Mutational analysis unveils the temporal and spatial distribution of G614 genotype of SARS-CoV-2in different Indian states and its association with case fatality rate of COVID-19

Ballamoole Krishna Kumar[1#*], Bakilapadavu Venkatraja[2#], Kattapuni Suresh Prithvisagar[1], Praveen Rai[1], Anusha Rohit[3], Madhura Nagesh Hegde[4], Indrani Karunasagar[1], Iddya Karunasagar[5*],

[1]Nitte (Deemed to be University), Nitte University Centre for Science Education and Research, (NUCSER), Division of Infectious Diseases, Deralakatte, Mangaluru-575018, India

[2]Department of Economics, Shri Dharmasthala Manjunatheshwara Institute for Management Development (SDMIMD), Mysore-570 011, India

[3]Department of Microbiology, The Madras Medical Mission, 4-A, Dr, Mogappair, Chennai, Tamil Nadu 600037, India

[4]Department of Information Science and Engineering, Sahyadri College of Engineering and Management, Mangaluru - 575007, India.

[5]Nitte (Deemed to be University), University Enclave, Medical Sciences Complex, Deralakatte, Mangaluru-575018, India

***Correspondence**

Krishna Kumar B., PhD
Division of Infectious Diseases, Nitte University Centre for Science Education and Research, Nitte (Deemed to be University), Deralakatte, Mangaluru-575018, India
Email: krishnakumarb@nitte.edu.in; Tel: +91-824 220 4304

Prof Iddya Karunasagar
Nitte (Deemed to be University), University Enclave, Medical Sciences Complex, Deralakatte, Mangaluru-575018, India
Email: iddya.karunasagar@nitte.edu.in; Tel: +91-Tel: 9481202750

 # contributed equally to this study

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Abstract

Pan genomic analysis of the global SARS-CoV-2 isolates has resulted in the identification of several regions of increased genetic variation but there is absence of research on its association with the clinical outcome. The present study fills the vacuum and does mutational analysis of genomic sequence of Indian SARS-CoV-2 isolates. Results reveal the existence of non-synonymous G614 spike protein mutation in 61.45% of the total study genome along with three other mutations. Further, temporal variation in the frequencies of G614 genotype in the country is observed. The examination of the probable association of G614 genotype with COVID-19 severity shows that CFR G614 genotype in India is positively and strongly correlated. It appears that the clinical outcome of the COVID-19 cases in India are significantly and adversely affected by the increasing trend in the G614 genotype; which needs to be addressed combining both laboratory experiments and epidemiological investigations.

Keywords: SARS-COV-2; COVID-19, Mutation, Spike protein, D614G

Introduction

Much like the world, India is fighting against an exponential growth of COVID-19 that started with the first imported case reported in late January 2020 from the Indian citizen travelled from Chinese city of Wuhan (Potdar et al., 2020). As of 21st July 2020, the country has recorded over 11, 55,190 positive cases involving 28,084 deaths due to COVID-19 (https://www.mygov.in/covid-19). The interim analysis of the genomic data on Indian isolates of SARS-CoV-2 shows the introduction of this virus to India from multiple sources such as China, Europe, USA, Canada and the Middle East (Potdar et al., 2020; Singh and Sharma 2020; Somasundaram et al., 2020). SARS-CoV-2 has a genome size of 29.9 kb which encodes four major structural proteins [spike (S), envelope (E), membrane (M), as well as nucleocapsid (N) protein], 16 non-structural proteins [nsp1–16] and five to eight accessory proteins (Wu et al., 2020). Based on the currently available pieces of evidence, it is noteworthy that the severity of COVID-19 differs significantly depending on the geographical locations and this could be due to amalgamation of different factors including genetics of the virus.

All RNA viruses are prone to acquire an extremely high rate of mutation in the genomic region, which provides a selective advantage to the virus for transmission and virulence. However, coronaviruses have been reported to have genetic proof-reading mechanisms and therefore, nucleotide sequence diversity in SARS CoV2 has been found to be very low (Fauver et al., 2020). Nevertheless, pan genomic analysis of the global SARS-CoV-2 isolates has resulted in the identification of several genomic regions of increased genetic variation, which suggests that the virus underwent evolutionary changes and diversified during the process of geographical dissemination. Acquisition of non-synonymous D614G (Aspartate to Glycine) mutation in C -terminal region of the spike protein is one such evolutionary change observed in the SARS-CoV-2 genome and has become the most dominant genotype (>70%)

by replacing D614G wild type strain in many countries and this mutation has been suggested to confer fitness advantage (Korber et al., 2020; 1. Biswas and Majumder 2020). In this study genomic sequence of Indian SARS-CoV-2isolates were examined for the temporal dynamics in the acquisition of D614G mutation and its probable relation to the case fatality rate among COVID-19 cases in India.

Materials and Methods

A total of 1375 SARS-CoV-2genome sequences available from the different Indian states were retrieved from GISAID (https://www.gisaid.org/) database on 6th July 2020. While retrieving the sequences the low coverage sequences with >5% Ns were excluded. It has been ascertained that all the retrieved sequences were mapped to reference genome of SARS-CoV-2deposited from Wuhan, China (GenBank Accession No. NC_045512.2) using Geneious Pro (V.8.0.5). The statistical data of daily reported cases and daily death toll (till June 30, 2020), case fatality rate (CFR) as per 7th July 2020 were obtained from COVID-19 India website (https://www.api.covid19india.org/). The mutation analysis was performed using COVID-19 genome annotator as per the method described by Mercatelli et al., (2020). Mutations observed in >1.5% of the total sequences were tabulated. As this study is focused on the temporal dynamics in the acquisition of D614G mutation in the Indian SARS-CoV-2 isolates, its existence was further confirmed by manually analyzing the alignment created using Geneious (V.8.0.5). The D614G mutation was analyzed state-wise and in time series at 15 days interval from 1st March 2020 to 30th June 2020. The percentage of D614G mutation was then correlated with CFR. Also, the percentage of G614 mutant genotype and CFR were subjected to linear regression analysis using least squares method.

Results and Discussion

The study identifies 47 key mutations spread over different coordinates of Indian SARS-CoV-2isolates, which includes 25 non synonymous, 19 synonymous and 3 mutations were extragenic in nature (Table S1). The most frequently occurring mutations among the genomes studied are represented in Fig 1 with NSP3:F106F followed by S: D614G from spike protein, 5ÚTR:241, NSP12b: P314L subunit of RdRp and so on. Among these, acquisition of non-synonymous D614G (Aspartate to Glycine) mutation positioned between the receptor binding domain and S1/S2 polybasic protease cleavage site of spike glycoprotein were observed in the 61.45 % (n= 845/1375) of the total study genome. We have also reported the simultaneous occurrence of three other mutations such as 5'UTR: 241 C > T (61.01%, 840/1375), NSP12b: P314L (58.18%, 800/1375) and NSP3 F106F (3037 C > T) (62.03%, 853/1375) silent mutation among the SASRS-CoV-2isolates which showed D614G variation in the study. From here onwards, SARS-CoV-2isolates harboring these four distinctive mutations will be referred as G614 genotype.

The genomic leader sequence at 5'UTR plays an important role in the viral replication and gene expression during discontinuous sub-genomic replication of RNA viruses. Previous *in silico* analysis has shown that mutation of 5'UTR: 241 from C to T enhances the binding of Transactive Response DNA binding protein (TARDBP) to 5’-UTR of SARS-CoV-2 genome that could enable the virus to multiply within the host. (Mukherjee and Goswami, 2020). NSP12b: P314L is another prominent mutation observed among the Indian SARS-CoV-2isolates; found in very close proximity to the drug binding region in the hydrophobic cleft of RNA dependent RNA polymerase (Gao et al., 2020; Pachetti et al., 2020). RdRp is a key enzyme involved in the replication of SARS-CoV-2and exists as a complex of nonstructural protein 12 and 8 (NSP12 and NSP8); which is also a potential target for nucleotide analog

antiviral inhibitors (Gao et al., 2020). 3037C>T is a silent mutation observed in the phosphoesterase, papain-like proteinase of SARS-CoV-2genome. Results obtained in this study are consistent with the previous findings where a simultaneous occurrence mutation in the different regions of the newly emerged mutant genotype of SARS-CoV-2has been reported (Korber et al., 2020). It is believed that, mutations in these structural and nonstructural proteins would have considerable impact on the replication of virus and their subsequent binding to the ACE2 receptor on the host cells. A spurt in the incidence of newly emerged G614 genotype was evident over wide geographical areas; afflicting an exceptionally high proportion of population. Studies have observed that there was dramatic increase in the incidence of SARS-CoV-2 carrying these distinctive mutations which may be due to the natural selection process (Korber et al., 2020; Pachetti et al., 2020). Based on data on RT-qPCR cycle threshold (CT) values, Korber et al., (2020) noted that G614 genotype infection leads to higher viral loads compared to D614, but no correlation with disease severity could be observed. On the other hand the functional study of G164 mutant utilizing retroviruses pseudotyped with G614 variant of spike protein showed greater infectivity potential compared to wildtype (Zhang et al., 2020).

Remarkably, none of the initial SARS-CoV-2 sequences from India had this distinctive D614G mutation; however, the first case of G614 genotype was detected in the early week of March from travelers arriving from European countries. From the analysis it is observed that there was a significant temporal variation in the frequencies of G614 genotype in the country as it contributed to 29.35% of the 109 genomes in March, 48.81% of the 338 genomes in April, 58.99% of the 656 available genome in May and 96.66% of 270 genomes in the month of June (Fig. 3). Our data indicates that in India, the transition from D614 to G614 genotype has grown progressively to become a dominant genotype and successfully established its niche in the country. Further, it is also evident that G614 genotype has a

varying spatial distribution in the different Indian states; the highest percentage (93.04 %) being reported from the state of Gujrat followed West Bengal, Maharashtra, Madhya Pradesh, Orissa and Karnataka (Fig. 3). It is also noticed that, G614 genotype was not reported in some of the Indian states as represented in the Fig. 3 does not mean that it's not prevailing because genome data from those regions are not sufficient to conclude its existence.

Phylogenetic tree based on amino acid sequences of spike protein for the selected isolates of India SARS-CoV-2were constructed using Nexstrain web tool (Fig 2). From the phylogeny it is apparent that, there are two major clusters of SARS-CoV-2in India based on the variation in the amino acid position 614. D614 is prominent in the reported cases of COVID-19 and newly detected G614 genotype circulating in the India similar to the situation in European countries (Pachetti et al., 2020). The transmission network analysis of the genomic data on Indian isolates of SARS-CoV-2shows that, the newly emerged G614 genotype that has reached India through travelers arriving from Europe and Middle East before establishing its prominent niche in the country (Fig 2). Overall observation made in this study suggests that G614 genotype has a pan India distribution and has steadily increased by replacing formerly introduced D614 genotype from Asian countries. But the distribution varied in different states in India (Fig 3). There were low numbers of sequences available from states like Andhra Pradesh (AP), Bihar (BH), Kerala (KL) and Ladakh (LD). Hence, we cannot draw any inference on the predominance of D614 in these states. But the difference between Delhi (DL) and Gujarat (GJ) is noteworthy. There were 214 sequences rom Delhi and 314 sequences from Gujarat. In Delhi, D614 predominated while in Gujarat, G614 predominated. Early hotspot in Delhi was a religious congregation involving travelers from Middle East as well as Asian countries and this could be reason for dominance of D614 in Delhi. But if S614 has a fitness advantage, it would have shifted the balance in favor of this genotype as pandemic advanced. This seems to be not the case at least in Delhi in India.

From the trends in data reported from India on COVID 19, it appears that CFR and incidence of G614 genotype is on the rise in the time path from the short to the long (Fig 3); but there is a paucity of evidences to prove the association among these factors. Korber et al (2020) noted that though viral loads were higher in infections with G614 genotype, there was no significant effect on hospitalization outcomes. Hence the study examines the probable association of D614G genotype with disease severity of COVID-19 across India through correlation analysis and the results are reported in Table 1. It appears that CFR is positively and strongly correlated with the increasing incidence of G614 genotype in different states of India. However, to predict the cause-effect relationship between CFR and level of G614 genotype, a liner regression model as presented in equation (1) has been used and the results are reported in Table 1.

$$CFR = \alpha + \beta\ G614 + \varepsilon \text{ ---------- } (1)$$

Where, CFR is COVID-19 case fatality ratio in India, α is the intercept of the equation, $\beta\ G614$ is beta coefficient of the deterministic variable of the model, ε is the error term. From the result, it appears that increasing incidence of G614 genotype reported from different states of India significantly contributes to higher fatality ratio among the COVID 19 positive cases. The beta of the regression coefficient is positive and statistically significant at 5% level. The model is a good fit as the r^2 is at the acceptable level. It indicates that 38% changes in CFR are defined by the regressor variable in the model i.e. G614 genotype, whereas the remaining 62% changes in CFR are controlled by exogenous factors that are outside the present model. The F-statistic of the model is statistically significant at 1% level indicating that the results generated from the model are reliable. Hence the result from the regression model provides evidence to support the theoretical perception that CFR is adversely affected by G614 genotype thereby predicting that increasing trend in the G614 genotype may have profound negative impact on the clinical outcome of the COVID-19 cases in India.

Conclusions

Even though there is a prima facie evidence on the infectivity potential of G614 mutant from the *in vitro* studies but how this mutation translates to clinical outcome of the COVID-19 needs to be addressed combining both laboratory experiments, clinical data and epidemiological investigations. In summary, this study unveils the genomic regions of genetic variation in the SARS-CoV-2 circulating in India, which is extensively dominated by G614 genotype with a strong correlation to CFR of COVID-19 posing enormous challenge for the effective prevention and management of COVID-19 cases in India.

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Conflict of Interest

No conflicts of interest in this study.

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Table 1. Model represents the association between D614G SNP in the SARS-COV2 genome and case fatality rate among the selected COVID-19 cases in India

A. Correlation Matrix				
	CFR	**D614G SNP**		
CFR	1	0.619854		
D614G SNP	0.619854	1		
B. OLS Regression Analysis *Dependent Variable: CFR*				
Particulars	**Coefficient**	**Std. Error**	**t-Statistic**	**Prob.**
C	1.031447	0.482582	2.137353	0.0507
Mutation	0.027799	0.009406	2.955566	0.0104
R-squared	0.384219	Mean dependent var		2.118750
Adjusted R-squared	0.340235	S.D. dependent var		1.538059
S.E. of regression	1.249302	Akaike info criterion		3.399516
Sum squared resid	21.85059	Schwarz criterion		3.496090
Log likelihood	-25.19613	Hannan-Quinn criter.		3.404462
F-statistic	8.735370	Durbin-Watson stat		2.054826
Prob (F-statistic)	0.010430			

Fig 1. Analysis shows the most frequently found nucleotide variations (both synonymous and non-synonymous) in the Indian SARS-COV-2isolates

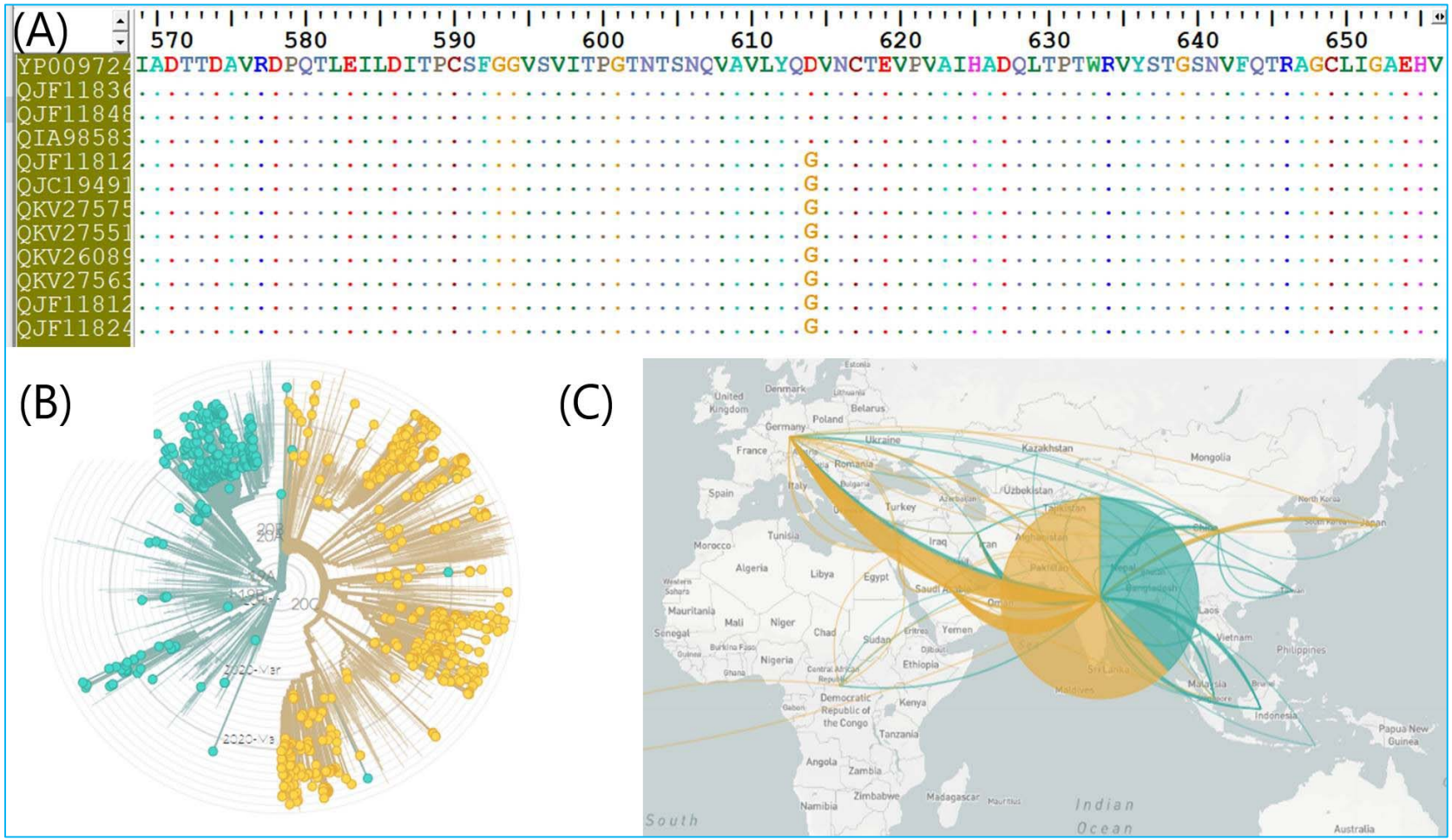
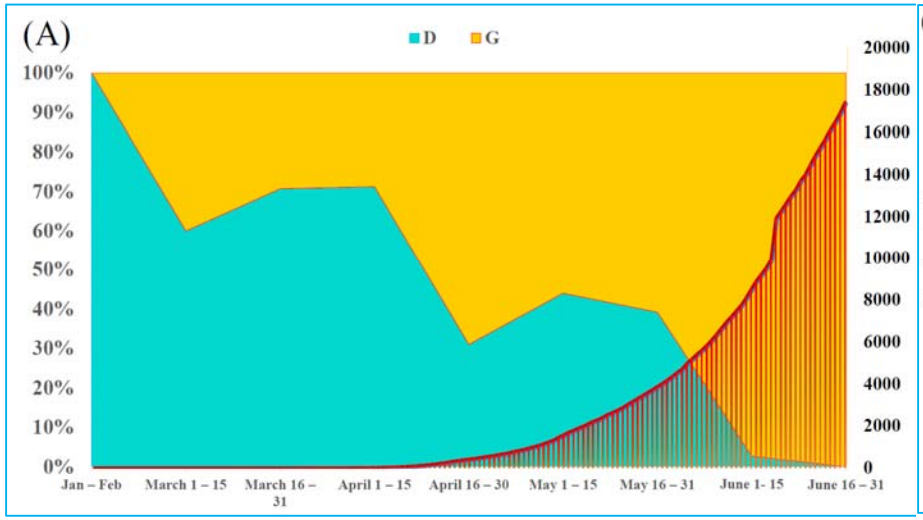
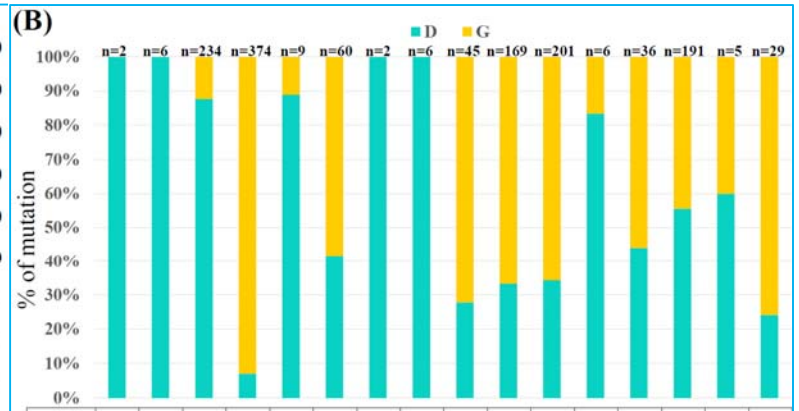


Fig 2. Analysis unveils presence of G614 genotype of SARS-CoV-2in Indian states. Fig (A) Amino acid alignment of the S protein showing the D614G mutation sites among the selected SARS-COV-2genomes and (Fig B) separation of two major clades in the SARS-COV2-circulating in India as observed by phylogenetic analysis. (Fig. C) Transmission map showing the introduction of G614 genotype to India.





State	AP	BH	DL	GJ	HR	KA	KL	LD	MH	MP	OR	RJ	TG	TN	UP	WB
Total Case	20019	12125	100823	36772	17504	25317	5622	1005	211987	15284	9526	20688	25273	114978	28636	22987
Total Death	239	97	3115	1960	276	401	27	1	9026	617	38	461	306	1571	809	779
CFR	1.2	0.8	3.1	5.3	1.6	1.6	0.5	0.1	4.3	4	0.5	2.2	1.1	1.4	2.8	3.4

Fig 3A. Temporal distribution of D614G genotype of SARS-CoV-2in India along with cumulative mortalities due to COVID-19 (Y1 axis represents percentage of D614G mutation and Y2 axis represent cumulative mortality). 3B. Distribution of D614G genotype in different state in India, total cases, mortality and case fatality rate in the states

[AP – Andhra Pradesh, BH – Bihar, DL – Delhi, GJ- Gujarat, HR – Haryana, KA – Karnataka, KL – Kerala, LD – Ladakh, MH – Maharashtra, MP – Madhya Pradesh, OR – Orissa, RJ – Rajasthan, TG – Telangana, TN – Tamil Nadu, UP – Uttar Pradesh, WB- West Bengal**]**

Table S1. List of mutation frequencies observed in genome of Indian SARS-CoV-2. The frequencies of mutations are calculated from 1383 SARS-CoV-2genomes

Sl. No	Protein mutation	SNP mutation	Type of mutation	Frequency	Name of the protein
1	NSP16:N298L	21550 AA > CT	NonSynonymous mutation	20	2'-O-ribose methyltransferase
2	NSP3:D455D	4084 C > T	Synonymous mutation	21	Predicted phosphoesterase, papain-like proteinase
3	NSP12b:V871I	16078 G > A	NonSynonymous mutation	21	RNA-dependent RNA polymerase
4	NSP3:P1103L	6027 C > T	NonSynonymous mutation	22	Predicted phosphoesterase, papain-like proteinase
5	S:T1273T	25381 A> C	Synonymous mutation	23	Spike
6	NSP4:A380V	9693 C > T	NonSynonymous mutation	27	Transmembrane protein
7	NSP13:L130L	16626 C> T	Synonymous mutation	29	Helicase
8	NSP4:D279N	9389 G> A	NonSynonymous mutation	36	Transmembrane protein
9	NSP2:G339S	1820 G > A	NonSynonymous mutation	36	Non-Structural protein 2
10	NSP14:T372I	19154 C > T	NonSynonymous mutation	37	3'-to-5' exonuclease
11	NSP3:E545E	4354 G > A	Synonymous mutation	39	Predicted phosphoesterase, papain-like proteinase
12	NSP3:S1285F	6573 C > T	NonSynonymous mutation	40	Predicted phosphoesterase, papain-like proteinase
13	ORF3a:L46F	25528 C > T	NonSynonymous mutation	40	ORF3a protein
14	NSP13:L92L	16512 A > G	Synonymous mutation	45	Helicase
15	3'UTR:29742	29742 G > A	Extragenic	46	NA
16	NSP3:S1197R	6310 C > A	NonSynonymous mutation	53	Predicted phosphoesterase, papain-like proteinase
17	NSP3:V527V	4300 G > T	Synonymous mutation	56	Predicted phosphoesterase, papain-like proteinase
18	NSP14:L177F	18568 C > T	NonSynonymous mutation	59	3'-to-5' exonuclease
19	S:T302T	22468 G > T	Synonymous mutation	60	Spike
20	3'UTR:29750	29750 C > T	Extragenic	60	NA
21	NSP2:Q496P	2292 A > C	NonSynonymous mutation	61	Non-Structural protein 2
22	NSP4:S76S	8782 C > T	Synonymous mutation	61	Transmembrane protein
23	NSP4:F121F	8917 C > T	Synonymous mutation	62	Transmembrane protein
24	N:S202N	28878 G > A	NonSynonymous mutation	66	Nucleocapsid protein
25	NSP14:L495L	19524 C > T	Synonymous mutation	67	3'-to-5' exonuclease
26	ORF8:L84S	28144 T > C	NonSynonymous mutation	69	ORF8 protein

27	S:L54F	21724 G > T	NonSynonymous mutation	73	Spike
28	NSP3:N305N	3634 C > T	Synonymous mutation	78	Predicted phosphoesterase, papain-like proteinase
29	NSP12b:N619N	15324 C > T	Synonymous mutation	78	RNA-dependent RNA polymerase
30	NSP1:L16L	313 C > T	Synonymous mutation	146	Leader protein
31	NSP3:A994D	5700 C > A	NonSynonymous mutation	147	Predicted phosphoesterase, papain-like proteinase
32	NSP3:C39C	2836 C > T	Synonymous mutation	155	Predicted phosphoesterase, papain-like proteinase
33	S:D294D	22444 C > T	Synonymous mutation	189	Spike
34	N:S194L	28854 C > T	NonSynonymous mutation	191	Nucleocapsid protein
35	N:RG203KR	28881 GGG > AAC	NonSynonymous mutation	269	Nucleocapsid protein
36	M:Y71Y	26735 C > T	Synonymous mutation	275	Membrane
37	NSP14:C279C	18877 C > T	Synonymous mutation	276	3'-to-5' exonuclease
38	ORF3a:Q57H	25563 G > T	NonSynonymous mutation	282	ORF3a protein
39	NSP6:L37F	11083 G > T	NonSynonymous mutation	301	Transmembrane protein
40	N:P13L	28311 C > T	NonSynonymous mutation	365	Nucleocapsid protein
41	S:Y789Y	23929 C > T	Synonymous mutation	367	Spike
42	NSP3:T1198K	6312 C > A	NonSynonymous mutation	383	Predicted phosphoesterase, papain-like proteinase
43	NSP12b:A88V	13730 C > T	NonSynonymous mutation	405	RNA-dependent RNA polymerase
44	NSP12b:P314L	14408 C > T	NonSynonymous mutation	800	RNA-dependent RNA polymerase
45	Leader sequence 5'UTR:241	C241T	Extragenic	840	NA
46	S:D614G	A23403G	NonSynonymous mutation	845	Spike
47	NSP3:F106F	3037 C > T	Synonymous mutation	853	Predicted phosphoesterase, papain-like proteinase