Clade GR and Clade GH Isolates in Asia Show Highest Amount of SNPs

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Abstract

Clades are monophyletic groups composed of a common ancestor and all its lineal descendants. As the propensity of virulence of a disease depends upon the type of clade the virus belongs to and it causes different fatality rates of disease in different countries, so the clade-wise analysis of SARS-CoV-2 isolates collected from different countries can illuminate the actual evolutionary relationships between them. In this study, 1566 SARS-CoV-2 genome sequences across ten Asian countries are collected, clustered, and characterized based on the clade they belong to. The isolates are compared to the Wuhan reference sequence (Accession no:$MN996528.1$) to identify the mutations that occurred at different protein regions. Structural changes in amino acids due to mutations lead to functional instability of the proteins. Detailed clade-wise functional assessments are carried out to quantify the stability and vulnerability of the mutations occurring in SARS-CoV-2 genomes which can shade light on personalized prevention and treatment of the disease and encourage towards the invention of clade-specific vaccines.

Keywords: COVID-19, clades, SARS-CoV-2, deleterious mutations, Asia

1. Introduction

Viruses have a remarkable capacity to adapt to new hosts and environments [1]. Mutations may lead to different phenotypic changes in them, which may lead to occur biodiversity. Phylogenies are frameworks for analysing biodiversity. Phylogenetic analysis based on sequence similarity is one of the very efficient way to do so [2]. However, it will be worth noting that due to the recent outbreak of pandemic COVID-19, people around the world are trying by every means to reach the origin, to get some ways of prevention and therapeutic pathways. Biodiversity is characterized by a continual replacement of branches in the tree of life, i.e. clade [3]. Evolutionary pressure on host immunodeficiency leads to different clades of viruses [4]. A clade is a group of highly related sequences that share a common ancestor. They can provide hypotheses about the actual evolutionary history of that group of sequences. Some clinical studies suggest that the proclivity of virulence of a disease depends upon the type of clade the virus belongs to [4]. Clade differences can result in varying degrees of pathology. Millions of gene regulatory elements are there which contribute heavily to the variation in gene expression of complex human traits and diseases [5].Determining mutation types influence a lot in gene regulation and is important for studying the role of regulatory variation in evolution. Genomic evolution helps a virus to escape host immunity [6, 7]. The clade-wise analysis of SARS-CoV-2 isolates collected from different countries can shed a light on the actual evolutionary history of the region or continent. In order to confirm the hypothesis in COVID-19 pathogenesis, it is highly recommended to make a thorough study of mutations occurring in SARS-CoV-2 isolates collected from different demographic areas and characterizing them based on the clades they come from [8]. A plethora of papers already have been published, where researchers have tried to study the virus isolates of SARS-CoV-2, which is solely responsible for the disease to occur in human [9, 10, 11, 12, 13]. Huge numbers of investigations are

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reported in order to find evolutionary relationships between SARS-CoV-2 and other corona-viruses and to determine the origin and molecular characteristics of SARS-CoV-2 [14, 15, 16]. Several works are also done on characterization and comparative analysis of structured and non-structured proteins of SARS-CoV-2 [17, 18]. According to Hassan et.al. [19] among all the accessory proteins of SARS-CoV-2, ORF3a plays an important role in virus pathogenesis, as it possesses various mutations which are linked with that of spike proteins.It is observed that due to its structural plasticity and high diversity, ORF8 plays important role in SARS-CoV-2 pathogenicity [20].Computational biology approaches are applied to investigate genomic and proteomic variations of S protein in SARS-CoV-2. In the paper authors find possibilities to design potential inhibitors against S protein [21]. Kumar et.al.stated the first observation on the deletion mutations in the C-terminal region of the envelope glycoprotein in India [22]. Some researchers have given more stresses on codon usage bias in SARS-CoV-2 rather than mutational trends [23]. Several investigations are carried out on characterizing the mutations of a particular country and even across the globe [24, 25, 26]. Researchers aimed too to analyse SARS-CoV-2 proteins modulating host immune response like type I interferon pathways [27]. People have tried to uncover the relation between hotspot mutations and viral pathogenicity [28]. Some research papers focused on characterizing B and T cell epitopes of certain proteins of SARS-CoV-2 which can help in vaccine development [29]. Phylodynamic analyses of SARS-CoV-2 genomes can provide insights into the roles of some relevant factors to limit the spread of the disease [30]. Priya and Shanker [31] have observed that the coevolutionary forces can increase the fitness of spike glycoprotein against ACE2 and increase the infectivity of SARS-CoV-2. According to Uddin et al. [32] antigenic epitopes of SARS-CoV-2 and SARS-CoV have highest level of similarities among them.SARS-CoV-2 is the seventh coronavirus to infect humans but the first HCoV which pandemic potential [33]. (Accession no:NC_045512) is the first SARS-CoV-2 sample SARS-CoV-2 sequence from Wuhan, and it is from clade 'O' [34]. Clade 'G' is the variant of the spike protein D614G which indicates significantly higher human host infectivity and better transmission efficiency to the virus. GH and GR are the most common offsprings of clade G. According to data from the public database of the Global Initiative on Sharing All Influenza Data (GISAID), three major clades of SARS-CoV-2 are clade G (variant of the spike protein S-D614G), clade V (a variant of the ORF3a coding protein NS3-G251), and clade S (variant ORF8-L84S) [34]. GR clade, carrying the combination of NSP3: F106F, Spike: D614G and Nucleocapsid: RG203KR mutations, whereas clade 'GH' represents the mutations NSP12b: P314L, S: D614G and ORF3a: Q57H. Different fatality rates observed in different countries may be the consequence of clade's differences in virulence. The spike protein of SARS-CoV-2 binds the host receptor angiotensin-converting enzyme 2 (ACE2) via receptor-binding domain (RBD). It is reported that immunization with SARS-CoV-2 receptor-binding domain (RBD) is able to induce clade-specific neutralizing antibodies in a host like mice [35]. In some cases vaccines are immunogenic and induced antibodies can neutralize homologous and heterogeneous viruses with different degrees of cross-reactivity [36]. Hence, in this present study, 1566 SARS-CoV-2 isolates from the Asian continent comprising 10 countries (India, Bangladesh, Pakistan, Srilanka, China, Japan, Malaysia, Iran, Thailand, and Saudi Arabia) are collected, clustered, and characterized based on the clade they belong to.

2. Methods and Materials

2.1. Collection of gene sequences of SARS-COV-2

One of the primary features of the investigation and analysis of the COVID-19 is availability of real-time data in global databases. To carry out the experiment We have collected 1566 isolates of SARS-CoV-2 from ten different Asian countries from the National Center for Biotechnology Information (NCBI) database (https://www.nih.gov/coronavirus) on October 20, 2020. The information about collected dataset are presented as Supplemental Materials in Table S1 and summarized in Table 1. In addition, we have collected the Reference Sequence (Accession no:$MN996528.1$) from the same Gene bank.However, information in detail about the dataset Collected sequences are then gone through preliminary screening for excluding noisy sequences. Here noise includes no mutations and the amino acid changes due to mutations specified by 'X'. Thus finally 1384 isolates are taken for further investigations.

Table 1: Collected SARS-CoV-2 genome sequences from COVID patients from ten different Asian Countries.

Country Name	# Associated Isolate	#Isolate in work
INDIA	570	565
THAILAND	227	104
BANGLADESH	231	231
IRAN	172	106
CHINA	189	189
JAPAN	96	96
SAUDI ARABIA	58	58
PAKISTAN	10	9
MALAYSIA	9	9
SRILANKA	4	4

Table 2: Clade-wise counting of isolates reported in 10 countries.

COUNTRY	G	GH	GR	L	S	O	V
THAILAND	12	0	0	1	39	51	1
BANGLADESH	6	13	208	1	1	2	0
SRILANKA	1	0	1	0	0	2	0
CHINA	2	0	1	64	48	71	3
JAPAN	2	5	71	13	2	3	0
INDIA	191	303	18	7	19	27	0
IRAN	32	1	0	4	0	69	0
MALAYSIA	0	1	0	0	0	8	0
PAKISTAN	0	1	5	2	0	1	0
SAUDI ARABIA	2	32	22	0	1	1	0

2.2. Methods

The present work aims to make a clade-wise classification and analysis of SARS-CoV-2 isolates of ten Asian countries. The isolates of each country are then compared with the reference sequence to find out the mutations that occurred. Clade-wise clustering of the given dataset is taken place. The observed mutations are then gone through different online software tools to investigate different biological functionalities that may change and affect the variants due to mutations. Here it is to be noted that we have used two web-based software tools (PROVEAN [37] and I-mutant [38]) for the aforesaid functional assessments.I-Mutant is a suite developed based on Support Vector Machine(SVM). ($\Delta\Delta G$>-0.5 Kcal/mol) indicates that the mutation can largely destabilize the protein, $\Delta\Delta G$ >0.5 Kcal/mol indicates about the strong stability and -0.5>=$\Delta\Delta G$>=0.5 Kcal/mol tells about weak effect of mutations. Isolates with a score equal to or below -2.5 are considered deleterious and scores above -2.5 are neutral. Lastly, we tracked the trend of mutations that occurred in the sequences of different clades.

3. Results and Discussions

3.0.1. Clade-wise clustering of SARS-CoV-2 strains taken as dataset from different countries

After excluding the noisy sequences finally 1371 isolates are found. Each strain belongs to a particular clade, so the isolates are clustered according to the clade from which they belong to. It has been observed that as a whole isolates of five clades (G, GH, GR, L, S, O, and V) are participated in those countries of the Asian continent. According to (Fig. 1) the order of the clade-wise participation of isolates is GH>GR>O>G>S>L>V. It is to be noted here that among the entire dataset taken Indian isolates hold a big amount of data. According to the country-wise view shown in Table 2 SARS-CoV-2 isolates of clade 'O' are present in the dataset of all countries and isolates of clade 'V' have been circulated only at China and Thailand. The country-wise analysis has a mixed result. In Srilanka,Thailand, China, Malaysia, and Iran the isolates are majorly from clade 'O'. India and Saudi Arabia have a prevalence of clade 'GH'. Pakistan, Bangladesh, and Japan have the prevalence of clade 'GR'. It indicates viral diversity regarding infection as the infection is transmitting from one country to another. Remarkable viral diversities are also present even in different regions within a country too.

3.1. Investigating trend of mutations in various clades

In this subsection firstly the positions of mutations are identified in each isolate and then it is aimed to calculate clade-wise percentage of mutations occurred in each country as shown in (Fig. 2).

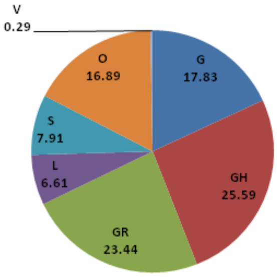


Figure 1: Calculate percentage of clades representing the isolates.

Secondly, a microscopic view has been given on clade-wise clustering of total mutations found in the whole dataset and calculating the protein-wise percentage of the mutations occurred according to the clades they belong to (Fig. 3).

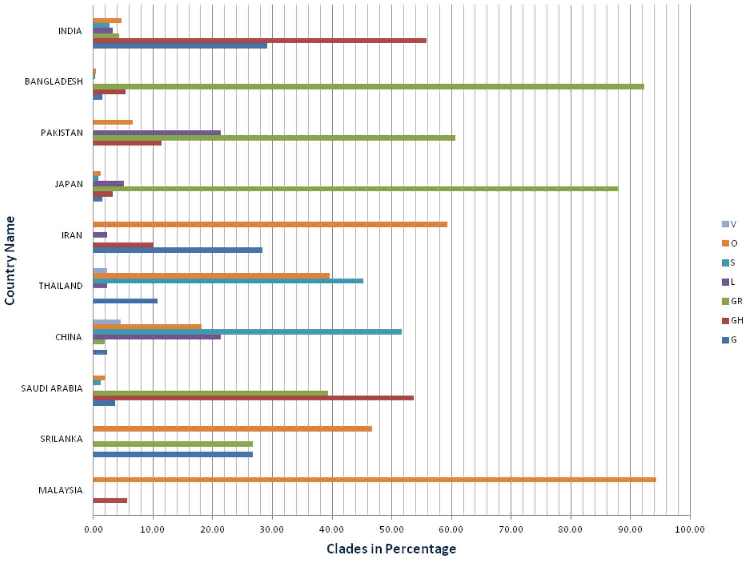
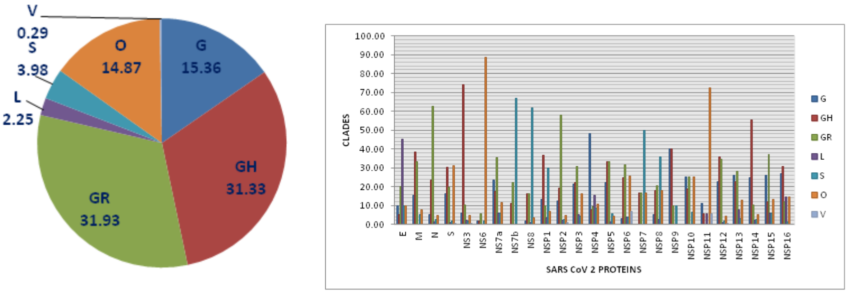


Figure 2: Calculating clade-wise percentage of mutations occurred in different countries.

According to (Fig. 2), India and Saudi Arabia have a prevalence of clade 'GH' (55.76%, and 53.69% respectively). Whereas, in Pakistan, Bangladesh, and Japan strains have a prevalence of clade 'GR' (60.66%, 92.33% and 88.06% respectively). Mutations have occurred in strains from Clade 'S' at China and Thailand (51.63% and 45% respectively). SARS-CoV-2 isolates of clade 'O' have significant participations in Iran, Srilanka and Malaysia (59.31%, 46.67% and 94.29% respectively). SARS-CoV-2 isolates of clades G, GH, GR, S, L, and O are circulating in India and Japan. Whereas, clades V, O, S, L, GR, and G are circulating at different regions of China and clades O, S, GR, GH, and G are circulated in Saudi Arabia. In Malaysia (clades O and GH) and Srilanka (clades O, GR, and G) the SARS-CoV-2 isolates do not have the viral diversities a lot.

Mutations refer to the virus to undergo certain changes which can lead to develop some new isolates after replications. Non-synonymous substitutions play a very significant role as this type of mutation makes change in amino acid. Alteration in amino acid causes structural change. With the aim of understanding the trend of non-synonymous mutations in different clades in the context of disease severity, a detailed protein-wise comparative analysis has been taken place. Mutations identified at different protein regions in all the isolates are shown at Table S2 in Supplementary file. To do so we have considered the total dataset as a whole. Clade-wise percentages of non-synonymous mutations at different protein regions are calculated. The clade-wise characterization of mutations of different proteins are shown in (Fig. 3).

According to the dataset taken, we have got 6665 numbers of non-synonymous mutations. We can observe at Table 3 that the chronological order of clades at per number of mutations taken place in whole dataset is GR>GH>G>O>S>L>V. It can be observed in (Fig. 3) that mutations are majorly



(a) a

(b) b

Figure 3: Quantitative analysis of clade-wise non-synonymous mutation. (a) Percentage of non-synonymous mutations found in different clades; (b) Clade-wise percentage of non-synonymous mutations occurred in different protein regions

Table 3: Clade-wise segregation of mutated data

PROTEIN	G	GH	GR	L	S	O	V
E	2	1	4	9	2	2	0
M	6	15	13	0	2	3	0
N	60	264	709	13	31	52	0
NS3	30	376	709	12	10	24	3
NS6	1	1	3	0	1	47	0
NS7a	4	3		1	1	2	0
NS7b	0	1		0	6	0	0
NS8	2	18	18	1	69	4	0
NSP1	4	11		1	9	2	0
NSP10	4	3	4	0	1	4	0
NSP11	2	1		1	0	13	1
NSP12	241	383	366	9	18	45	3
NSP13	24	21	26	7	3	12	0
NSP14	49	109	20	4	5	10	0
NSP15	22	10	31	5	5	11	0
NSP16	20	23	9	11	1	11	0
NSP2	52	82	245	9	11	20	4
NSP3	109	114	156	27	21	82	0
NSP4	50	8	10	16	9	11	0
NSP5	16	24	24	1	4	3	0
NSP6	3	25	32	4	4	26	7
NSP7	0	1	1	0	3	1	0
NSP8	2	7	8	1	14	7	0
NSP9	4	4	1	0	1	0	0
S	317	583	384	18	34	599	1
TOTAL MUTATION	**1024**	**2088**	**2128**	**150**	**265**	**991**	**19**

taken place at isolates of clades GH and GR which are 31.33% and 31.93% of respectively. Samples of clade V have been affected rarely (0.29%). Clade-wise distribution of mutations in each protein does not have a very similar trend(s). Although the majority of proteins mutated are either of clade GR (N, NS7a, NSP2, NSP6, NSP13, and NSP15) or GH (M, NS3, NSP1, NSP12, NSP14, and NSP16) but clade G also has large numbers of mutations in some proteins(S, NS6, and NSP11). Isolates of Clade L and clade G here have got mutations maximum only in protein E and NSP4 respectively. In proteins NS7b, NS8, and NSP7 of the isolates from clade S have maximum distributions of non-synonymous mutations. In the isolates of clade G, GR, and O number of mutations at NSP10 are equal. Mutations are also equally distributed in protein NSP5 of clades GH and GR.

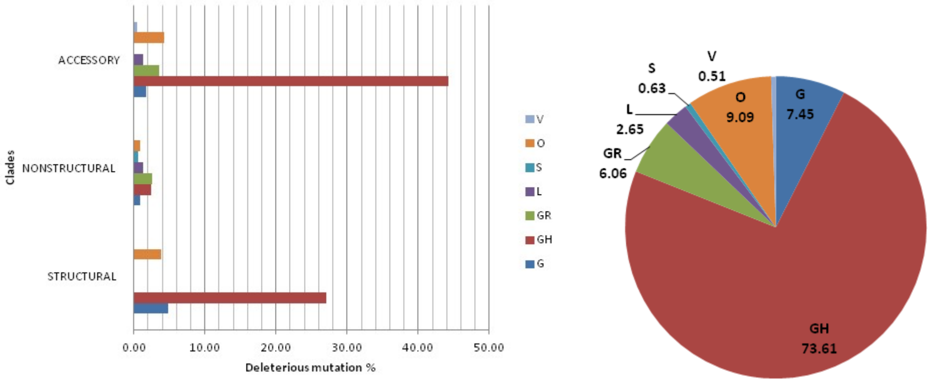
3.2. Quantitative assessment of functional changes occur due to mutations

Structural changes in amino acids due to mutations lead to create functional instability of the isolates themselves, cause vulnerable diseases and even increase the magnitude of virulence. In this subsection, we have tried to find the impact of single point mutations on the biological function of proteins of each isolates through the light of PROVEAN (Protein Variation Effect Analyzer) score, which may be deleterious or neutral [39]. We have also calculated the change in Gibbs free energy ($\Delta\Delta G$) occur due to single point mutations as the difference in folding free energy change between wild type and mutant protein ($\Delta\Delta G$) is considered as an impact factor of protein stability changes [40]. The motivation here is to understand the effect of those mutations on protein stability. The quantitative analysis will give an insight into the probable mutations that occur in a particular clade and the magnitude of virulence

Table 4: Investigate the deleterious mutations in total dataset.

PROTEIN	DELETERIOUS MUTATION	PROVEAN SCORE
S	D936Y	-2.60
S	L752R	-3.84
S	A288P	-2.80
S	C851W	-10.16
S	D820N	-4.67
S	E725K	-3.75
S	G648R	-4.093
S	G669R	-3.58
S	L533K	-2.62
S	L916F	-3.74
S	P863H	-5.12
S	R905S	-5.62
S	S758I	-4.13
S	S875F	-3.37
S	T716P	-3.347
S	T874P	-5.59
S	V534G	-3.12
N	S193I	-2.76
N	S194L	-4.27
N	R191L	-3.27
N	S180L	-3.40
PROTEIN	DELETERIOUS MUTATION	PROVEAN SCORE
NS3	Q57H	-3.286
NS3	G251V	-8.58
NS3	D155Y	-6.83
NS3	G172C	-6.75
NS3	G172V	-6.76
NS3	N257D	-3.286
NS3	G251V	-8.58
NS3	W45R	-8.87
NS7a	E95K	-2.61
NS7a	P45L	-10
NS7a	V104F	-2.83
NS8	P38R	-8.5
NSP2	T85I	-4.09
NSP2	F368V	-2.69
NSP2	G339S	-3.13
NSP2	P129S	-3.17
NSP2	E309A	-3.33
NSP3	A1914D	-3.8
NSP3	P1558L	-4.532
NSP4	L111S	-1.54
NSP4	G309C	-6.73
NSP5	P108S	-4.53
NSP5	I106S	-4.41
NSP5	L75F	-3.79
NSP5	N142L	-4.3
NSP5	R279C	-6.46
NSP6	G277S	-6
NSP6	H64N	-2.97
NSP12	D303G	-6.05
NSP1	W161L	-7.8
NSP13	R392C	-2.73
NSP14	T113I	-4.25
NSP15	M330T	-5.62
NSP16	R287I	-2.54
NSP16	P12S	-7.73
NSP16	G77R	-7.35
NSP12	T803I	-3.55
NSP12	M124I	-3.048
NSP12	P227S	-6.00
NSP12	S607I	-2.83
NSP12	A97V	-3.61

of them. It is to be noted that here we have excluded the mutations which are occurred only once.The deleterious mutations are shown in Table 4. It is observed at Table 4 that if we consider the dataset as a whole, then among structural proteins the mutations occurred in spike protein(s) are more deleterious than others. Among the accessory proteins, NS3 is affected the most. NSP2, NSP5, and NSP12 are the non-structural proteins that have most of the deleterious mutations that occurred. Furthermore, we have calculated clade-wise percentage of deleterious mutations that occurred in different protein regions. To do so, we have segregated each deleterious mutation occurred in ten different countries along with their clades Table 5. The (Fig. 4) shows the protein regions that are mostly affected by the deleterious mutations. Maximum deleterious mutations occurred in structural and accessory proteins belong to clade GH. Most of the deleterious mutations in non-structural protein regions are occurred in the isolates of both clades GH and GR. The isolates of clade V are rare and only found in the isolates of China and Thailand, but interestingly it is observed that few of deleterious mutations are also enlisted there. (Fig.4) depicts the fact that most of the deleterious mutations take place in amino acid sequences of clade GH. It is reported that the human genome may carry large numbers of deleterious mutations which as a whole make a significant contribution to fatal diseases. Identification and analysis of deleterious mutations can shade lights on personalized treatment and medicine [41]. Hence, the identification of these kinds of mutations in SARS-CoV-2 isolates and their impacts on the host body seek attention of virologists.



(a) (b)

Figure 4: Quantification of clade-wise deleterious non-synonymous mutation. (a) Clade-wise percentage of deleterious non-synonymous mutations in different protein regions; (b) Clade-wise calculations of deleterious non-synonymous mutations of total data set

Table 5: Clade-wise clustering of deleterious mutations in total dataset.

MUTATION	G	GH	GR	L	S	O	V	MUTATION	G	GH	GR	L	S	O	V
S193I	1	7	0	0	0	0	0	V104F	0	2	0	0	0	0	0
S194L	21	190	0	0	0	2	0	P38R	0	0	3	0	0	0	0
R191L	2	0	0	0	0	0	0	W161L	1	1	0	0	0	0	0
S180L	1	1	0	0	0	0	0	R392C	0	0	2	0	0	0	0
D936Y	1	1	0	0	0	0	0	T113I	0	0	0	1	0	4	0
L752R	0	0	0	0	0	2	0	M330T	0	0	2	0	0	0	0
A288P	0	0	0	0	0	3	0	R287I	0	0	3	0	0	0	0
C851W	1	0	0	0	0	1	0	P12S	0	0	0	4	0	0	0
D820N	0	0	0	0	0	2	0	G77R	0	0	0	3	0	1	0
E725K	0	0	0	0	0	2	0	T85I	0	0	0	0	0	0	0
G648R	1	0	0	0	0	3	0	F368V	1	1	0	0	0	0	0
G669R	0	0	0	0	0	4	0	G339S	0	0	0	1	0	0	0
L533K	0	0	0	0	0	2	0	P129S	0	0	3	0	0	0	0
L916F	1	0	0	0	0	1	0	E309A	1	1	0	0	0	0	0
P863H	4	15	0	0	0	0	0	A1914D	2	3	0	0	0	0	0
R905S	2	0	0	0	0	0	0	P1558L	0	0	3	2	0	2	0
S758I	2	0	0	0	0	0	0	L111S	0	0	0	0	5	0	0
S875F	1	0	0	0	0	2	0	G309C	1	2	0	0	0	0	0
T716P	0	0	0	0	0	2	0	P108S	0	0	7	0	0	0	0
T874P	0	0	0	0	0	3	0	I106S	0	0	2	0	0	0	0
V534G	0	0	0	0	0	2	0	L75F	1	2	0	0	0	0	0
Q57H	12	342	7	0	0	7	0	N142L	0	5	0	0	0	0	0
G251V	0	0	0	0	0	5	4	R279C	0	2	0	0	0	0	0
D155Y	0	2	5	0	0	0	0	G277S	0	2	0	0	0	0	0
G172C	0	0	3	0	0	0	0	H64N	1	1	0	0	0	0	0
G172V	0	0	3	0	0	0	0	D303G	0	0	2	0	0	0	0
N257D	1	1	0	0	0	0	0	T803I	0	0	2	0	0	0	0
W45R	0	0	0	7	0	0	0	M124I	0	0	3	0	0	0	0
E95K	1	1	0	0	0	0	0	P227S	0	0	2	0	0	0	0
P45L	0	2	0	0	0	0	0	S607I	0	0	2	0	0	0	0
A97V	0	0	0	3	0	22	0								

Table 6 gives us a microscopic view of the severity of the mutations that occurred in the dataset taken. In 82% of deleterious mutations protein stability has been decreased due to single point mutation. It is already observed that maximum mutations have occurred in the isolates which belong to clade GH. Out of 18 deleterious mutations happened in isolates from clade GH in 15 isolates (S194L, D936Y, P863H, W161L, F368V, E309A, A1914D, G309C, L75F, H64N, Q57H, N257D, E95K, P45L, V104F) stability have been decreased due to mutations. It can be observed at Table 7 that due to mutations majorly amino acid Glutamine and Serine are affected. Glutamine(Q) has been changed to Histidine(H), and Serine(S) changed to Leucine(L). The result may indicate that in Asian countries SARS-CoV-2 isolates responsible for COVID-19 majorly belong to the clades GR and GH. Among them mutations that occurred in isolates of clade GH are deleterious in nature, so have an impact on the biological function of proteins. The mutations also change the structural stability of proteins by making changes in free energy(ΔG).

Table 6: Investigating Stability of deleterious mutations in total dataset.

Mutation type	Mutation	Stability	ΔΔG	Mutation type	Mutation	Stability	ΔΔG
S194L	Deleterious	Decreased	-0.47	G77R	Deleterious	Decreased	-0.65
D936Y	Deleterious	Decreased	-0.35	T85I	Deleterious	Decreased	-0.91
L752R	Deleterious	Decreased	-1.39	F368V	Deleterious	Decreased	-1.74
C851W	Deleterious	Decreased	-0.09	G339S	Deleterious	Decreased	-1.2
D820N	Deleterious	Decreased	-1.25	P129S	Deleterious	Decreased	-1.82
E725K	Deleterious	Decreased	-0.35	E309A	Deleterious	Decreased	-0.96
G648R	Deleterious	Decreased	-0.6	A1914D	Deleterious	Decreased	-0.68
G669R	Deleterious	Decreased	-0.14	P1558L	Deleterious	Decreased	-0.36
L533K	Deleterious	Decreased	-1.81	L111S	Deleterious	Decreased	-2.29
L916F	Deleterious	Decreased	-1.12	G309C	Deleterious	Decreased	-1.07
P863H	Deleterious	Decreased	-1.44	P108S	Deleterious	Decreased	-1.73
R905S	Deleterious	Decreased	-1.18	I106S	Deleterious	Decreased	-2.24
T716P	Deleterious	Decreased	-0.8	L75F	Deleterious	Decreased	-1.09
T874P	Deleterious	Decreased	-0.22	R279C	Deleterious	Decreased	-0.67
V534G	Deleterious	Decreased	-2.029	G277S	Deleterious	Decreased	-1.3
Q57H	Deleterious	Decreased	-0.9	H64N	Deleterious	Decreased	-0.03
G251V	Deleterious	Decreased	-0.54	D303G	Deleterious	Decreased	-0.9
G172C	Deleterious	Decreased	-0.83	M124I	Deleterious	Decreased	-0.53
G172V	Deleterious	Decreased	-0.41	P227S	Deleterious	Decreased	-1.49
N257D	Deleterious	Decreased	-0.21	S193I	Deleterious	Increased	-0.29
W45R	Deleterious	Decreased	-1.05	R191L	Deleterious	Increased	-0.26
E95K	Deleterious	Decreased	-0.58	S180L	Deleterious	Increased	-0.12
P45L	Deleterious	Decreased	-0.71	A288P	Deleterious	Increased	-0.29
V104F	Deleterious	Decreased	-1.47	S758I	Deleterious	Increased	0.43
P38R	Deleterious	Decreased	-0.9	S875F	Deleterious	Increased	0.66
W161L	Deleterious	Decreased	-0.51	D155Y	Deleterious	Increased	0.21
R392C	Deleterious	Decreased	-1.29	N142L	Deleterious	Increased	-0.05
T113I	Deleterious	Decreased	-0.43	T803I	Deleterious	Increased	0.18
M330T	Deleterious	Decreased	-1	S607I	Deleterious	Increased	0.37
R287I	Deleterious	Decreased	-0.66	A97V	Deleterious	Increased	-0.31
P12S	Deleterious	Decreased	-1.29				

Table 7: Amino Acid changed due to deleterious mutations taken place.

MUTATION	COUNT	MUTATION	COUNT
Q>H	368	M>I	3
S>L	215	P>R	3
A>V	25	R>I	3
P>H	19	S>F	3
P>S	16	C>W	2
G>R	12	D>G	2
G>V	12	D>N	2
S>I	12	E>A	2
P>L	9	E>L	2
T>I	9	F>V	2
D>Y	9	H>N	2
W>R	7	I>S	2
G>C	6	L>K	2
A>D	5	L>R	2
L>F	5	L>S	2
N>L	5	M>T	2
T>P	5	N>D	2
E>K	4	R¿>L	2
R>C	4	R>S	2
A>P	3	V>F	2
G>S	3	V>G	2

4. Conclusions

The in silico analysis performed in this study states that the isolates in ten Asian countries are from clades G, GH, GR, L, S, O, and V. It indicates the diversity of the infection indeed. O, GH, and GR are the most widely affected ancestors of isolates among them. But when there is a talk about mutations, 31.93% of total mutations have taken place in the isolates of clade GR, and 31.33% of the mutations from GH. Hence, number of mutations are really high in the isolates belong to both the clades. When clades G, GH, and GR traversed almost in all countries specified here, the isolates of clade V are affected rarely. The most frequently mutated amino acids are Glutamine and Serine. In most of the cases glutamine is changed into Histidine and serine is changed to Leucine. It is to be noted that both the mutations are deleterious and the isolates of clade GH carry the major deleterious mutation load

(44.19% of the total dataset). The majority of mutations taken place in the isolatess of clade GH are deleterious in nature. 82% of deleterious mutations are unstable and so their biological functions are affected. As a whole in this present work, the investigation provides us clade-wise characteristics of the SARS-CoV-2 isolates of the Asian continent. When reported research papers shed the light on development of clade-specific vaccines [35], our analysis can encourage drug designers for development of customized drugs or vaccines for Asian continent in order to combat COVID-19.

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Competing interests

The authors declare no competing interests.

Supporting information

Table S1. Country-wise specifications of isolates with the clades they belong to.

Table S2. Identifying mutations at different protein regions of the dataset taken.