Original Article

Occurrence of Recurrent Mutations in SARS-CoV-2 Genome and its Implications in the Drug Designing Strategies

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Abstract

Objectives: The objective of this study was to explore mutational sites in the genome of SARS-CoV-2 of Indian isolates through multiple sequence alignment and comparing it with the reference sequence of Wuhan SARS-CoV-2. These mutational studies generate crucial insights in designing of antiviral agents against COVID-19 infections.

Methods: Sequence downloaded from NCBI virus database and multiple sequence alignment done using CLUSTAL Omega online platform, viewed using Jalview.

Results: A total of 64 recurrent mutations were detected from Indian isolates of SARS-CoV-2 which were phylogenetically distant from that of Wuhan type.

Conclusion: Occurrence of new mutations at different locations supports viral evolbability and provides a crucial insight into the development of effective vaccine and drug against COVID-19.

Keywords: Pandemic; SARS-CoV-2; COVID-19; Wuhan; Mutations

Introduction

SARS-CoV-2 is a single stranded, (+) sense RNA virus and the etiolating agents of present pandemic COVID-19. This pandemic emerged from Wuhan, China, is a highly contagious disease, spreading rapidly across the globe [1]. Its transmission occurs primarily through human to human contacts or droplets of an infected person. Due to absence of proper medication against this pandemic more than millions of people getting life threatening risk daily. Its outbreak has been reported from almost all 5 major continents. By 26 September 2020, WHO has reported 32,981,206 confirmed positive cases of COVID-19, including 996,589 deaths worldwide.

Frequent mutations altered protein structure of these RNA viruses by altering their amino acid sequences. Due to high rates of mutation, members of RNA viruses showed high genetic variability, consequently, acquiring drug resistance [2]. The genome size of SARS-CoV-2 is approximately30 kb which encodes 14 open reading frame (ORFs and), including 29 proteins as well as four structural proteins like S (spike), E (envelope), M (membrane), and N (nucleocapsid) proteins. Inspite of these proteins, viral genome contain 16 non structural proteins (nsp) including 9 accessory proteins [3,4].

Previous studies have documented that adaptive potential of these RNA viruses must be given weightage while designing antiviral therapeutics. In the present study, various recurrent mutations have been find out observed in the viral protein sequence of Indian isolates and compared with those of corresponding Wuhan isolate as a reference sequence [4]. The outcomes findings of present work suggest that mutational analysis of this contagious virus might be useful to develop new antiviral therapeutics against COVID-19.

Methods

The protein sequences of SARS-CoV-2 ORF1ab polyprotein, 7096 amino acid long were downloaded from NCBI virus database. Those sequences which were submitted from India in the month of August, 2020 were selected and used further for analysis. A reference sequence of Wuhan SARS-CoV-2 (Accession number YP_009724389) [4]. ORF1ab polyprotein was also downloaded to be used as a reference for mutational analysis.

The sequences downloaded were aligned using CLUSTAL Omega online server which performs alignment with HMM profiling [5]. For the alignment, Wuhan type SARS-CoV-2 was used as a reference to detect the variation. The aligned files were viewed using Jalview and recorded along with accession number to detect the mutation occurring in the Indian SARS-CoV-2 protein sequence with respect to Wuhan type SARS-CoV-2.

A phylogenetic tree was also constructed using CLUSTAL Omega which uses the aligned files to detect the relationship among different isolates as well as reference isolate.

Results

A total of 21 sequences were downloaded for ORF1ab polyprotein, which was submitted from India in the month of August, 2020 (including one reference). The accession number along with geographic location is as shown in table 1. The aligned files were viewed in Jalview to detect the mutational variants. A total of 64 point mutations were detected in the overall ORF1ab polyprotein of SARS-CoV-2 isolates of India. The position of mutation as well as the wild type and mutated amino acid sequence as shown in table 2.

Table 1: Details of SARS-CoV-2 sequences released from India in the month of August 2020.

Accession	Release_Date	Protein	Geo_Location	Collection_Date
QNL35816	2020-08-29T00:00:00Z	ORF1ab polyprotein	India: Ahmedabad	6/18/2020
QNL35828	2020-08-29T00:00:00Z	ORF1ab polyprotein	India: Ahmedabad	6/18/2020
QNL35840	2020-08-29T00:00:00Z	ORF1ab polyprotein	India: Ahmedabad	6/25/2020
QNL35852	2020-08-29T00:00:00Z	ORF1ab polyprotein	India: Ahmedabad	6/25/2020
QNL35864	2020-08-29T00:00:00Z	ORF1ab polyprotein	India: Ahmedabad	6/25/2020
QNL35876	2020-08-29T00:00:00Z	ORF1ab polyprotein	India: Ahmedabad	7/27/2020
QNL35888	2020-08-29T00:00:00Z	ORF1ab polyprotein	India: Ahmedabad	7/27/2020
QNL35900	2020-08-29T00:00:00Z	ORF1ab polyprotein	India: Ahmedabad	7/27/2020
QNL35912	2020-08-29T00:00:00Z	ORF1ab polyprotein	India: Ahmedabad	7/27/2020
QNL35924	2020-08-29T00:00:00Z	ORF1ab polyprotein	India: Adajan	6/12/2020
QNL35936	2020-08-29T00:00:00Z	ORF1ab polyprotein	India: Adajan	6/12/2020
QNL35948	2020-08-29T00:00:00Z	ORF1ab polyprotein	India: Mehsana	7/6/2020
QNL35960	2020-08-29T00:00:00Z	ORF1ab polyprotein	India: Mehsana	7/6/2020
QNL35972	2020-08-29T00:00:00Z	ORF1ab polyprotein	India: Mehsana	7/6/2020
QNL35984	2020-08-29T00:00:00Z	ORF1ab polyprotein	India: Modasa	7/9/2020
QNL35996	2020-08-29T00:00:00Z	ORF1ab polyprotein	India: Modasa	7/9/2020
QNL36008	2020-08-29T00:00:00Z	ORF1ab polyprotein	India: Modasa	7/9/2020
QNL36560	2020-08-29T00:00:00Z	ORF1ab polyprotein	India: Ahmedabad	6/25/2020
QIA98563	2020-08-18T00:00:00Z	ORF1ab polyprotein	India: Kerala State	1/30/2020
QIA98573	2020-08-18T00:00:00Z	ORF1ab polyprotein	India: Kerala State	1/31/2020

The phylogenetic analysis showed the occurrence of all the Indian isolates on different subtrees with respect to Wuhan type (Figure 1). This deviation is the result of the point mutation occurring in the sequences and hence providing a multifaceted nature to the virus. This also helps in evading the host immune response and is a barrier in vaccine as well as antiviral therapeutics development.

Table 2: Mutational locations after multiple sequence alignment of SARS-CoV-2 full length protein sequences with position and sequence.

S.No.	Accession No.	Position of mutation	Wild sequence	Mutated sequence
1.	QNL35912	33	D	M
2.	QNL35816	346	T	I
3.	QNL35828	519	G	S
4.	QIA98573	671	I	T
5.	QIA98563	671	I	T
6.	QIA98573	692	S	T
7.	QIA98573	718	R	G
8.	QNL35924	814	T	A
9.	QNL35936	814	T	A
10.	QNL36560	928	D	E
11.	QNL35840	930	D	Y
12.	QNL35876	1125	G	C
13.	QNL36560	1189	S	R
14.	QNL36560	1203	I	N
15.	QNL35888	1596	P	S
16.	QNL35900	1596	P	S
17.	QIA985731	1930	F	V
18.	QIA985731	1931	V	L
19.	QIA985731	1968	V	G
20.	QNL36560	2120	K	N
21.	QIA98563	2144	K	S
22.	QIA58973	2617	E	Q
23.	QIA98563	2870	E	H
24.	QIA98563	2872	S	K
25.	QIA98563	3049	A	D
26.	QIA98563	3118	N	K
27.	QIA98563	3119	D	V
28.	QIA98563	3128	M	R
29.	QIA98563	3129	M	R
30.	QIA98563	3134	T	A

31.	QNL36560	3306	I	V
32.	QNL35816	3353	K	R
32.	QNL35828	3395	P	L
33.	QNL35972	3459	T	M
34.	QIA98573	3760	F	I
35.	QIA98573	3846	G	C
36.	QIA98573	3888	W	S
37.	QNL36560	3993	R	C
38.	QNL35972	4065	T	I
39.	QNL36560	4326	C	R
40.	QIA98573	4404	C	S
41.		4429	I	N
42.	QIA98563	4437	F	Y
43.	QNL36560	4514	Y	F
44.	QNL35828	4533	T	I
45.	QIA98563	4718	F	I
46.	QNL35924	4746	V	L
47.	QNL35926	4746	V	L
48.	QNL36560	4790	V	L
49.	QIA98573	4798	A	V
50.	QIA98563	4798	A	V
51.	QNL36560	4880	I	S
52.	QNL35816	5272	V	I
53.	QIA98573	5974	M	A
54.	QIA98573	6305	G	A
55.	QNL36008	6449	T	I
56.	QIA98563	6545	P	T
57.	QNL35840	6678	K	C
58.	QNL35948	6958	T	R
59.	QNL35960	6958	K	R
60.	QIA98573	6987	W	S
61.	QIA98573	7054	G	A
62.	QNL36008	7036	Q	H
63.	QNL36560	7093	L	M
64.	QNL35912	7092	V	A

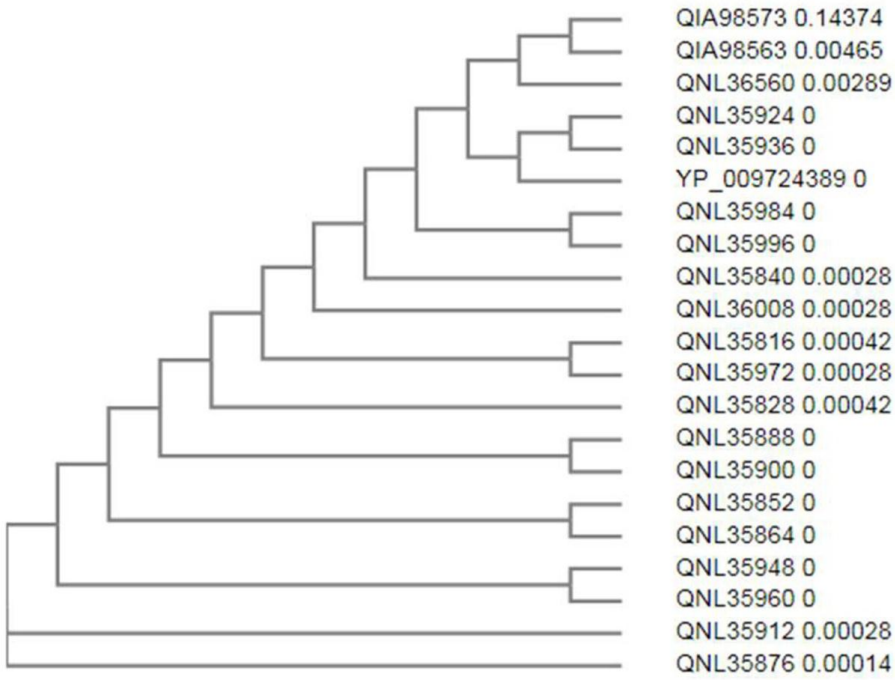

Figure 1: Phylogenetic tree showing Wuhan type and Indian type SARS-CoV-2 on different clusters.

Discussion

RNA viruses such as SARS-CoV-2 have very high potential to exhibit point mutation which is utmost beneficial for these viruses to adapt and evolve in rapidly changing environmental conditions which is responsible for its transmission worldwide [2,3]. These viruses can survive in dynamic environment of hosts because mutation brings natural selection and selecting those traits of viruses which are essential for their survival [9].

The results of present study revealed the presence of total 64 point mutations in 20 isolates of SARS-CoV-2 from India submitted in the month of August. These Coronavirus variants clustered in the different subtree to that of Wuhan SARS-CoV-2 and therefore, expressed their variability and rapid evolution with lapse of time [10]. Unstable protein structures are formed as results of mutagenic alterations caused by this RNA virus which helps in evasion of the host defense [11]. Recurrent mutations are big hurdle in the designing of antiviral drugs, moreover some drugs like chloroquine, remdesvir and favipiravir were found at some extent effective in treatment of COVID-19 infections. Our findings shown in this study, addresses a snap shot in time of a changing situation.

Conclusion

Occurrence of new mutations at different locations supports viral evolvability and provides a crucial insight into the development of effective vaccine and drug against COVID-19.

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