RESEARCH ARTICLE

The popular ^{3675}SGF deletion in Nsp6 domain of ORF1ab polyprotein of SARS-CoV-2 was spread into alpha, beta, gamma, iota and omicron variants except highly infectious and death promoting delta variant

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ABSTRACT

The SARS-CoV-2 was appeared in mid-2020 but the first RNA sequence was available since December, 2020. The higher transmission and disease severity were seen since acquisition of D614G dominant point mutation in spike protein as well as P4715L point mutation in the RNA-dependent RNA polymerase (RdRp). The next event occurred in B.1.1.7 Alpha variant was acquisition of N501Y point mutation and 69HV two AAs deletion in the spike protein. Interestingly, 3675SGF three AAs deletions were also occurred in *ORF1ab* polyprotein in B.1.1.7 variants. The appearance of notorious Delta variant in Mid-2021 was shocking as too many deaths happened worldwide. The Delta variants (B.1.617.2 and AY.X) acquired 157FR two AAs deletion instead 69HV in B.1.1.7 variant. Here, we showed that Delta variant was spread worldwide but has no 3675SGF deletion. Moreover, 3675SGF deletion was found in all Omicron variants which were appeared in December, 2021 and its subvariants BA.2.75, BA.5.2.1.7 (BF.7), BQ.1, BQ.1.1 and XBB.1.5 which were spreading now worldwide with mild infections. Thus, Delta variant has very complete *ORF1ab* protein (9096 AAs) to attend high titre and to infect more human lung cells. Further, 119DF deletion in *ORF8* activator protein and 157FR deletion in spike might be contributed to higher pathogenicity in Delta including P2046L and P2287S mutations in nsp3 main protease, P4715L and G5063S mutations in RdRp and P6128S and A6319V mutations in nsp14 Ribonuclease. Further, complete attenuation of Delta corona virus spread recently likely due to herd immunity and wide spread inoculation with COVID-19 vaccine.

Key Words: *SARS-CoV-2, Delta variant, SGF deletion, Nsp6, ORF1ab polyprotein, Higher transmission, High titre COVID-19*

INTRODUCTION

The COVID-19 virus is a ~30kb single-stranded positive-sense large RNA virus which infects human lung cells to cause corona disease since 2020 [1]. COVID-19 is related to six different coronaviruses like CoV-229E, CoV-HKU1, CoV-OC43, CoV-NL63, SARS-CoV, and Middle East Respiratory Syndrome Coronavirus (MERS-CoV) which were known since 2003-2012 [2,3]. Within two years the coronavirus acquired many mutations and deletions with the difference in its pathogenicity and severity to cause death [4-8]. Five VOCs of SARS-CoV-2 mainly caused millions of death worldwide and named B.1.1.7 (Alpha; U.K.), B.1.351 (Beta; South Africa), P.1 (Gamma; Brazil), B.1.617.2 (Delta; India), and B.1.1.529 (Omicron; USA, Africa). Analysis suggested that spike protein (*1273AA*) of COVID-19 had gone through extensive mutations and deletions than large polyprotein *ORF1ab* (7096aa) which was degraded into sixteen polypeptides like RNA topoisomerase (nsp2), proteases (nsp3 and nsp5), RNA-dependent RNA polymerase (nsp12), RNA helicase-capping methyltransferase (nsp13), RNases (nsp14 and nsp15) and 2'-uridine methyltransferase (nsp16) [9-13]. The different peaks of VOCs appeared at different times and stayed for some time but a new wave appeared due to the antigenic shift of COVID-19 usually due to spike protein mutations [14,15]. Here, we showed that more severe form of COVID-19, Delta coronaviruses have no deletion mutation in the ORF1ab polyprotein (9096 AAs).

MATERIALS AND METHODS

We searched PubMed to get an idea of published papers on ORF1ab and also searched the SARS-CoV-2 NCBI database using BLAST-N and BLAST-X search methods to get sequences. Multi-alignment of protein was done by MultAlin software and multi-alignment of DNA by CLUSTAL-Omega software, EMBL-EBI [15-20]. The *ORF1ab* mutants were obtained by BlastN search of deletion boundary of 60-100nt sequence and then analyzing the sequences with 95%-100% similarities [21]. The other *ORF1ab* mutants were detected by Blast-N search and Blast-X- search with selected deletion boundaries. The hairpin structure of 120-200nt sequence was done by Oligo Analyzer 3.1 software (Integrated DNA Technologies). The protein 3-D structure was determined by SWISS-Model software with normal vs. mutant peptides [22,23].

RESULTS

Multi-alignment was a powerful tool to compare DNA, RNA, and protein sequences from different sources to see mutations and deletions of RNA viruses like SARS-CoV-2. The multi-alignment of 30kb genomes gave 100 pages of data and thus we only showed the representative area with deletions and point mutations (Figure 1). There were hundreds of silent mutations genome-wide but unless there was an amino acid change, title effects might occur. Figure 2 showed a multi-alignment portion of different COVID-19 sequences where SGF deletion (5'-TCTGGTTTT-3') occurred. We had given accession numbers, type of variant, and date of coronavirus isolation from patients starting Wuhan virus (B.0 variant) which was available first in December 2020. The SGF deletion was not prominent in early lineages like B.1 and B.1.2, as well as higher variants like B.1.160, B.1.389, B.1.177, B.1.177.17, B.1.177.218, B.1.416, B.1.429 including B.1.1.37, B.1.1.301 and B.1.1.317 but surprisingly B.1.1.7 variant and B.1.526 variant, had SGF deletion. More surprising fact there was no SGF deletion in B.1.617.2 Delta variant (accession numbers: OL475604, OL745448, OU877926, OK465723, OM045751, OQ121361, OQ119975, OQ119976, OQ121585, OQ121550, OQ099094 and OQ099096) (Figure 2). The total amino acids of *ORF1ab* polyprotein varied in different important COVID-19 variants and subvariants. For example, Wuhan=9096 AAs as also Delta variant. But three amino acid deletions (3675SGF) were found in ORF1ab protein (nsp6 protein region) of Alpha and Omicron BA.2/BA.5 (ORF1ab=7093 AAs) but at the same region 3674LSG deletion, as well as extra 2083S deletion, were found in Omicron BA.1 coronavirus (*ORF1ab*=7092 AAs). Whereas, extra three amino acids (141KSF) deletions were found in Omicron BA.4 variant

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(*ORF1ab*=7090 AAs) and such change was utilized to detect BA.4 Omicron variant by BLAST-2 alignment with oligonucleotide selected at the deletion boundary [24]. We multi-aligned 40 sequences of Omicron BA.1, BA.2, BA.4, and BA.5 subvariants including B.1.1.529 to demonstrate SGF deletion happened in all these Omicron variants including recently appeared B.2.75, BF.7, BQ.1 and XBB.1 (Figure 2). Truly, before the appearance of Omicron variants, B.1.617.2 and AY.X were the major coronavirus population but no SGF deletion was detected! On the other hand, all Omicron populations carried such SGF deletion! Such special distribution of SGF deletion suggested some way Omicron was derived from B.1.1.7 (Alpha) population or related B.1.526 (Iota) and P.1 (Gamma) but not B.1.351 (Beta). We thought B.1.1.7 may the source of the Omicron BA.1 variant because 69HV deletion in the spike appeared in B.1.1.7 first but Omicron BA.2 had no such 69HV deletion and that way such a variant may be created from P.1 or B.1.526 variant. However, the creation of Omicron~20 mutations in the RBD domain of spike protein likely happened through a thousand recombination and point mutations.

Figure 3 demonstrated the composite data of multi-alignment to demonstrate other minor deletions and point mutations in the *ORF1ab* like at the 82GHVMV locus (accession no. OP827777) and 141KSF locus (accession no. OP591969) in the *nsp1* protein and other sites like silent T>C mutation at 11269 positions. Now the question arises then what may be other factors for the severe pathogenicity of the Delta variant? We BLAST-2 aligned spike protein of Wuhan and Delta to see the important mutations as shown in Figure 4. We found two important mutations L452R (450R in Delta) and T478K (476K in Delta) in the RBD domain of the spike. At least the L452 mutation was found involved in immune escape and higher transmission [25, 26]. However, major spike mutations (T19R, T95I, G142D, E156G) and deletions (157FR) were located in the NH2 terminus (1-180 AAs) (Figure 4).

We also demonstrated the 119DF deletion in the Delta variant (Figure 5). Such deletion in Delta sequences found in the database suggested

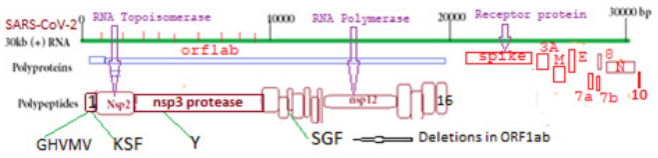

Figure 1) *Structure of SARS-CoV-2 and localization of deletions in the ORF1ab polyprotein. Note that spike protein was highly deleted and mutated than ORF1ab protein and more deletions were also reported in N, ORF7a, ORF7b, and ORF8 small proteins.*

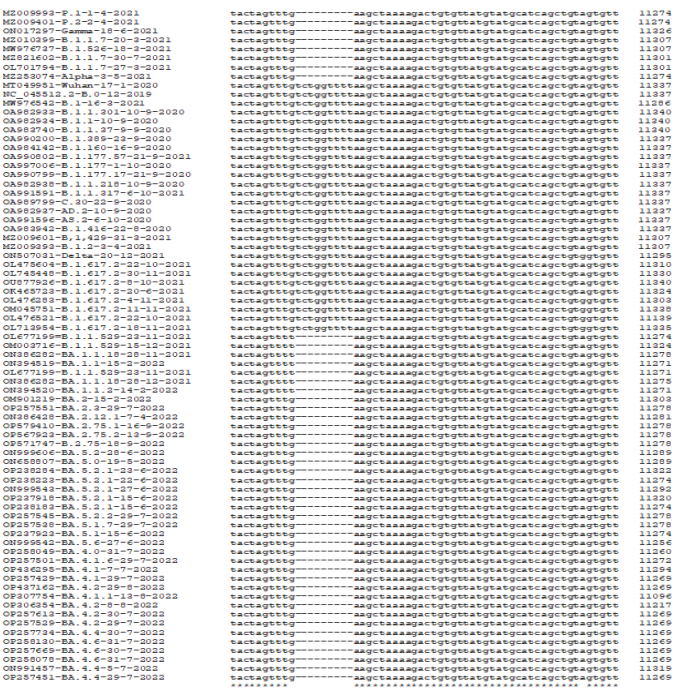

Figure 2) *Multi-alignment to show the 3675SGF deletion was found in Alpha, Beta, Gamma, and Iota variants as well as in all Omicron subvariants but not in Delta variants. (Alpha=B.1.1.7; Beta=B.1.351; Gamma=P.1; Iota=B.1.526; Delta=B.1.617.2 and AY.103; Omicron=BA.1, BA.2, BA.4, BA.5, BF.7, XBB.1.5, BQ.1, BN.1 etc).*

Figure 3) *Detection of ORF1ab other deletions in SGF deletion SARS-CoV-2 mutants as well as T>C silent mutation (GAT=GAC=D; no AA change) at 11269 positions.*

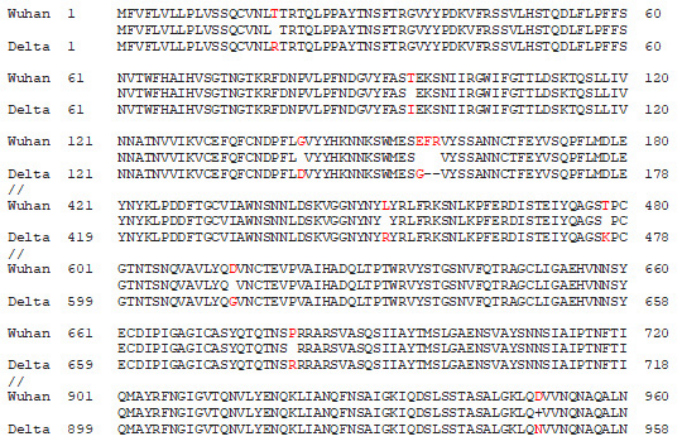

Figure 4) *The Spike protein mutations (nine AAs point mutations and two AAs deletions) in the delta variant. The sequences were derived from accession numbers NC_045512.2 (Wuhan; protein id: YP_009724390) and OM542166 (Delta; protein id: UKM99895). Parts of the alignment were shown. Major changes were found in 1-180 AAs and in the RBD domain L452R and T478K two important mutations needed for higher pathogenicity. The important 157FR two AAs deletions were shown with adjacent E156G point mutation. The D614G dominant mutation was also shown that involved in higher transmission in presence of important R4715L RdRp mutation in ORF1ab polyprotein (not shown here).*

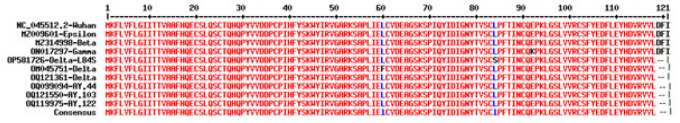

Figure 5) *The delta variant had important 119DF two AAs deletion at the end of the ORF8 protein which might be involved in an increase in pathogenicity. Note that another VOC B.1.1.7 COVID-19 also had truncated ORF8 protein in its genome but other VOCs like Beta, Gamma, and Epsilon, etc had normal length ORF8 protein.*

truncated non-functional ORF8 trans-activator protein which interacted with many accessory cellular proteins like histones, MHC-1, and interferons [27-29]. Interestingly, ORF8 protein was also truncated in B.1.1.7 variant which also caused severe corona diseases (data not shown). In Figure 6, we showed the two important mutations (P4715L and G5063S) in the RNA-dependent RNA Polymerase enzyme required for higher transmission and disease severity. Similarly, BLAST-2 similarity between Wuhan and Delta *ORF1ab* proteins showed two important mutations (P2046L and P2287S) in the nsp3 main protease. We know alterations of two Proline might be responsible for efficient protease activities needed for better viral assembly. We also showed the two important mutations (P6128S and A6319V) in the nsp14 ribonuclease of the Delta variant where the specificity of nuclease to host specific mRNAs might be changed. In truth, virus-host interactions select the disease severity, viral immunity, and virus clearance (Figures 7a and 7b).

We attempted to analyze the 3-D model structure of the SGF mutant of nsp6 protein but we found no crystal structure of normal nsp6 was solved yet. However, running SWISS-Model we found a mere 20% homology at the C-terminus with a hypothetical protein TT1805 from Thermus thermophilus HB8 and *E. coli* or *S. aureus* ribosomal protein S16 (ID: 6q97.1.P and 5t7v.1.F respectively) and model was incomplete (data not shown). Pandey et al gave some model structures of nsp6 protein with seven transmembrane domains using AlphaFold software (Figure 8) [30]. The study indicated in tissue culture cells that SGF-deleted nsp6 may form better phagosomes in the replication

complex with or without nsp3 and nsp4 proteins [31]. Thus, more work is necessary to solve the function of SGF deletion mutants. Nevertheless, we speculated the other mutations in RdRp, protease, and ribonuclease of the Delta variant to attend high titer, higher transmission, and possibly high risk of life.

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RNA Polymerase                                    P4715L
Wuhan  14101  ATTGTGCAAACTTTAATGTTTTATTCTCTACAGTGTTCCCACCTACAAGTTTTGGACCAC  14160
              ||||||||||||||||||||||||||||||||||||||||||| ||||||||||||||||
Delta  14332  ATTGTGCAAACTTTAATGTTTTATTCTCTACAGTGTTCCCACTTACAAGTTTTGGACCAC  14391
RNA Polymerase      G5063S
Wuhan  15181  GTGGCGGTTCACTATATGTTAAACCAGGTGGAACCTCATCAGGAGATGCCACAACTGCTT  15240
              ||||| ||||||||||||||||||||||||||||||||||||||||||||||||||||||
Delta  15412  GTGGCAGTTCACTATATGTTAAACCAGGTGGAACCTCATCAGGAGATGCCACAACTGCTT  15471
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Figure 6) *BLAST-2 alignment of 266-21555 Wuhan sequence (NC_045512.2) with Delta sequence (accession no. OM542166). Two mutations (P4715L and G5063S) were shown for RNA-dependent RNA Polymerase involved in higher transmission and disease severity. Total of 20 mutations were found in the ORF1ab gene but few were silent as in RNA topoisomerase T>C mutation (AAT=AAC) at 2049 coding Asparagine.*

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Wuhan  2041  CEDLKPVSEEVVENPTIQKDVLECNVKTTEVVGDIILKPANNSLKITEEVGHTDLMAAYV  2100
             CEDLK VSEEVVENPTIQKDVLECNVKTTEVVGDIILKPANNSLKITEEVGHTDLMAAYV
Delta  2041  CEDLKLVSEEVVENPTIQKDVLECNVKTTEVVGDIILKPANNSLKITEEVGHTDLMAAYV  2100

Wuhan  2101  DNSSLTIKKPNELSRVLGLKTLATHGLAAVNSVPWDTIANYAKPFLNKVVSTTTNIVTRC  2160
             DNSSLTIKKPNELSRVLGLKTLATHGLAAVNSVPWDTIANYAKPFLNKVVSTTTNIVTRC
Delta  2101  DNSSLTIKKPNELSRVLGLKTLATHGLAAVNSVPWDTIANYAKPFLNKVVSTTTNIVTRC  2160

Wuhan  2161  LNRVCTNYMPYFFTLLLQLCTFTRSTNSRIKASMPTTIAKNTVKSVGKFCLEASFNYLKS  2220
             LNRVCTNYMPYFFTLLLQLCTFTRSTNSRIKASMPTTIAKNTVKSVGKFCLEASFNYLKS
Delta  2161  LNRVCTNYMPYFFTLLLQLCTFTRSTNSRIKASMPTTIAKNTVKSVGKFCLEASFNYLKS  2220

Wuhan  2221  PNFSKLINIIIWFLLLSVCLGSLIYSTAALGVLMSNLGMPSYCTGYREGYLNSTNVTIAT  2280
             PNFSKLINIIIWFLLLSVCLGSLIYSTAALGVLMSNLGMPSYCTGYREGYLNSTNVTIAT
Delta  2221  PNFSKLINIIIWFLLLSVCLGSLIYSTAALGVLMSNLGMPSYCTGYREGYLNSTNVTIAT  2280

Wuhan  2281  YCTGSIPCSVCLSGLDSLDTYPSLETIQITISSFKWDLTAFGLVAEWFLAYILFTRFFYV  2340
             YCTGSI CSVCLSGLDSLDTYPSLETIQITISSFKWDLTAFGLVAEWFLAYILFTRFFYV
Delta  2281  YCTGSISCSVCLSGLDSLDTYPSLETIQITISSFKWDLTAFGLVAEWFLAYILFTRFFYV  2340
```

Figure 7a) *BLAST-2 alignment between ORF1ab protein of Wuhan (NC_045512.2) and Delta (OM542166) showing two mutations (P2046L and P2287S) in the nsp3 main protease.*

```
Wuhan  6121  KYFVKIGPERTCCLCDRRATCFSTASDTYACWHHSIGFDYVYNPFMIDVQQWGFTGNLQS  6180
             KYFVKIG ERTCCLCDRRATCFSTASDTYACWHHSIGFDYVYNPFMIDVQQWGFTGNLQS
Delta  6121  KYFVKIGSERTCCLCDRRATCFSTASDTYACWHHSIGFDYVYNPFMIDVQQWGFTGNLQS  6180

Wuhan  6181  NHDLYCQVHGNAHVASCDAIMTRCLAVHECFVKRVDWTIEYPIIGDELKINAACRKVQHM  6240
             NHDLYCQVHGNAHVASCDAIMTRCLAVHECFVKRVDWTIEYPIIGDELKINAACRKVQHM
Delta  6181  NHDLYCQVHGNAHVASCDAIMTRCLAVHECFVKRVDWTIEYPIIGDELKINAACRKVQHM  6240

Wuhan  6241  VVKAALLADKFPVLHDIGNPKAIKCVPQADVEWKFYDAQPCSDKAYKIEELFYSYATHSD  6300
             VVKAALLADKFPVLHDIGNPKAIKCVPQADVEWKFYDAQPCSDKAYKIEELFYSYATHSD
Delta  6241  VVKAALLADKFPVLHDIGNPKAIKCVPQADVEWKFYDAQPCSDKAYKIEELFYSYATHSD  6300

Wuhan  6301  KFTDGVCLFWNCNVDRYPANSIVCRFDTRVLSNLNLPGCDGGSLYVNKHAFHTPAFDKSA  6360
             KFTDGVCLFWNCNVDRYP NSIVCRFDTRVLSNLNLPGCDGGSLYVNKHAFHTPAFDKSA
Delta  6301  KFTDGVCLFWNCNVDRYPVNSIVCRFDTRVLSNLNLPGCDGGSLYVNKHAFHTPAFDKSA  6360
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Figure 7b) *BLAST-2 alignment between Wuhan (protein id. YP_009724389) and Delta (protein id. UKM99893) ORF1ab proteins resulted in fifteen AAs changes. The P6128S and A6319V two AAs changes in the Ribonuclease (nsp14; 5926 AAs-6452 AAs)) were shown.*

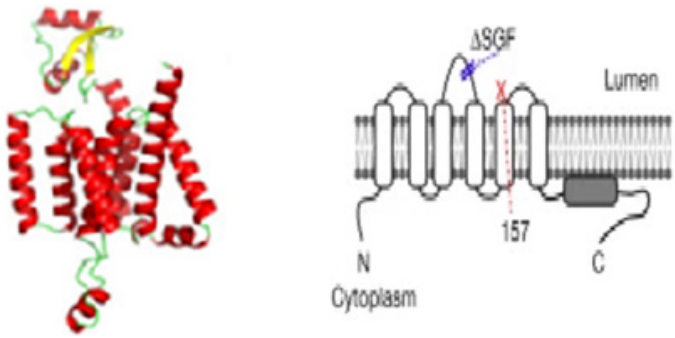

Figure 8) *Model structure of the nsp6 protein generated by AlphaFold-2 and the transmembrane helix was predicted through the THHMM server as no 3-D crystal structure of nsp6 solved yet [30, 31].*

DISCUSSION

The Alpha, Beta, Gamma, and Delta coronaviruses were spread maximum at certain time points but were diminished due to viral herd immunity as well as rapid worldwide inoculation with spike vaccine which had shown to be best protective with high antibody titer [32]. We demonstrated a few genetic changes that surely gave high titer and severe disease patterns by Delta coronaviruses (Figure 2 and Figure 3). Such viruses form syncytia with human lung cells through ACE-2 receptors destroying cells [33]. However, the Omicron virus spread was maximum now and earlier human sera infected with Delta, Alpha, Beta, and Gamma corona viruses failed to cure Omicron virus-infected patients [34]. Surely, a clear demonstration of the absence of SGF deletion in the nsp6 protein of Delta coronaviruses was an important observation. The nsp6 protein forms smaller phagosomes at the host membrane of lung cells for virus assembly and internalization and such an autophagy process is important for the virus life cycle [35]. Similarly, ORF8 protein 119DF deletion mutant in Delta coronavirus was another important fact (Figure 5). Many small accessory proteins like nsp6, nsp9, ORF3, ORF7a, and ORF8 were involved in important functions in COVID-19 spread and viral immunity, and more and more work and Database analysis needed for coronavirus control [36-38]. Interestingly, more and more spliced mRNAs were detected in COVID-19-infected cells and more small trans-activator proteins may be discovered soon demonstrating the clear mechanism of coronavirus disease [39]. The complete structure of *ORF1ab* polyprotein contributed to higher transmission with higher titer in the Delta variant causing high fatality. If terminal 2 AAs deletion (219DF) in the ORF8 protein of Delta variant has any effect on pathogenicity is not clear yet. The deletions of amino acids Asp119 and Phe120 in ORF8 of the delta variant resulted in structural instability of ORF8 dimer (PDB ID:7JTL) caused by disruption of hydrogen bonds and salt bridges as revealed by structural analysis and MD simulation studies [40].

CONCLUSION

MHC-1 interactions may be hindered causing a better immune response by the host.

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