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Prevalent mutations in the Delta variants of SARS CoV2 circulating in India

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ABSTRACT: In this study SARS-CoV-2 genome encoding for spike protein of Delta lineage circulating upto December 2022 in India were downloaded from NCBI and GISAID and aligned against the reference sequence MN908947.3. The mutational profiles of these sequences were estimated using online software available. The most frequent non-synonymous mutational events were D614G, L452R, T478K, P681R, D950N, T19R, G142D, A222V, T95I and V1061V.

Key words: Delta variants, mutations, SARS-CoV-2

Viruses mutate with due course of time and the mutational rate differs in viruses (Gralinski and Menachery, 2020; Tang *et al.*, 2020). The maximum mutational rate is exhibited by RNA viruses (Gralinski and Menachery, 2020; Tang *et al.*, 2020) and that is why RNA viruses exhibit high adaptability to the environmental conditions. In RNA viruses the mutations arise due to the lack of proof-reading mechanism of RNA polymerase and due to recombination between two viral lineages. Although most common mutations found are neutral, however, some mutations may affect the replication of virus and its infectivity (Loewe and Hill, 2010; Alexander *et al.*, 2017; Gralinski and Menachery, 2020; Tang *et al.*, 2020).

In India, the first case of COVID-19 was found on January 27th, 2020 in Kerala. SARS-CoV-2 infection throughout many countries has resulted in infection of people with diverse immunological strength, age, sex and environmental condition which has imposed selection pressure on SARS-CoV-2. Therefore, new emerging variants of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) poses threat to the global health.

Mutations in the variants of interest (VOIs) and Variants of concern (VOCs) are associated with increased transmissibility (Plante *et al.*, 2021) and reduced neutralization by antibody (Planas *et al.*,

2021). The genome analysis of the SARS CoV-2 strains has revealed that the mutations are distributed heterogeneously in the genome of SARS-CoV-2 (Dearlove *et al.*, 2020; van Dorp *et al.*, 2020). The spike protein of SARS CoV-2 has accumulated several mutations which have increased affinity to human ACE2 receptor (Luan *et al.*, 2021). Hence, it becomes imperative to carry on the regular genomic sequencing and surveillance of SARS CoV-2 cases. Therefore, the main aim of this study was to study the mutations prevalent in the spike protein of SARS CoV-2 Delta variants circulating in India.

MATERIALS AND METHODS

Genome sequences retrieval and alignment

A total of 423, SARS-CoV-2 complete, and high coverage viral genome sequences of Delta variants were downloaded from Global Initiative on Sharing All Influenza Database (GISAID) platform and National Center for Biotechnology Information (NCBI). Only viruses affecting human hosts were selected and low-quality sequences having sequences more than 5% NNNs and other ambiguous characters were removed using BioEdit 7.2 software. Only full-length sequences including reference sequence MN908947.3 having more than 29000 nucleotides were included in the analysis.

Multiple sequence alignment and lineage prediction

Sequences of SARS-CoV-2 Delta variants from NCBI & GISAID were downloaded and consensus sequences were aligned in FASTA format with the reference sequence of the SARS-CoV-2 Wuhan-Hu-1 isolate (GenBank accession number MN908947.3) using Multiple Alignment using Fast Fourier Transform (MAFFT) (Katoh *et al.*, 2002). The sequences were aligned with default setting of gap open penalty 1.53, gap extension penalty 0.123 and were downloaded in FASTA format. The SARS-CoV-2 lineages were predicted using Pangolin COVID-19 lineage assigner (<https://pangolin.cog-uk.io/>) (Rambaut *et al.*, 2020).

Mapping nucleotide substitutions in the genome and expressed proteins of SARS Co-V2

The mutations across the genome of aligned sequences were mapped with the reference sequence of the SARS-CoV-2 Wuhan-Hu-1 isolate (GenBank accession number MN908947.3) using an online software described previously (Mercatelli *et al.*, 2021).

RESULTS AND DISCUSSION

Mutations prevalent in the spike protein of Delta variants

The 423 genomes of Delta variants were subjected

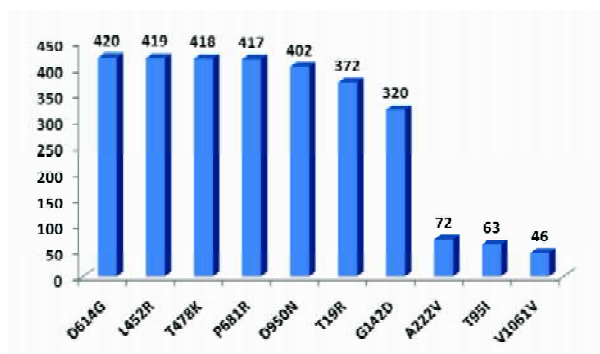


Fig 1: Frequency of non-synonymous substitutions in the Delta variants of SARS-CoV-2 circulating in India

Table 1: Percentage prevalence of top ten mutations amongst the 423 Delta variants of

SARS CoV-2			
S No	Mutation	Number of mutations	Percentage prevalence
1	D614G	420	99.29
2	L452R	419	99.05
3	T478K	418	98.81
4	P681R	417	98.58
5	D950N	402	95.03
6	T19R	372	87.94
7	G142D	320	75.65
8	A222V	72	17.02
9	T95I	63	14.89
10	V1061V	46	10.87

to online software as described previously and the mutations were annotated. The most common non-synonymous mutations present in decreasing order of frequency were D614G, L452R, T478K, P681R, D950N, T19R, G142D, A222V, T95I and V1061V. The majority of Delta variants exhibited D614G, L452R, T478K, P681R, D950N mutations which were prevalent at 99.29%, 99.05%, 98.81%, 98.58% and 95.03%, respectively. Other mutations T19R, G142D, A222V, T95I, V1061V were prevalent at rate of 87.94%, 75.65%, 17.02%, 14.89% and 10.87%, respectively. The frequencies of these mutations have been depicted in Fig. 1 and Table 1.

The mutations E471Q, E484K, E484Q, L452R and N501Y are responsible for high transmissibility and virus escape mutants from convalescent sera (Deng *et al.*, 2021, Jangra *et al.*, 2021). The RBD is a 273 amino acid segment of the whole Spike protein which stretches from residue 319 to 591 (Lan *et al.*, 2020). In the spike protein, eleven non-synonymous substitutions viz. E471Q, E484K, E484Q, L452R, N501Y, S477G, S477I, S494L, T478K, T478R and V483F were present in the receptor binding motif (RBM) of Receptor binding domain (RBD). All these mutations lied in the RBM which stretches from 438 to 506 amino acid location of RBD of spike protein which subsequently binds to human ACE-2 receptor.

CONCLUSION

The above study reveals that it is imperative to

monitor the mutations in the RBM of Spike protein of SARS-CoV-2 to monitor the transmissibility of SARS-CoV-2 so that appropriate measures may be taken from public health point of view.

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