y652), P683H (H678), N766K (K761), D798Y (Y793), N858K (K853), Q956H (H951), N971K (K966), and L983F (F978) [in sate values for omicron virus positions [23-26]. Interestingly, N501Y dominant mutation in B.1.1.7 was found in omicron BA.1, BA.4 and BA.5 including other related variants like BQ.1 and BF.7. The nsp1 protein is 180 amino acids and such protein has deleted in some corona virus strains [27]. Recent data suggested that Nsp1 protein could inhibit all cellular antiviral defence mechanisms that would depend on the expression of host factors, interferon-gamma and IL-6 [28-32]. It was found that amino acid residues K164 and H165 of Nsp1 from both SARS-CoV and SARS-CoV-2 were necessary for ribosome interaction as revealed by Cryo–Electron Microscopy of in vitro–reconstituted various Nsp1-40S and Nsp1-80S complexes. The Nsp1 C-terminus bound to mRNA tunnel inhibiting mRNA entry and protein synthesis blocking the retinoic acid inducible gene-I dependent innate immune responses that would otherwise facilitate clearance of the infection [33-36]. The SARS-CoV-2 escapes direct NK cell killing through Nsp1-mediated downregulation of ligands for NKG2D [37]. The mRNA degradation function of nsp1 protein was reported [38,39]. Further, nsp1 is a potent translational inhibitor [40,41]. The nsp1 protein also inhibits cellular mRNA synthesis and directs viral protein synthesis [42-44]. The deletions hotspot in the nsp1 protein are thus very interesting. We demonstrated in this article that 141KSF deletion in nsp1 protein was occurred in mostly omicron BA.4 variants whereas some deletion hotspot was located at 59 amino acids (AAs) upstream of 141KSF deletion site which we called 82GHVMV locus where 2-5 AAs deletions were found in some SARS-CoV-2 variants [45-47].

Methods

We searched PubMed to get idea on published papers on nsp1protein (www.ncbi.nlm.nih.gov/pubmed). The SARS-CoV-2 sequences were down loaded from SARS-CoV-2 database (NCBI, NIH, USA). We also searched NCBI BLAST search using BLAST-N and BLAST-X search methods to get sequences [48]. Multi-alignment of protein was done by MultAlin software and multi-alignment of DNA by CLUSTAL-Omega software, EMBL-EBI [49-51]. The ORF1ab mutants was obtained by BlastN search of deletion boundary of 60-100nt sequence and then analyzing the sequences with 95-100% similarities [52,53]. The other ORF1ab mutants were detected by Blast-N search and Blast-X search with selected deletion boundaries. Hairpin structure of ~ 120-200nt sequence was done by OligoAnalyzer 3.1 software (Integrated DNA Technologies). The protein 3-D structure was determined by SWISS-Model software with normal vs. mutant peptides [54-58].

Results

We made multi-alignment of coronavirus genomes to find specific deletions in the ORF1ab genes and few oligonucleotides at the deletion boundaries of ^{82}GHVMV, ^{141}KSF and ^{3675}SGF deletions of ORF1ab protein as shown in (Table 1). The KSF deletion oligo (5'-tgg cca tag gta cgg cgc cga tct aga ctt agg cga cga gc ttg gca ctg a-3'), GHVMV deletion oligo (5'-acg ttc gga tgc tcg aac tgc acc tca tga gct ggt agc aga act cga agg cat t-3') and SGF deletion oligo (5'-aat tac aga aga ggt tgg cca tag ttt gaa gct aaa aga ctg tgt tat gta tgc atc ag-3') gave very informative on the ORF1ab deletion mutants (>5000 sequences) in the NCBI database. The GHVMV-KSF oligo gave >995 sequences with both ^{82}GHVMV plus ^{141}KSF deletion in the nsp1 protein. A 63nt deletion from nt. 27695-27768 at the junction of ORF7a gene 3'-end and ORF7b gene 5'-end was found in accession no. OM766944. A 26nt deletion at the 3'-UTR (nt. 29733-29759) of SARS-CoV-2 genome was found (5'-gag gcc acg cgg agt acg atc gag tg-3') in different GHVMV mutants (accession numbers OP200462, BS004962, OX271963, ON956441, OP258049, ON414598, and ON766944) (Figure 2). We BLAST-N searched using SGF-1st and SGF-2nd oligos to trap 3675SGF deletion mutants and 10 sequences (five 1st SGF and five 2nd SGF) were aligned using NC_045512.2 as standard. We found that SGF 1st and 2nd deletion oligos selected sequences had all 3675SGF deletions (data not shown) but two sequences (acc. nos. OK040080 and OP591969) had 141KSF deletion whereas one (acc. no. OP827777) had 84VMV three AA deletion instead 82GHVMV (Figure 3A). The ratio of SGF: KSF: GHVMV deletions in ORF1ab protein estimated approximately 10:2:1. Isolated sequences were mostly Omicron corona virus variants with 31ERS N-protein deletion except accession numbers

MZ223360 and OL369199, which had 69HV and 212L deletions but no 31EPS insertion and designated as pre-omicron BA.1 variant. Surprisingly, the sequence OK040080 had 31ERS deletion in N-protein and 141KSF deletion in ORF1ab whereas no 24LPP or 69HV deletions in spike indicating it was either BA.1/BA.2 or BA.4/BA.5 but omicron pre-BA.4. On the contrary, the sequence OP591969 had 141KSF deletion in ORF1ab and 24LPP plus 69HV deletions in spike and was omicron BA.4 variant (Figure 3B).

Table 1: *Sequences of the deletion boundary oligonucleotides.*

GHVMV-KSF oligo	5'-cgttcggatgctcgaactgcacctcatgagctggtagcagaactcgaaggcatt cagtacggtcgtagtggtgagacacttggtgtccttgtccctcatgtgggcgaaat accagtggcttaccgcaaggttcttcttcgtaagaacggtaataaaggagctggtg gccataggtacggcgccgatctagacttaggcgacgagcttggcactgatcctt-3'	>1000
KSF oligo	5'-tggccataggtacggcgccgatctagacttaggcgacgagcttggcactga-3'	>5000
GHVMV oligo	5'-acgttcggatgctcgaactgcacctcatgagctggtagcagaactcgaaggcatt-3'	>5000
1st SGF oligo	5'-GACATGGTTGATACTAGTTTGAAGCTAAAAGACTGTGTTATGTAT-3'	>250
2nd SGF oligo	5'- GATATGGTTGATACTAGTTTGAAGCTAAAAGACTGTGTTATGTAT-3'	>10000

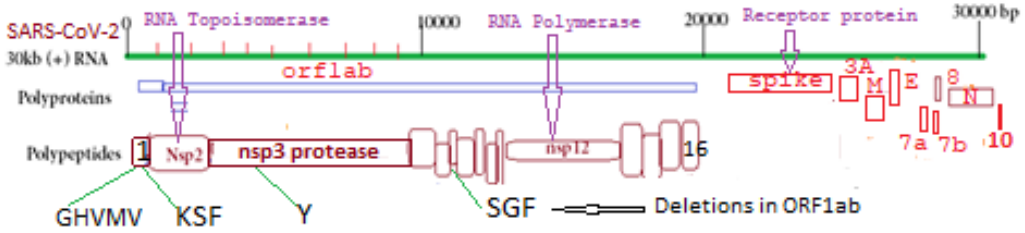

Figure 1: *Structure of SARS-CoV-2 and localization of deletions in the ORF1ab polyprotein. Note that spike protein was highly deleted and mutated than ORF1ab protein and more deletions were also reported in N, ORF7a, ORF7b, ORF8 small proteins.*

```
Accession no. Date of isolation      Nsp1 region of ORF1ab gene
NC_045512.2-12-2019      acgttcggatgctcgaactgcacctcatggtcatgttatggttgagctggtagcagaact 540
OP200462-23-7-2022       acgttcggatgctcgaactgcacctcat--------------gagctggtagcagaact 453
BS004962-7-8-2022        acgttcggatgctcgaactgcacctcat----GHVMV------gagctggtagcagaact 471
OX271963-31-7-2022       acgttcggatgctcgaactgcacctcat--------------gagctggtagcagaact 525
ON956441-20-6-2022       acgttcggatgctcgaactgcacctcat--------------gagctggtagcagaact 471
OP258049-31-7-2022       acgttcggatgctcgaactgcacctcatggtcat---VMV---gagctggtagcagaact 481
ON414598-21-4-2022       acgttcggatgctcgaactgcacctcatggtcatgttatggttgagctggtagcagaact 501
ON766944-31-5-2022       acgttcggatgctcgaactgcacctcatggtcat--VM--gttgagctggtagcagaact 484
OM766944-3-2-2022        acgttcggatgctcgaactgcacctcat---GHV---gtggttgagctggtagcagaact 500
ON159588-3-2022          acgttcggatgctcgaactgcacctcat--------------gagctggtagcagaact 473
ON972497-6-3-2022        acgttcggatgctcgaactgcacctcat----GHVMV------gagctggtagcagaact 485
                         ****************************              ******************
  /120nt/
NC_045512.2-12-2019      tggccatagttacggcgccgatctaaagtcatttgacttaggcgacgagcttggcactga 720
OP200462-23-7-2022       tggccataggtacggcgccgatcta---KSF---gacttaggcgacgagcttggcactga 624
BS004962-7-8-2022        tggccataggtacggcgccgatctaaagtcatttgacttaggcgacgagcttggcactga 651
OX271963-31-7-2022       tggccataggtacggcgccgatctaaagtcatttgacttaggcgacgagcttggcactga 705
ON956441-20-6-2022       tggccataggtacggcgccgatctaaagtcatttgacttaggcgacgagcttggcactga 651
OP258049-31-7-2022       tggccataggtacggcgccgatcta---KSF---gacttaggcgacgagcttggcactga 652
ON414598-21-4-2022       tggccataggtacggcgccgatctaaagtcatttgacttaggcgacgagcttggcactga 681
ON766944-31-5-2022       tggccataggtacggcgccgatctaaagtcatttgacttaggcgacgagcttggcactga 664
OM766944-3-2-2022        tggccatagttacggcgccgatctaaagtcatttgacttaggcgacgagcttggcactga 680
ON159588-3-2022          tggccatagttacggcgccgatctaaagtcatttgacttaggcgacgagcttggcactga 653
ON972497-6-3-2022        tggccatagttacggcgccgatctaaagtcatttgacttaggcgacgagcttggcactga 665
                         ********* ***************          ****************************
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Figure 2: *Multi-alignment of SARS-CoV-2 GHVMV mutant sequences showing the deleted regions in the nsp1 gene of ORF1ab as compared to Wuhan sequence (NC_045512.2).*

Citation: Chakraborty AK (2023) The [82]GHVMV and [141]KSF Deletions in the Nsp1 Protein of ORF1ab Polyprotein Favour the Creation of Immune-Weak SARS-CoV-2. SunText Rev Virol 3(2): 137.

Figure 3A: *Multi-alignment to show unusual Spike protein deletions in SGF mutants of SARS-CoV-2. The sequences were derived by BLAST-N search of database with 1st SGF oligo and 2nd SGF oligo (see, table-1).*

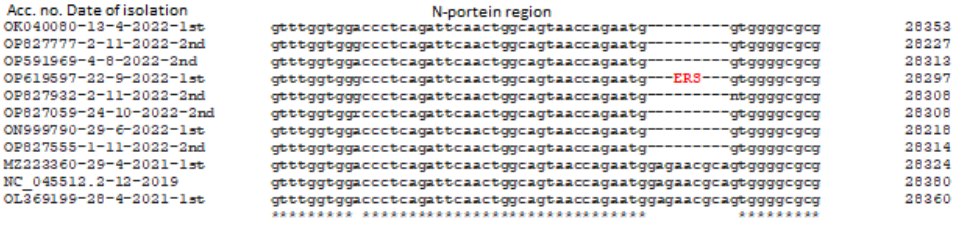

Figure 3B: *Multi-alignment of N-protein region showing [31]ERS deletion in many [3675]SGF mutants that were Omicron variants.*

Figure 4A: *Multi-alignment of GHVMV oligo selected sequences to demonstrate all Omicron isolates with [82]GHVMV, [3675]SGF deletions but [141]KSF. The accession number ON408727 was obtained from GHVMV-KSF oligo selected sequence where [141]KSF deletion was shown (acc. no. ON408727).*

Citation: Chakraborty AK (2023) The [82]GHVMV and [141]KSF Deletions in the Nsp1 Protein of ORF1ab Polyprotein Favour the Creation of Immune-Weak SARS-CoV-2. SunText Rev Virol 3(2): 137.

Figure 4B: *Multi-alignment of spike gene region of SARS-CoV-2 [82]GHVMV oligo-selected sequences. The ON408727 was pre-BA.4 variant with[141]KSF deletion (not shown here) and [24]LPP deletion but no [69]HV deletion in spike. Whereas, OP809949 sequence was BA.2 variant with [24]LPP deletion but no [69]HV deletion (acc. no. OP809949). Rest [82]GHVMV deletion mutants were BA.5 variants (OP741434, BS0005416, OX272297 etc.). All [82]GHVMV mutants had [31]ERS N-protein deletion and 26nt 3'UTR deletion (data not shown).*

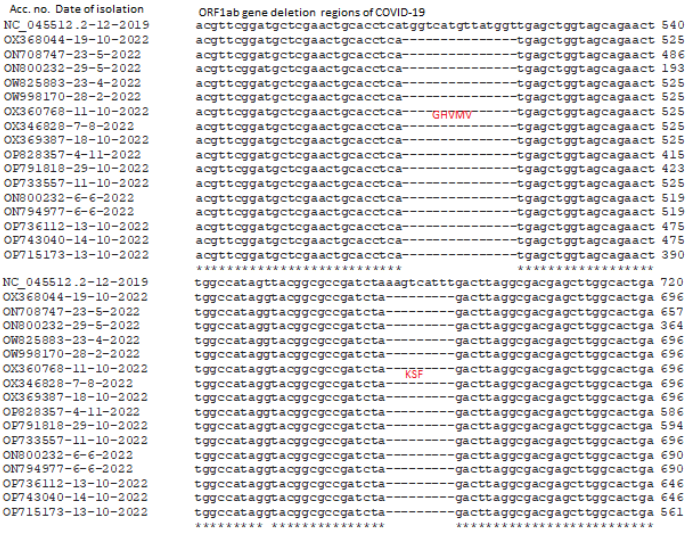

Figure 5A: *Multi-alignment of GHVMV-KSF oligo selected SARS-CoV-2 sequences showing both [82]GHVMV and [141]KSF deletions in all nsp1 proteins from February-November, 2022. Parts of the alignment with ORF1ab deletions were showed.*

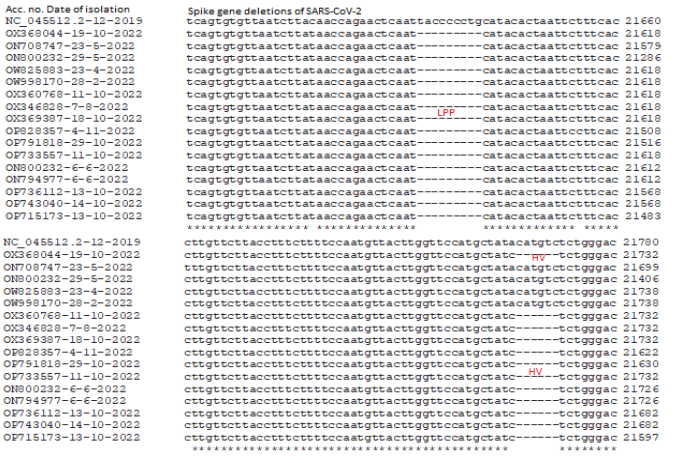

Figure 5B: *Multi-alignment of GHVMV-KSF oligo selected sequences showing spike gene 24LPP and 69HV deletions for Omicron variants. Most COVID-19 were omicron BA.4 subvariants with 141KSF-deletion in ORF1ab gene and four were BA.2 variants (ON708747, ON800232, OW825883,*

and OW998170) those had 24LPP deletion but no 69HV deletion in spike. All sequences carried N501Y and D614G in the spike mutations as well as P4517L mutation in RdRp domain of ORF1ab polyprotein (data not shown).

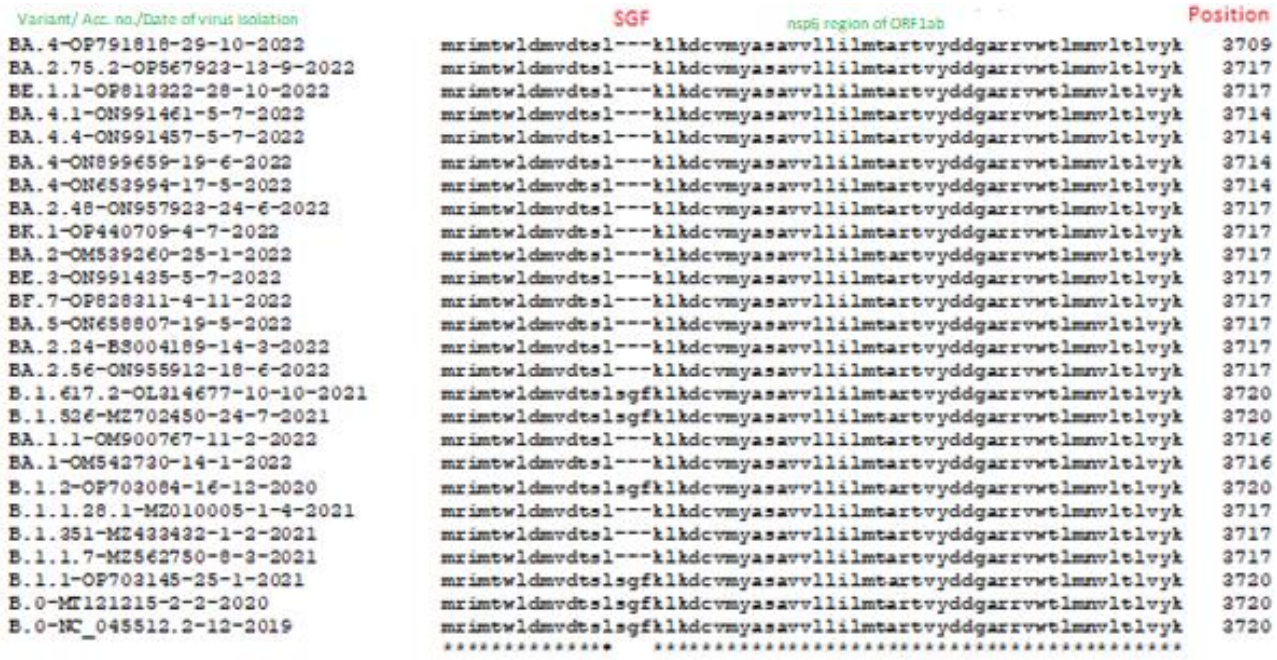

Figure 6A: *Multi-alignment to demonstrate the penetration of 3675SGF mutants in different corona virus variants with time. Surprisingly, 3675SGF deletion found in Alpha and Omicron variants but not in Delta variant (accession no. OL314677) and Iota variant (accession no. MZ702450). Such deletion was not also found in B.1.1 and B.1.2 early variants with D614G dominant spike mutation suggesting the 3675SGF three AA deletion was appeared in B.1.1.7 (Alpha) and related P.1 (Gamma) and B.1.351 (Beta) variants after June, 2020 but very much spread into Omicron variants in 2022. New omicron isolates BF.7, BK.1, BA.2.75.2, BQ.1.1 and BE.1.1 also had 3675SGF deletions.*

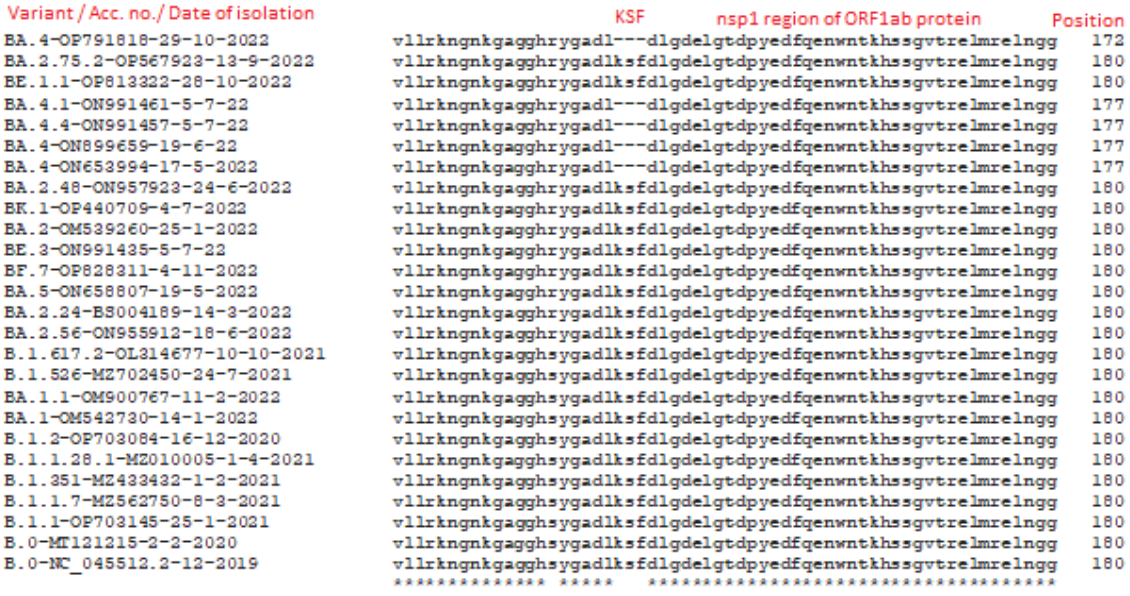

Figure 6B: *Multi-alignment as in figure-5A to demonstrate 141KSF deletion was found in only Omicron BA.4 subvariants. Note that 3675SGF deletion was found in all Omicron variants (BA.1, BA.2, BA.4 and BA.5) including B.1.1.7 (Alpha), B.1.1.28.1 (Gamma) and B.1.351 (Beta) variants. Similarly, 82GHVMV deletions carried recently into different Omicron variants only (not shown here).*

Citation: Chakraborty AK (2023) The ^{82}GHVMV and ^{141}KSF Deletions in the Nsp1 Protein of ORF1ab Polyprotein Favour the Creation of Immune-Weak SARS-CoV-2. SunText Rev Virol 3(2): 137.

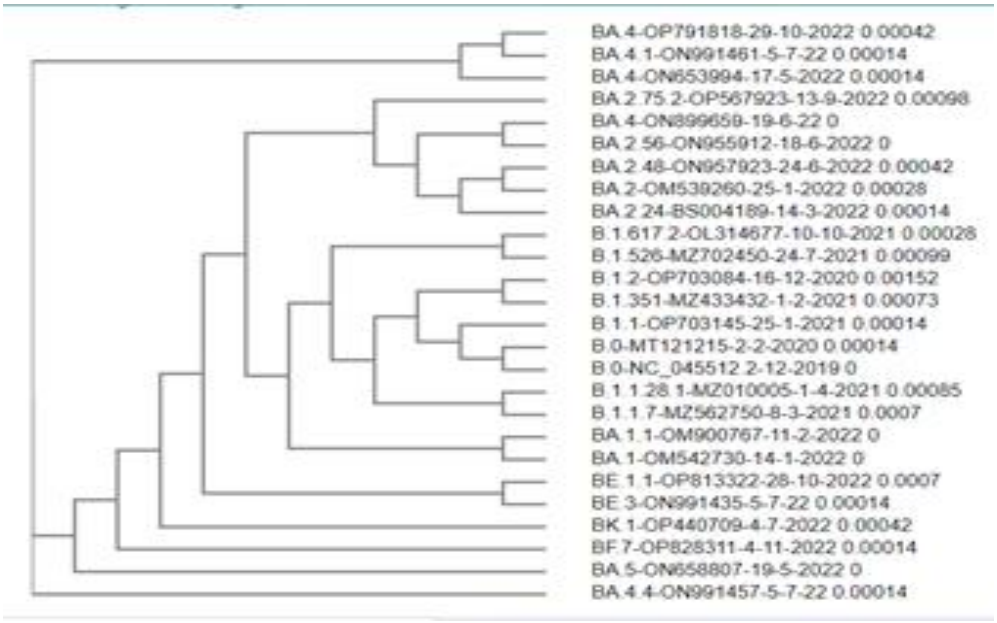

Figure 6C: *CLUSTAL-Omega Phylogenetic analysis to demonstrate relation among the COVID-19 variants. It was found that BK.1, BF.7 and BA.5 were related whereas B.1.617 and B.1.526 were closer. Further, BA.2.75.2 had common mutation similar to BA.4 variant and B.1.1.7 had some close relation to B.1.1.28.1 (P.1).*

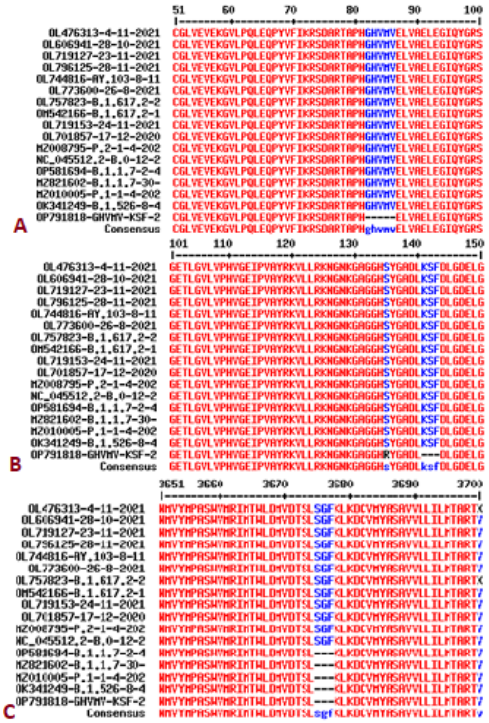

Figure 7: *Demonstration that 3675SGF deletion was not prominent in Delta variant (C) but found in Alpha, Beta, Gamma and Iota variants which appeared in early 2021. Similarly, the 82GHVMV (A) and 141KSF (B) deletions were Omicron corona virus specific and were appeared in 2022. Parts of the alignment with only deletion sites were shown here.*

Citation: Chakraborty AK (2023) The [82]GHVMV and [141]KSF Deletions in the Nsp1 Protein of ORF1ab Polyprotein Favour the Creation of Immune-Weak SARS-CoV-2. SunText Rev Virol 3(2): 137.

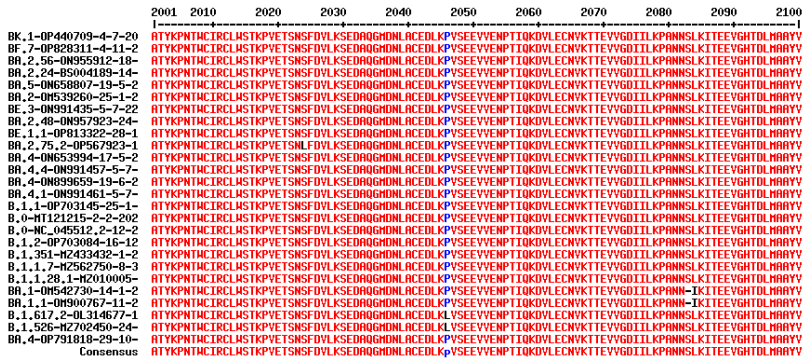

Figure 8A: *Multi-alignment of different SARS-CoV-2 ORF1ab protein sequences to detect of one amino acid deletion (2083S) in the nsp3 (C3 protease) domain of Omicron BA.1 and BA.1.1 variants.*

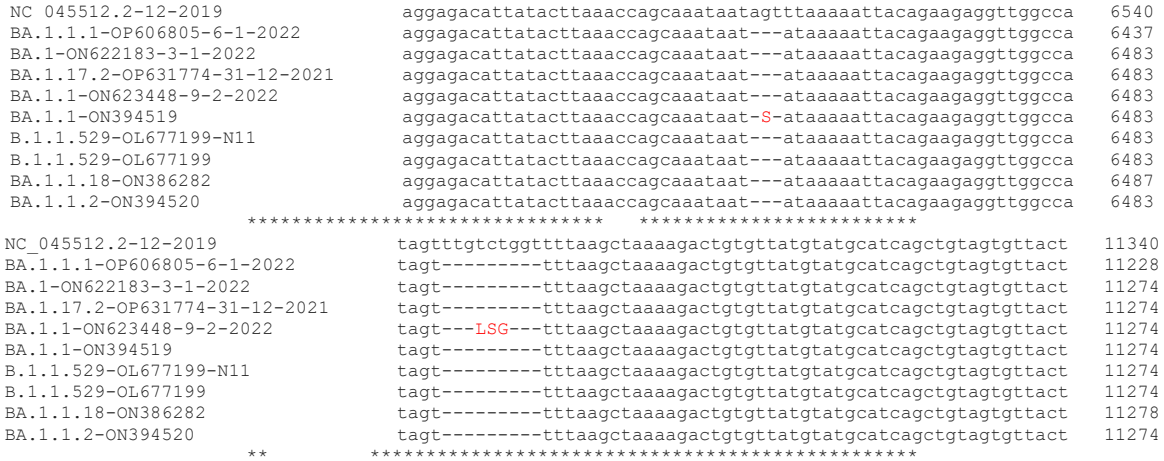

Figure 8B: *Demonstration of 2083S deleted SARS-CoV-2 genomes were Omicron BA.1 variant specific. Here, 3674LSG deletion happed in the same locus instead of 3675SGF deletion found in BA.2, BA.4 and BA.5 Omicron variants. The B.1.1.529 variant was BA.0, the renamed primary Omicron variant detected in December, 2021.*

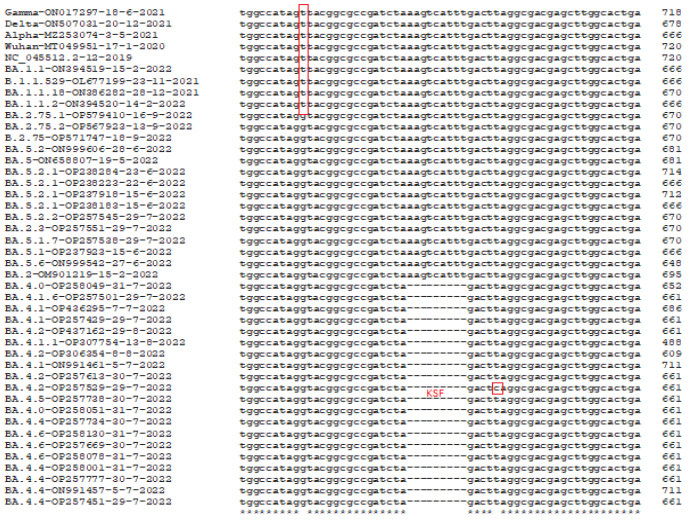

Figure 9: *Multi-alignment to demonstrate the 3675SGF deletions in all Omicron variants (figure-5) but 141KSF deletion only in Omicron BA.4 variant. The 3675GHVMV deletion was rare and only a "VMV" three AAs deletion found in BA.4 variant here (accession no. OP258049). Part of the alignment was shown.*

Citation: Chakraborty AK (2023) The [82]GHVMV and [141]KSF Deletions in the Nsp1 Protein of ORF1ab Polyprotein Favour the Creation of Immune-Weak SARS-CoV-2. SunText Rev Virol 3(2): 137.

Acc. no/Country/variant/date of isolation	SGF	ORF1ab protein region	Position
OP567923-BA.2.75.2-13-9-2022	mrimtwldmvdtsl---klkdcvmyasavvllilmtartvyddgarrvwtlmnvltlvyk		3717
OP791818-GHVMV-KSF-2022	mrimtwldmvdtsl---klkdcvmyasavvllilmtartvyddgarrvwtlmnvltlvyk		3709
ON991461-BA.4.1-5-7-22	mrimtwldmvdtsl---klkdcvmyasavvllilmtartvyddgarrvwtlmnvltlvyk		3714
OM539260-BA.2-25-1-2022	mrimtwldmvdtsl---klkdcvmyasavvllilmtartvyddgarrvwtlmnvltlvyk		3717
OP828311-BF.7-4-11-2022	mrimtwldmvdtsl---klkdcvmyasavvllilmtartvyddgarrvwtlmnvltlvyk		3717
OM542166-Delta-19-12-2021	mrimtwldmvdtslsgfklkdcvmyasavvllilmtartvyddgarrvwtlmnvltlvyk		3720
OL476313-USA-4-11-2021	mrimtwldmvdtslsgfklkdcvmyasavvllilmtartxyddgarrvwtlmnvltlvyk		3720
OL606941-USA-28-10-2021	mrimtwldmvdtslsgfklkdcvmyasavvllilmtartvyddgarrvwtlmnvltlvyk		3720
MW181828-India-6-5-2020	mrimtwldmvdtslsgfklkdcvmyasavvllilmtartvyddgarrvwtlmnvltlvyk		3720
MZ010005-Gamma-1-4-2021	mrimtwldmvdtsl---klkdcvmyasavvllilmtartvyddgarrvwtlmnvltlvyk		3717
MW937195-USA-30-03-2021	mrimtwldmvdtsl---klkdcvmyasavvllilmtartvyddgarrvwtlmnvltlvyk		3717
MW889937-USA-11-03-2021	mrimtwldmvdtsl---klkdcvmyasavvllilmtartvyddgarrvwtlmnvltlvyk		3717
MW937198-USA-05-04-2021	mrimtwldmvdtsl---klkdcvmyasavvllilmtartvyddgarrvwtlmnvltlvyk		3717
MZ433432-Beta-1-2-2021	mrimtwldmvdtsl---klkdcvmyasavvllilmtartvyddgarrvwtlmnvltlvyk		3717
MW932096-USA-27-03-2021	mrimtwldmvdtsl---klkdcvmyasavvllilmtartvyddgarrvwtlmnvltlvyk		3717
OK341249-Iota-8-4-2021	mrimtwldmvdtsl---klkdcvmyasavvllilmtartvyddgarrvwtlmnvltlvyk		3717
MW889941-USA-16-03-2021	mrimtwldmvdtslsgfklkdcvmyasavvllilmtartvyddgarrvwtlmnvltlvyk		3720
OM542730-BA.1-14-1-2022	mrimtwldmvdts---fklkdcvmyasavvllilmtartvyddgarrvwtlmnvltlvyk		3716
MW889932-USA-10-03-2021	mrimtwldmvdtslsgfklkdcvmyasavvllilmtartvyddgarrvwtlmnvltlvyk		3720
MW356786-Taiwan-4-10-2020	mrimtwldmvdtslsgfklkdcvmyasavvllilmtartvyddgarrvwtlmnvltlvyk		3720
MT582499-Germany-28-02-2020	mrimtwldmvdtslsgfklkdcvmyasavvllilmtartvyddgarrvwtlmnvltlvyk		3720
MW425719-USA-22-6-2020	mrimtwldmvdtslsgfklkdcvmyasavvllilmtartvyddgarrvwtlmnvltlvyk		3720
MZ008795-Zeta-1-4-2021	mrimtwldmvdtslsgfklkdcvmyasavvllilmtartvyddgarrvwtlmnvltlvyk		3720
MW555786-India-30-06-2020	mrimtwldmvdtslsgfklkdcvmyasavvllilmtartvyddgarrvwtlmnvltlvyk		3720
MW365067-Chile-11-05-2020	mrimtwldmvdtslsgfklkdcvmyasavvllilmtartvyddgarrvwtlmnvltlvyk		3720
MT240479-Pakistan-4-3-2020	mrimtwldmvdtslsgfklkdcvmyasavvllilmtartvyddgarrvwtlmnvltlvyk		3720
MT578017-Bangla-23-5-2020	mrimtwldmvdtslsgfklkdcvmyasavvllilmtartvyddgarrvwtlmnvltlvyk		3720
MT738101-Brasil-13-03-2020	mrimtwldmvdtslsgfklkdcvmyasavvllilmtartvyddgarrvwtlmnvltlvyk		3720
MT628700-HongKong-30-3-2020	mrimtwldmvdtslsgfklkdcvmyasavvllilmtartvyddgarrvwtlmnvltlvyk		3720
MW056033-Spain-05-08-2020	mrimtwldmvdtslsgfklkdcvmyasavvllilmtartvyddgarrvwtlmnvltlvyk		3720
MT814698-Egypt-19-7-2020	mrimtwldmvdtslsgfklkdcvmyasavvllilmtartvyddgarrvwtlmnvltlvyk		3720
MT246489-USA-14-3-2020	mrimtwldmvdtslsgfklkdcvmyasavvllilmtartvyddgarrvwtlmnvltlvyk		3720
MT079853-China-22-01-2020	mrimtwldmvdtslsgfklkdcvmyasavvllilmtartvyddgarrvwtlmnvltlvyk		3720
NC_045512.2-China-12-2019	mrimtwldmvdtslsgfklkdcvmyasavvllilmtartvyddgarrvwtlmnvltlvyk		3720
MT066156-Italy-30-01-2020	mrimtwldmvdtslsgfklkdcvmyasavvllilmtartvyddgarrvwtlmnvltlvyk		3720

Figure 10: *Multi-alignment of ORF1ab proteins of different COVID-19 variants since 2019 to 2022 to demonstrate the 3675SGF deletion occurred in early 2021. Worldwide data suggested that no 3675SGF as well as 141KSF deletion was detected during 2019-2020.*

	2083V	
Gamma-ON017297-18-6-2021	tgtaggagacattatacttaaaccagcaaataatagtttaaaaattacagaagaggtttg	6535
Delta-ON507031-20-12-2021	tgtaggagacattatacttaaaccagcaaataatagtttaaaaattacagaagaggttgg	6495
Alpha-MZ253074-3-5-2021	tgtaggagacattatacttaaaccagcaaataatagtttaaaaattacagaagaggttgg	6483
Wuhan-MT049951-17-1-2020	tgtaggagacattatacttaaaccagcaaataatagtttaaaaattacagaagaggttgg	6537
NC_045512.2-12-2019	tgtaggagacattatacttaaaccagcaaataatagtttaaaaattacagaagaggttgg	6537
BA.1.1-ON394519-15-2-2022	tgtaggagacattatacttaaaccagcaaataata---taaaaattacagaagaggttgg	6480
B.1.1.529-OL677199-23-11-2021	tgtaggagacattatacttaaaccagcaaataata-V-taaaaattacagaagaggttgg	6480
BA.1.1.18-ON386282-28-12-2021	tgtaggagacattatacttaaaccagcaaataata---taaaaattacagaagaggttgg	6484
BA.1.1.2-ON394520-14-2-2022	tgtaggagacattatacttaaaccagcaaataata---taaaaattacagaagaggttgg	6480
BA.2.75.1-OP579410-16-9-2022	tgtaggagacattatacttaaaccagcaaataatagtttaaaaattacagaagaggttgg	6487
BA.2.75.2-OP567923-13-9-2022	tgtaggagacattatacttaaaccagcaaataatagtttaaaaattacagaagaggttgg	6487
B.2.75-OP571747-18-9-2022	tgtaggagacattatacttaaaccagcaaataatagtttaaaaattacagaagaggttgg	6487
BA.5.2-ON999606-28-6-2022	tgtaggagacattatacttaaaccagcaaataatagtttaaaaattacagaagaggttgg	6498
BA.5-ON658807-19-5-2022	tgtaggagacattatacttaaaccagcaaataatagtttaaaaattacagaagaggttgg	6498
BA.5.2.1-OP238284-23-6-2022	tgtaggagacattatacttaaaccagcaaataatagtttaaaaattacagaagaggttgg	6531
BA.5.2.1-OP238223-22-6-2022	tgtaggagacattatacttaaaccagcaaataatagtttaaaaattacagaagaggttgg	6483
BA.5.2.1-OP237918-15-6-2022	tgtaggagacattatacttaaaccagcaaataatagtttaaaaattacagaagaggttgg	6529
BA.5.2.1-OP238183-15-6-2022	tgtaggagacattatacttaaaccagcaaataatagtttaaaaattacagaagaggttgg	6483
BA.5.2.2-OP257545-29-7-2022	tgtaggagacattatacttaaaccagcaaataatagtttaaaaattacagaagaggttgg	6487
BA.2.3-OP257551-29-7-2022	tgtaggagacattatacttaaaccagcaaataatagtttaaaaattacagaagaggttgg	6487
BA.5.1.7-OP257538-29-7-2022	tgtaggagacattatacttaaaccagcaaataatagtttaaaaattacagaagaggttgg	6487
BA.5.1-OP237923-15-6-2022	tgtaggagacattatacttaaaccagcaaataatagtttaaaaattacagaagaggttgg	6483
BA.5.6-ON999542-27-6-2022	tgtaggagacattatacttaaaccagcaaataatagtttaaaaattacagaagaggttgg	6465
BA.2-OM901219-15-2-2022	tgtaggagacattatacttaaaccagcaaataatagtttaaaaattacagaagaggttgg	6512
BA.4.0-OP258049-31-7-2022	tgtaggagacattatacttaaaccagcaaataatagtttaaaaattacagaagaggttgg	6469
BA.4.1.6-OP257501-29-7-2022	tgtaggagacattatacttaaaccagcaaataatagtttaaaaattacagaagaggttgg	6481
BA.4.1-OP436295-7-7-2022	tgtaggagacattatacttaaaccagcaaataatagtttaaaaattacagaagaggttgg	6503
BA.4.1-OP257429-29-7-2022	tgtaggagacattatacttaaaccagcaaataatagtttaaaaattacagaagaggttgg	6478
BA.4.2-OP437162-29-8-2022	tgtaggagacattatacttaaaccagcaaataatagtttaaaaattacagaagaggttgg	6478
BA.4.1.1-OP307754-13-8-2022	tgtaggagacattatacttaaaccagcaaataatagtttaaaaattacagaagaggttgg	6305
BA.4.2-OP306354-8-8-2022	tgtaggagacattatacttaaaccagcaaataatagtttaaaaattacagaagaggttgg	6426
BA.4.1-ON991461-5-7-2022	tgtaggagacattatacttaaaccagcaaataatagtttaaaaattacagaagaggttgg	6528
BA.4.2-OP257613-30-7-2022	tgtaggagacattatacttaaaccagcaaataatagtttaaaaattacagaagaggttgg	6478
BA.4.2-OP257529-29-7-2022	tgtaggagacattatacttaaaccagcaaataatagtttaaaaattacagaagaggttgg	6478
BA.4.5-OP257738-30-7-2022	tgtaggagacattatacttaaaccagcaaataatagtttaaaaattacagaagaggttgg	6478
BA.4.0-OP258051-31-7-2022	tgtaggagacattatacttaaaccagcaaataatagtttaaaaattacagaagaggttgg	6478
BA.4.4-OP257734-30-7-2022	tgtaggagacattatacttaaaccagcaaataatagtttaaaaattacagaagaggttgg	6478
BA.4.6-OP258130-31-7-2022	tgtaggagacattatacttaaaccagcaaataatagtttaaaaattacagaagaggttgg	6478
BA.4.6-OP257669-30-7-2022	tgtaggagacattatacttaaaccagcaaataatagtttaaaaattacagaagaggttgg	6478
BA.4.6-OP258078-31-7-2022	tgtaggagacattatacttaaaccagcaaataatagtttaaaaattacagaagaggttgg	6478
BA.4.4-OP258001-31-7-2022	tgtaggagacattatacttaaaccagcaaataatagtttaaaaattacagaagaggttgg	6478
BA.4.4-OP257777-30-7-2022	tgtaggagacattatacttaaaccagcaaataatagtttaaaaattacagaagaggttgg	6478
BA.4.4-ON991457-5-7-2022	tgtaggagacattatacttaaaccagcaaataatagtttaaaaattacagaagaggttgg	6528
BA.4.4-OP257451-29-7-2022	tgtaggagacattatacttaaaccagcaaataatagtttaaaaattacagaagaggttgg	6478

Figure 11: *Multi-alignment to demonstrate that 2083Y deletion in nsp3 was occurred in Omicron BA.1 variant only but not in Omicron BA.2, BA.4 and BA.5 subvariants as well as other deadly variants like Alpha, Beta and Delta variants.*

Citation: Chakraborty AK (2023) The ^{82}GHVMV and ^{141}KSF Deletions in the Nsp1 Protein of ORF1ab Polyprotein Favour the Creation of Immune-Weak SARS-CoV-2. SunText Rev Virol 3(2): 137.

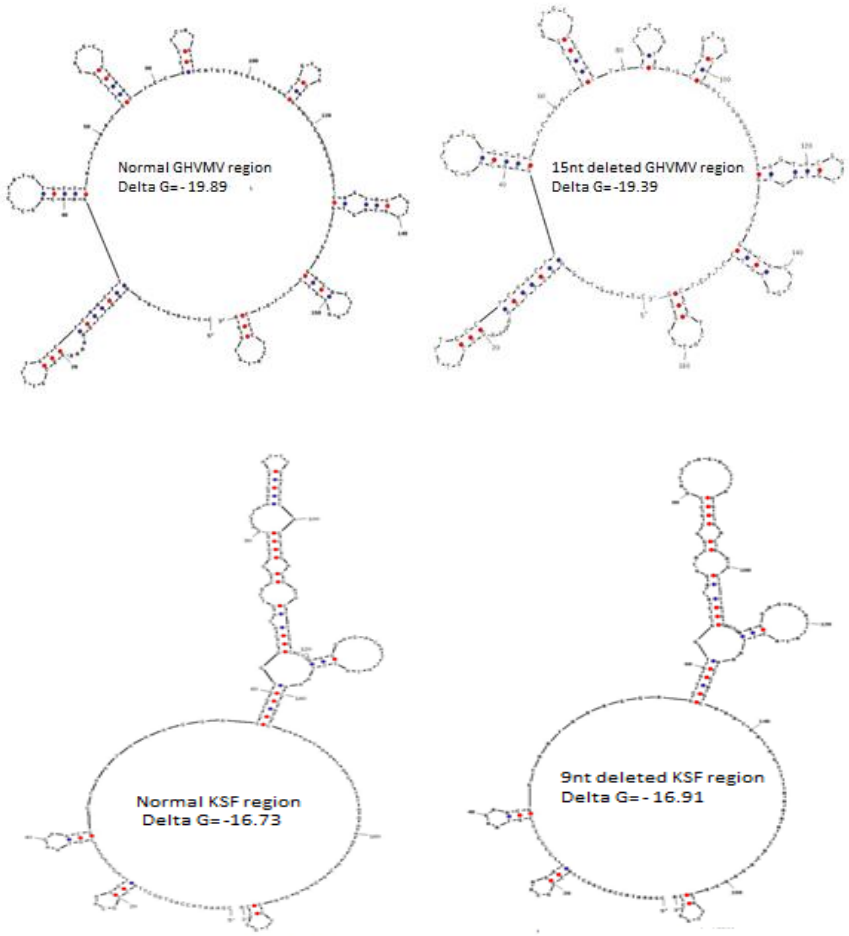

Figure 12: *Secondary hairpin structures in the 82GHVMV (upper) and 141KSF (lower) deletion boundary sequences of the nsp1 gene. In GHVMV locus, the distance between hairpin 4 and hairpin 5 was changed while in KSF locus, a very stiff hairpin was found where the size of nob was increased in 141KSF deletion variants.*

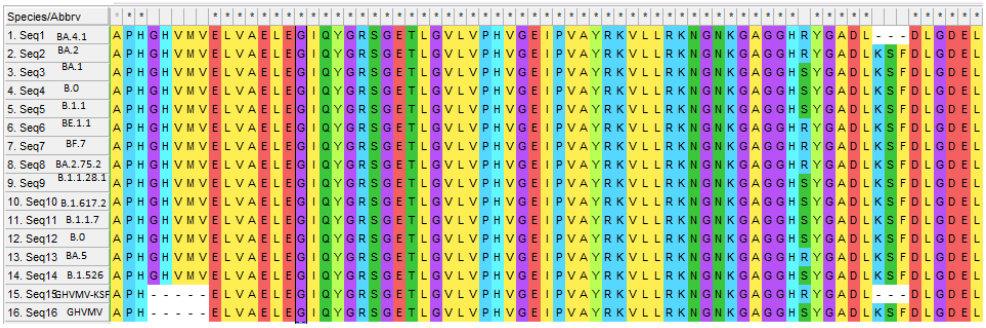

Figure13: *The 180 amino acids Nsp1 protein multi-alignment (MEGA v.11) to detect 82GHVMV and 141KSF deletions in 16 different SARS-CoV-2 variants.*

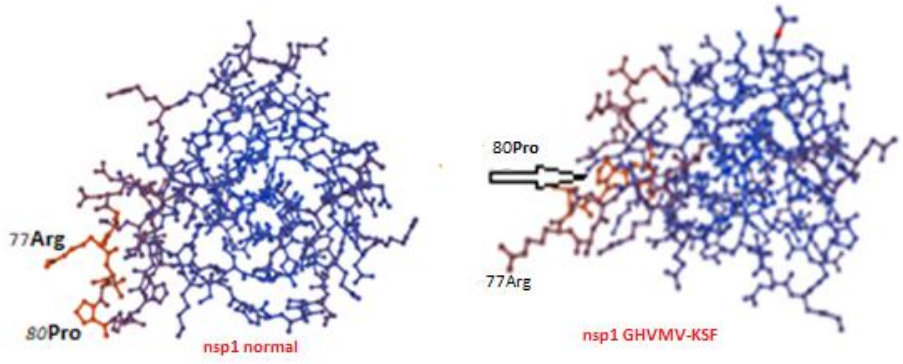

Figure 14: *Model structure of normal nsp1 protein and GHVMV-KSF deletion nsp1 mutant. Profound changes in 3-D structure was found although Ramchandran plot suggested 98.18% favoured in normal nsp1 protein over 98.1% in deletion mutant.*

The sequences, OP619597, OP827932 and OP827059 had 24LPP and 69HV deletions but no 141KSF deletion in ORF1ab and were omicron BA.5 variants. The sequence ON999790 had 24LPP in spike but no 69HV deletion and was omicron BA.2 variant. This data confirmed the heterogeneous population of corona viruses in different 3675SGF deletion mutants which appeared early. Multi-alignment of GHVMV oligo selected eight sequences demonstrated all recent Omicron isolates with ^{3675}SGF deletions but no ^{141}KSF deletion (Figure 4A). The 24LPP and 69HV spike deletions suggested all were omicron corona virus (Figure 4B). Further, we aligned GHVMV-KSF deletion oligo selected sixteen sequences to show such sequences had both ^{82}GHVMV plus ^{141}KSF deletion in the nsp1 protein including 3675SGF (ORF1ab), 31ERS (N-protein), 24LPP (spike) and 26nt 3'UTR deletions (Figure 5A). Thus, mostly were omicron BA.4 sub-variants with 141KSF-deletion in ORF1ab gene and four were BA.2 variants (ON708747, ON800232, OW825883, and OW998170) those had 24LPP deletion but no 69HV deletion (Figure 5B). All sequences carried dominant N501Y and D614G non-sense point mutation in the spike as well as P4517L mutation in RdRp domain of ORF1ab polyprotein (data not shown). Interestingly, the ORF7a gene hotspot deletion and mutation sites were affected in the few GHVMV-KSF oligo selected Omicron corona viruses. Among them, a 112nt large deletion detected in accession number OP791818 at nucleotide position 27547. While 20nt deletion detected in accession number OP828357 at nucleotide 27546 and 12nt (5'-ttt act ctc caa-3') deletion detected in accession number OX368044 at nucleotide 27679 while mere 3nt (5'-tac-3') deletion detected in accession number OX369387 at nucleotide 27681 (data not shown). We multi-aligned different corona virus variants to demonstrate the spread of 82GHVMV, 141KSF and 3675SGF deletions in different variants with time (Figure 6A). Most viruses since early 2021 had 3675SGF deletion except most deadly Delta variant whereas ^{141}KSF nsp1 deletion appeared in early 2022 in omicron BA.4 variants (Figure 6B). We performed the CLUSTAL-omega phylogenetic analysis to show relation among the sixteen different corona virus lineages to confirm Delta variant was unique sub-variant (Figure 6C). The COVID-19 B.1.617.2 and B.1.526 variants were closely related having no 3675SGF deletion in nsp6 protein. Similarly, BF.7, BK.1, BE.1.1 were related to BA.5 variant. Whereas B.1.1.7 (Alpha), B.1.351 (Beta) and B.1.1.28.1 (Gamma) were closely related with abundant ^{3675}SGF deletion as reported earlier. Further, to conclude that ^{3675}SGF deletion was not carried in Delta variant, we selected B.1.617.2 and AY.103 Delta corona virus sequences but did not detected any ^{82}GHVMV, ^{141}KSF and ^{3675}SGF deletions (Figure 7). However, KSF deletion only happened in omicron BA.4 variants as we demonstrated earlier. Importantly, we detected one amino acid deletion in the omicron BA.1 and BA.1.1 variants at 2083S of ORF1ab polyprotein as demonstrated in (Figure 8A). We multi-aligned B.1.1.529, BA.1, BA.1.1, BA.1.1.2, BA.1.1.18 sub-variants sequences to conclude that 2083S deletion was indeed BA.1 variant specific (Figure 8B). In figure-9, we extensively showed the ^{141}KSF deletion was associated with only omicron BA.4 variants. To trap the early date ^{3675}SGF deletion, we aligned sequences from different countries to demonstrate that early 2021 was date time for appearance of such deletion when no KSF deletion was found (Figures 9-10). Further we showed that the ^{2083}Y deletion was found in omicron BA.1 variant only but not in omicron BA.2, BA.4 and BA.5 sub-variants as well as other deadly variants like Alpha, Beta and Delta variants (Figure 11). Further, we analysed the hairpin structures of ~250nt sequences surroundings ^{82}GHVMV and ^{141}KSF deletion sites. Demonstrated that special hairpin nob-like structure altered in ^{82}GHVMV locus and a stiff long hairpin in ^{141}KSF locus also slightly changed (Figure 12). Such hairpin structures may explain the reason of deletions involving recombination enzymes like RNA topoisomerase (nsp2) or other cellular recombination enzymes. We used MEGAV.11 software to align 14 nsp1 sequences and only one sequence was BA.4.1 variant with ^{141}KSF deletion (Figure-13). The Seq15 was GHVMV-KSF oligo selected sequence whereas

the Seq16 was GHVMV oligo selected sequence. A S135R mutation in nsp1 was found in Omicron variants (BA.2, BE.1.1, BF.7, BA.2.75.2, BA.4.1 and BA.5) but not in Alpha, Gamma or Delta variants. Interestingly, GHVMV oligo selected sequence (accession no. ON972497) has no such mutation but with GHVMV-KSF oligo selected one (accession no. OP200462) which was BA.4 variant. Model structure (SWISS-MODEL) of normal nsp1 protein and GHVMV-KSF deletion mutant suggested a profound change in 3-D residues although Ramachandran plot suggested 98.18% favoured in normal nsp1 protein over 98.1% in GHVMV-KSF deletion mutant (Figure 14). The Clash Score increased in mutant from 0.00 to 1.77 whereas Mol Probity Score change from 0.51 to 0.96 based on published nsp1 model structures (PDB: 7K3N and 6ZMI). We showed in figure-14 how in mutant nsp1 protein Proline 80 residue was hidden and Arginine 77 residue was protruded in deleted nsp1 protein. Likely such changes may lower the binding efficiency of nsp1 protein to human 80S ribosome complex to inhibit host protein synthesis.

Discussion

We clearly demonstrated that Delta corona virus variant has no ^{82}GHVMV, ^{141}KSF, ^{2083}Y and ^{3675}SGF deletions. Further we clearly showed that among the four deletions described, the SGF deletion was appeared first in B.1.1.7 during early 2021. Similarly, ^{2083}Y, a single amino acid deletion was specific for Omicron BA.1 variant whereas KSF deletion was specific for omicron BA.4 variant and both were appeared in early, 2022. While deletion in the GHVMV locus was limited and only appeared in Omicron variants. The nsp1 is a hotspot of deletion and may be target drug design. We have clearly demonstrated the deletions and dominant point mutations in the ORF1ab gene that gave 7096 AA protein which on proteolysis produced 16 polypeptides (nsp1-nsp16) with diverse functions. In majority of corona virus population, the most frequent and common mutation like T265I (C1059T) in nsp2 RNA topoisomerase, P323L(C17747T) in RdRp, D614G (A23403G) in spike, Q57H (G25563T) in ORF3a and L84S (T28144C) in ORF8, were detected [59]. Khalid et al reported the insertion of TTT at 11085 creating one extra amino acid (F) to the NSP6 protein at amino acid position 38. The mutations and deletions were ubiquitous but analysis of 20 or more sequences sometime might give erroneous data and only desired portion of the multi-alignment data was presented [60]. The nsp6 protein has 7 putative trans-membrane helices and binds to TANK binding kinase 1 (TBK1) and suppresses the phosphorylation of interferon regulatory factor 3 (IRF3) thereby, lowering the Type I interferon response; to evade host defences. The point mutations were also important in different domains of ORF1ab polyproteins. The nsp13 RNA helicase-rRNA methyltransferase P504L and Y541C mutations were documented in samples before April, 2020 [61]. Different five mutations; T265I in nsp2, T1246I in nsp3 protease, G3278S in nsp5 proteinase, L3606F in nsp6 and P4715L in RdRp were found common in corona viruses analysed from six geographical locations; Africa, Asia, Europe, North America, Oceania and South America [24]. Other than SGF (3675-3677) deletion of nsp6, the F3760 and MVD (3669-3671) deletions were also reported. A YHFRELGVV (4738- 4746) deletion in the RNA dependent RNA polymerase or N389, GLNDNL (445-450), V649, T770, C784 deletions in the RNA topoisomerase were reported by same group [62]. Quite surprising 6 and 10 amino acids deletions were reported in spike protein at 365 and 679 positions respectively (accession nos. MT621560 and MT370992 respectively). Thus, deletion and point mutation in most RNA viruses were universal although we were unable to show such mutation in the RNA polymerase enzyme except P4715L. Importantly, recent Omicron virus 24LPP deletion in spike and 31ERS deletion in N-protein were very important in regulating COVID-19 immune-function and replication. We do not know the consequence of 26nt 3'-UTR deletion as we detected in many Omicron variants. It assumed then that such deletions might be lowering the SARS-CoV-2 overall pathogenicity. The Alpha variant N501Y mutation increased transmission and most importantly D614G mutation found in all variants since March, 2020 which made corona virus deadly. The Omicron corona virus 20-25 mutations over Wuhan corona virus in the RBD domain of spike absolutely gave COVID-19 immune-escape character and a repeated-infections even after 2-3 doses of vaccine intake were reported worldwide. If the nsp1 82GHVMV and 141KSF deletions in the nsp1 protein in Omicron variants has any relation to spike 24LPP or 31ERS N-protein deletions was not known [59]. Molecular modelling suggested that nsp1 deletions might have negative impact of its trans-activator or moderator function with host genes. Similarly, we do not know, why is the 2083Y deletion in nsp3 protease of Omicron corona virus was BA.1 variant specific? Fisher et al. reported that 3675SGF deletion in nsp6 affected the virus replication machinery as reduced virus titre was found [31]. It appeared that 3675SGF deletion was not granted in Delta variants (AY.103, B.1.617.2) (figure-9 and figure-11). However, we found a popular corona virus Delta variant characteristic of 157FR two amino acids deletion in spike protein and 119DF deletion in the ORF8 protein (data not shown). The 3675GHVMV deletion in the nsp1 protein was found very limited with only few hundred in the database and 141KSF deletion in the same protein was very much abundant in Omicron BA.4. Variant and subvariants (figure-7 and figure-8). Sosnowski et al. demonstrated that conserved key residues in the amino-terminal half of the NSP1 protein were essential for evasion to the inhibitory effect of NSP1 on translation [43,47]. Fisher et al demonstrated the multifunctional role of nsp1 to shut off cellular

protein synthesis, to degrade mRNAs and to block cellular interferon response [31]. We presumed a hairpin nob-like structures located at the nsp1 locus regulated such deletions (figure-14). Further, model structure clearly demonstrated the impact of such 8 AAs deletions in the nsp1 protein changing its overall 3-D structure. Taken together, we demonstrated the distribution of COVID-19 ORF1ab major deletions since December 2019 to December 2022 in different variant and sub variants which was never explored [63]. Most vivid example was, such deletion was not detected in Corona virus Delta variant which was impacted society in a horrible way with million deaths between May, 2021 to December, 2021. Surely, we have to explore the most recent BA.2.75, BA.4.6, BA.5.2.1, BF.7 and BE.1.1 lineages if any new deletion to appear changing epidemic spread of corona virus infections [64-65].

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Competent interest

The author declares no conflict of interest. This paper uses only computer-generated data analysis using SARS-CoV-2 Database.

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