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Research Article

In Silico Characterisation of Severe Acute Respiratory Syndrome Coronavirus-2 (SARS-CoV-2) based on the Spike Protein Gene

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Abstract

Objectives: The Coronavirus Disease 2019 (COVID-19) caused by SARS-CoV-2 has been the current global pandemic concern. With a high transmission rate, especially through direct contact, this disease spreads from person to person, and this has in turn led to a huge number of infections on a global scale.

Methods: In present study, comparative genomic analysis was performed using 151 gene sequences of the viral spike protein retrieved from NCBI and along with its translated nucleotide sequences using MEGAX software. Variation in the nucleotide and amino acid positions were identified.

Results: Our analysis revealed that 22 nucleotide variations observed in positions 13, 141, 162, 233, 284, 328, 455, 459, 716, 773, 784, 882, 1686, 1715, 1749, 1841, 2031, 2076, 2383, 2520, 2533, 3300 and 17 amino acid variations observed in position 5, 54, 78, 90, 95, 152, 153, 239, 258, 262, 572, 583, 614, 684, 677, 795 and 845. Further, phylogenetic analysis was used to uncover the patterns of spread of the virus across the affected countries. Although, certain strains showed patterns of transmission within communities, a vast majority revealed an evident mosaic pattern.

Conclusion: The data obtained provides a clear understanding of variations in the nucleotide and translated nucleotide sequences, which can be targeted towards drug designing and to study evolutionary analysis.

Keywords: *In silico,* SARS-CoV-2, Spike protein gene, mutation, Multiple Sequence Alignment, Variation

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The SARS-CoV-2 (Severe Acute Respiratory Syndrome-Coronavirus-2), the causative agent of COVID-19 is found to be similar to Middle East Respiratory Syndrome-Coronavirus (MERS-CoV) and Severe Acute Respiratory Syndrome-Coronavirus (SARS-CoV). This virus has led to infecting over 223 countries worldwide with over 133 million (133,552,774) confirmed cases and 2 million (2,894,295) confirmed deaths as of World Health Organisation (WHO) reports on 10th April, 2021.[1] The mortality rate of SARS-CoV-2 lies between 1-35% and is similar to SARS-CoV and MERS-CoV during the year 2003 and 2012 respectively.[2] With a higher infectivity rate than its mortality rate, COVID-19 finds itself easily unfurling across six continents in the form of droplets, sneezing and cough from one individual to another.[3,4] The disease is primarily characterized by fever, sore throat, common cold, fatigue, lack of smell and taste. People having comorbidity such as heart disease, diabetes or chronic lung disease may further develop severe symptoms including pneumonia and acute respiratory distress syndrome. A few people also develop asymptomatic conditions of the disease.[5,6]

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The Coronavirus also designated as 'Severe Acute Respiratory Syndrome Coronavirus 2' (SARS-CoV-2) is a positive-sense single stranded RNA virus belonging to Order *Nidovirales*, Family *Coronaviridae* and Subfamily *Coronavirinae*.[7] The subfamily is further divided as *Alphacoronavirus* and *Betacoronavirus* infecting the mammals; and *Gammacoronavirus* and *Deltacoronavirus* infecting the birds respectively.[8,9] These viruses have a genome size of 26-32kb encoding 4 structural proteins [spike glycoprotein (S), envelope glycoprotein (E), matrix protein (M), nucleocapsid protein (N)] and 8 accessory proteins (3a, 3b, p6, 7a, 7b, 8b, 9b and orf14)] (Fig 1).[10] Their genome is protected through a layer of capsid proteins, or nucleocapsid, which forms a covering. This viral nucleocapsid possesses a helical symmetry, an atypical feature of positive-sense RNA virus which is further surrounded by an envelope glycoprotein layer.[11]

Among the 4 structural proteins, spike glycoprotein is highly essential for the virus to enter the host *via* interaction with host cellular receptors like Angiotensin Converting Enzyme-2 (ACE-2).[12,13] S protein belongs to class I viral transmembrane protein and has 1160 to 1400 amino acids. It is a trimer found on the surface of virus which gives it a crown-like appearance. It has 2 ectodomains, S1 and S2. S1 enables host receptor binding and S2 helps in fusion process. S1 is further subdivided into N-terminal domain (NTD), central receptor binding domain (RBD) and a C-terminal domain (CTD). This S1 CTD has receptor binding motif (RBM) and S1 trimer stalks itself upon the trimeric S2 stalk. There are 27 amino acid substitutions seen in 1273 amino acid stretch sequence. Of these, 6 are from RBD and 4 from RBM at CTD of S domain. These rapid substitutions depict the rapid changes in virus evolution.[14-16]

SARS-CoV-2 shares 89% similarity with SARS-CoV and MERS-CoV. SARS-CoV-2 consists of 12 functional open reading frames (ORFs) with the GC content of 38%.[17] The gene order: 5'cap-5'UTR-ORF1a-ORF1b-S-ORF3a-E-M-ORF6a-ORF7a-ORF7b-ORF8-N-ORF10-3'UTR-polyA tail.[18,19] Among these genes, ORF1ab contains the maximum number (21290 nucleotides) of nucleotides than the others. ORF1a-1b are arranged in the form replicase and helicase followed by 4 structural proteins.[20] The whole genome encodes a long polyprotein consisting of accessory proteins of 7096 residues long besides the structural proteins. These accessory proteins enable in viral replication, transcription, protein processing and response to antiviral actions.[21-23]

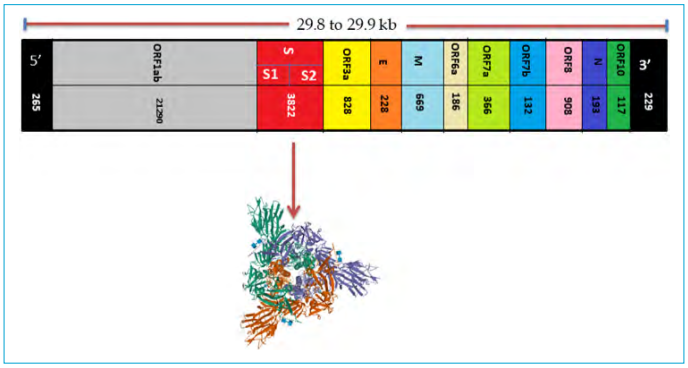

Figure 1. Schematic representation of SARS-CoV-2 genome. This figure represents the gene order from 5' to 3' end along with the nucleotide length of each gene respectively. The red arrow indicates the three-dimensional view of spike glycoprotein.

The spike protein is a vital target molecule for the production of DNA and attenuated vaccines, being the SARS-CoV-2 key protein responsible for infection, entry into host and pathogenesis. Here, we are performing a sequence analysis of the viral spike protein gene to enable clearer understanding of the regions easily variable both in nucleotide and translated nucleotide sequences. This data could be used to carefully modify those nucleotide or amino acid positions while preparing a remedy that could account for an enhanced protection from the SARS-CoV-2. Therefore, this present study which is carried out to identify the variation, mutations and conserved sequences present in the 151 nucleotide and amino acids of spike protein gene of SARS-CoV-2, is greatly important. Phylogenetic analysis of 151 sequences from different geographical regions worldwide was carried out to recognize the trend of nucleotide and amino acid variation and identify the different strains of SARS-CoV-2 present in studied sequences.

Methods

Retrieval of Sequences and Alignment

A total of 151 complete sequences of spike protein gene of SARS-CoV-2 along with the reference sequence were retrieved from the NCBI Database (www.ncbi.nig.gov) (Table 1). The nucleotide sequences were downloaded in FASTA format ensuring that they were all 3822 bp in length (complete sequences). After retrieving, the sequences were then aligned using Multiple Sequence Alignment program CLUSTAL_W using MEGA X software.[24] The aligned sequence files were used for further analysis.

MEGA Software

Molecular Evolutionary Genetics Analysis (MEGA) software was initially (in the 1900s) programmed for gene sequence analysis. Its recent version, MEGA X, enables whole genome sequencing (both DNA and protein) *via* Pairwise or Multiple Sequence Alignment with CLUSTAL_W program. The Molecular Evolutionary Genetics Analysis (MEGA) software provides tools to conduct automatic and manual sequence alignment and includes a large repertoire of programs for

assembling sequence alignments, estimating genetic distances and diversities, inferring evolutionary trees, computing time trees, inferring ancestral sequences and testing selection. In addition, this software has a bootstrap tree construction and model selection further enabling phylogenetic analysis.

Nucleotide and Amino acid Variation

In order to detect nucleotide variations in the spike protein gene sequences, we performed MSA of highly accurate and continuous assemblies of sequences. Variations were then curated manually from aligned sequences by individually hand-picking and using software display tools. The nucleotide variations of each of the 151 sequences are mentioned in Table 2. To overcome the problem of degeneracy of codon, variations in amino acid sequences were also determined and noted with respect to all positions in the alignment by robust analysis. Only those variations that were able to make a credible change in any amino acid residue within the protein sequences were counted and labelled as mutations.

Multiple Sequence Alignment (MSA) and Phylogenetic Analysis

To understand the homology and evolutionary relationship between these 151 retrieved sequences along with reference genome, we used MEGA X software (www.megasoftware.net)[24] to carry out MSA. This tool was preferred since it is one of the most cited tools used for evolutionary analysis in diverse biological fields. Multiple sequence alignment, following sequence retrieval, was performed using CLUSTAL W of MEGA X software with default parameters. This aligned sequence file was further analysed. Phylogeny was inferred using the Maximum Likelihood Method and the Tamura-Nei Model[25] – for Nucleotide Sequence Alignment, while Maximum Likelihood Method and a JTT matrix-based model[26] – was used for amino acid sequence alignment, both at 1000 bootstrap level in MEGA X. The Phylogenetic Trees thus created for both the alignments were then visualized in interactive Tree of Life (iTOL) (Fig. 2 and Fig. 3).[27]

Results

Complete 151 gene sequences of the SARS-CoV-2 spike gene along with the reference sequence were retrieved from NCBI Database randomly to avoid sample bias across the globe. Among the 150 sequences considered, partial sequences and incomplete sequences were manually excluded from the analysis to clearly understand the precise variations in nucleotide and translated nucleotide sequences. Of the 151 sequences, 91 sequences were from

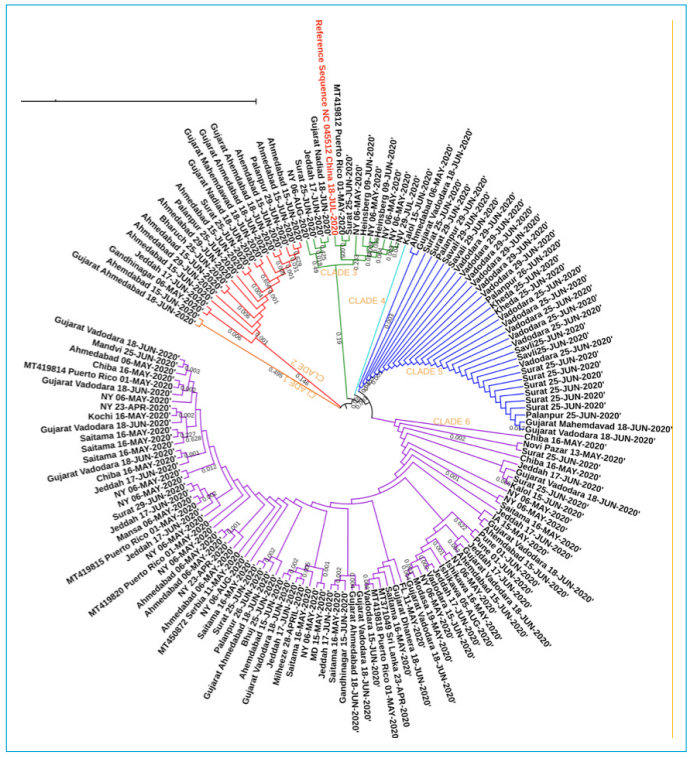

Figure 2. Evolutionary analysis of nucleotide sequence of Spike protein Gene by Maximum Likelihood method. The evolutionary history was inferred by using Maximum Likelihood method and Tamura-Nei model.[25] This analysis involves 151 nucleotide sequences. There was a total of 3822 positions in the final dataset. Evolutionary analyses were conducted in MEGA X.[24]

India, 27 sequences from United States of America (USA), 15 sequences from Japan, 11 sequences from Saudi Arabia, 2 sequences each from Serbia and Germany, 1 sequence each from China, Netherlands and Sri Lanka. While NCBI had over 4000 sequences deposited when we were conducting the study, we confined our analysis to 151 complete sequences since many sequences detected were incomplete and partial. Only the spike gene was retrieved as this was identified and confirmed for pathogenesis.

MEGA X software is a user-friendly software enabling the alignment of both DNA and protein sequences. The nucleotide variations and amino acid variation positions selected sequences are mentioned in table 2 and table 3 respectively. The phylogenetic tree, also made using MEGA X software, aids us in understanding the common ancestor and how these sequences varied with time. This in turn permits the study of an evolutionary analysis as well. On performing MSA, we obtained 22 nucleotide variations in positions 13, 141, 162, 233, 284, 328, 455, 459, 716, 773, 784, 882, 1686, 1715, 1749, 1841, 2031, 2076, 2383, 2520, 2533, 3300 (Table 2). We also found 17 amino acid variations in position 5, 54, 78, 90, 95, 152, 153, 239, 258, 262, 572, 583, 614, 684, 677, 795 and 845 (Table 3). This observation re-

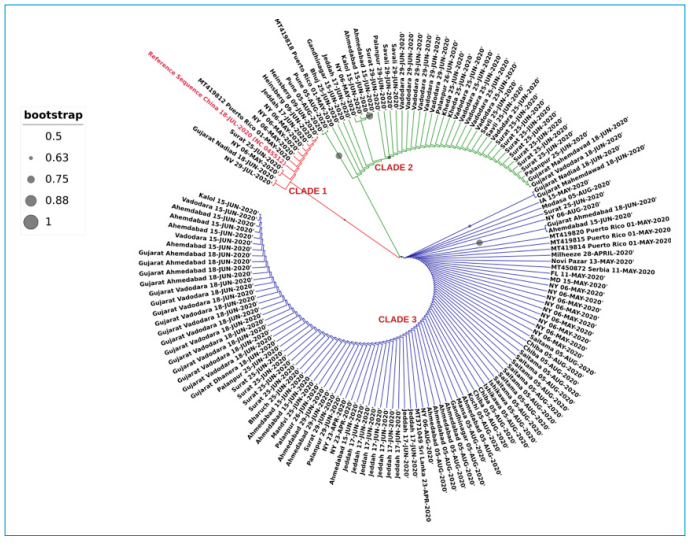

Figure 3. Evolutionary analysis by Maximum Likelihood method. The evolutionary history was inferred by using the Maximum Likelihood method and JTT matrix-based model.[26] The tree with the highest log likelihood (-3898.48) is shown. Bootstrap Values of more than 50% are represented on branches as grey dots with sizes corresponding to the respective Bootstrap Values. Initial tree(s) for the heuristic search were obtained automatically by applying Neighbor-Join and BioNJ algorithms to a matrix of pairwise distances estimated using the JTT model, and then selecting the topology with superior log likelihood value. The tree is drawn to scale, with branch lengths measured in the number of substitutions per site. This analysis involved 151 amino acid sequences (including the Reference Sequence). There were a total of 1273 positions in the final dataset. Evolutionary analyses were conducted in MEGA X.[24]

vealed that N-terminal region of the spike protein gene is more prone to mutations whereas C-terminus region of the spike protein gene was found to be conserved. Out of 17 variations occurred in the studied amino acid sequences, 14 variations were observed in the S1 subunit of spike protein gene.

Phylogenetic analysis was carried out using nucleotide and amino acid sequences of spike protein gene of SARS-CoV-2. The phylogenetic tree was built using Maximum Likelihood method and Tamura-Nei model.[25] All the sequences retrieved from NCBI, belonging to different countries were sketched, and grouped into six and three clades based on their respective nucleotide and amino acid sequences (Fig. 2 and Fig. 3). In a closure view of nucleotide based phylogenetic tree, the studied isolates were grouped in 22 clades (Fig. 2). However, 17 clade groups were seen based on the amino acid sequences. As the origin of SARS-CoV-2 and its subsequent rapid transition from an epidemic to pandemic are still clouded in ambiguity, it was interesting to try to identify the pattern of viral spread across various geographical locations. We discovered that, in both the nucleotide and amino acid alignments, the reference sequence (isolated from Wuhan, China) clustered closely with strains from Gujarat and Puerto Rico, followed by several different strains from the USA.

Discussion

SARS-Coronavirus-2, the unexpectedly relentless virus, has been extending its tentacles all over the world uncaring of borders and confinements. Since the declaration of the disease as a pandemic by WHO, many countries have implemented complete lockdowns, sealing of international borders, and instructing people to step out only during emergencies. Since it is transmissible via contact and respiratory fluids,[30] it has emerged as one of biggest factors posing public health risk. In the first four months of 2021 many countries facing the second wave of virus and found to be more severe than the first one. Coronavirus is also seen to affect the nervous system of individuals.[29] The current speedy transmission and worldwide spread of SARS-CoV-2 have raised life-threatening questions about the drastic evolution and its adaptation. The RNA genome of virus is prone to mutations, recombination's deletions and they are attacking different hosts having diverse strength of the immune response, which is responsible for variation in the genome of the viruses. In the present study, comparative genomic analysis was used to identify the conserved sites and major hotspots. Vaccines designed by considering these variations, could cut losses in terms of time and expenditure that might incur during the vaccine production. Similar studies have also been found, conducted in Dengue virus,[32] Saint Louis encephalitis virus,[33] Rotavirus,[34] H1N1 Influenza A virus,[35] Zika virus[36] and Coronavirus.[37] The viral particle exhibits 76-78% similarity with its ancestor- SARS-CoV.[31]

The envelope of SARS-CoV-2 consisting of trimeric spike protein in the S1 domain (14–685 amino acid residues), is responsible for the binding to the ACE2 receptor of the host (Fig. 4). We infer that the major 14 amino acid variations observed in our studied sequences may thus account for the strain variation in the domain (Walls et al., 2020., Yan R et al., 2020). The first step of viral infection is the binding of the virus particle to receptor present of the host cell. The virus has to therefore recognise specific receptor to enter into the host and this being a crucial step and found to be one of the key targets for drug designing. The amino acid variation identified in the present study is useful for drug design, diagnostics and vaccine development programs (Huang et al., 2020). In our study, variations from only the spike gene sequences were identified as the major gene involved pathogenesis and entry of virus in human host. We found 22 nucleotide variations in the positions: 13, 141,

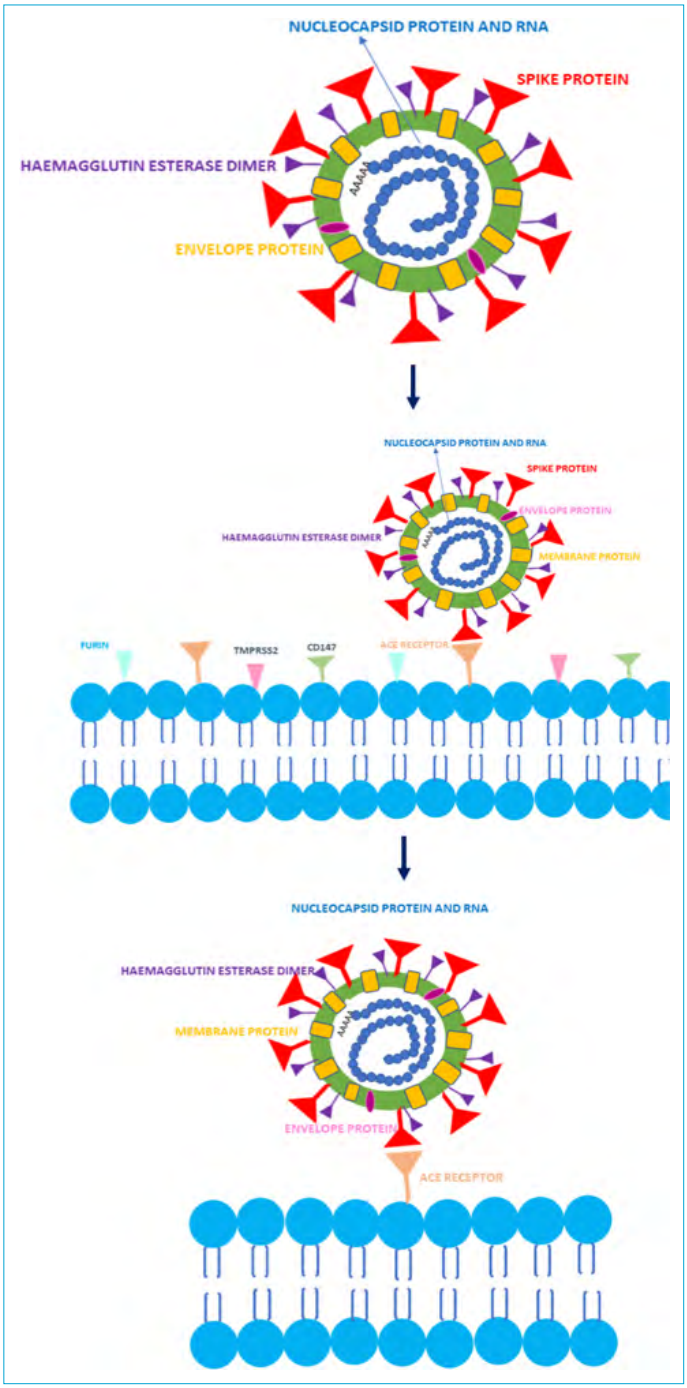

Figure 4. Schematic representation of attachment of the Spike protein of SARS-CoV-2 and ACE-2 receptor on the human lung.

162, 233, 284, 328, 455, 459, 716, 773, 784, 882, 1686, 1715, 1749, 1841, 2031, 2076, 2383, 2520, 2533, and 3300 (Table 3). To our surprise, many of these nucleotide variations contributed to synonymous variations in aligned amino acid sequences. We found 17 amino acid variations in the positions: 5, 54, 78, 90, 95, 152, 153, 239, 258, 262, 572, 583, 614, 684, 677, 795 and 845 (Table 3) when we translated the nucleotide sequence of spike protein gene into amino acid sequence. The code degeneracy restricts the all-nucleotide variation into the amino acid variations. C- terminal region of the spike protein was found to be conserved and very few variations were observed in this region. Further studies might be required to ensure a clearer understanding especially during vaccine preparation.[39-41]

A similar kind of study conducted by the Syed et al., 2020 involved MSA of 320 whole sequences and spike protein sequences and they found 483 new variations in the whole genome in SARS-CoV-2. This included 25 synonymous mutations and 1 deletion in spike protein of SARS-CoV-2. Of these 26 variations, 12 were present in NTD and 6 variations in RBD of spike protein. They also found 22 amino acid variations when compared with SARS-CoV-2, whereas in our study we found 17 variations in the spike proteins gene. This observation revealed the need for screening a larger number spike protein genes of SARS-CoV-2, and might be helpful to understand in variation at both levels i.e., nucleotide and amino acid. The above-mentioned observation shows that this might affect the receptor recognition of virus during host viral interactions and the phylogeny analysis reveals that the present SARS-CoV-2 is closely similar to the bat corona virus. Phylogenetic analysis also reveals that some strains belonging to a particular geographical area (Ahmedabad, Vadodara, Surat and Palanpur from Gujarat, and Saitama, Chiba and Ishikawa from Japan) clustered closely with one another indicating viral spread due to transmission between people in nearby communities. However, similar patterns in other strains were rather rare as other strains from Gujarat and Japan notably clustered with those belonging to Serbia or Heinsberg. Our observations revealed that, a state or country contains more than one type of strains of SARS-CoV-2.

Furthermore, most of the strains from a single state (such as Gujarat) or from a single country (USA or Japan), clustered distantly from one another, and instead showed relatedness with strains from other geographical locations. This was interesting to note as the absence of connection among strains from neighbouring regions or countries might suggest that each strain was brought in by the travel of infected individuals to different countries or regions. This in turn might have caused the development of a mosaic pattern of phylogenetic placements. Similar studies reporting mosaic pattern of phylogeographical distribution have also been conducted.[28] Studies on phylogeny help us to comprehend pathogenesis and design potential inhibitors -therapeutic drugs, besides vaccines and anti-viral therapies.[38] The 2017 avian like H1N1 lineages was found to be similar to 2009 pandemic H1N1 lineages in parallel to SARS-CoV-2 being similar to MERS-CoV and SARS-CoV viruses. Such a phylogeny analysis could help to design anti-

SARS-CoV-2 antibodies with SARS-CoV-2 spike protein particularly targeting the spike protein gene to apprehend the trending variation and cross-reactivity. Consistent studies and constant monitoring of the SARS-CoV-2 spike protein gene is of immense importance for subsequent novel drug development, newer diagnostics and protection against this deadly COVID-19 crisis.

Conclusion

In the present study, we were able to identify both the nucleotide and amino acid variation in different 22 and 17 positions respectively from 151 SARS-CoV-2 spike protein sequences. Since, these positions occupy the active site of epitope-based vaccines and most drug targets, their inclusion, or careful modifications could be beneficial in many ways. The data extracted in this present study will be useful for the further drug designing and modification, development of serological and molecular based diagnostics tools, evolution and variation studies and could finally be implemented in vaccine development programs.

Disclosures

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Table 1. Table indicating the accession number, name of the isolate, name of the gene, location (country, state, district), and the published date of the 150 spike proteins along with the reference sequence at top as extracted from NCBI Database

Sr. No	Accession No.	Isolate Name/number	Name of gene	Host	Country: State: District	Date
1. (Ref. Seq.)	NC_045512.2	Wuhan-Hu-1	S	Homo sapiens	China	18-07-20
2	MT419820	SARS-CoV2/human/USA/PR-CDC-S11/2020	S	Homo sapiens	Puerto Rico	01-05-20
3	MT419818	SARS-CoV2/human/USA/PR-CDC-S9/2020	S	Homo sapiens	Puerto Rico	01-05-20
4	MT419815	SARS-CoV2/human/USA/PR-CDC-S6/2020	S	Homo sapiens	Puerto Rico	01-05-20
5	MT419814	SARS-CoV2/human/USA/PR-CDC-S5/2020	S	Homo sapiens	Puerto Rico	01-05-20
6	MT419812	SARS-CoV2/human/USA/PR-CDC-S3/2020	S	Homo sapiens	Puerto Rico	01-05-20
7	MT396266	SARS-CoV2/mink/NLD/1/2020	S	Mustela lutreola	Netherlands: Milheeze	28-04-20
8	MT459979	SARS-CoV2/human/SRB/Novi Pazar-363/2020	S	Homo sapiens	Serbia: Novi Pazar	13-05-20
9	MT450872	SARS-CoV2/human/SRB/KV26/2020	S	Homo sapiens	Serbia	11-05-20
10	MT472624	SARS-CoV2/human/USA/FL-CDC-7619/2020	S	Homo sapiens	USA: FL	15-05-20
11	MT472626	SARS-CoV2/human/USA/IA-CDC-8200/2020	S	Homo sapiens	USA: IA	15-05-20
12	MT434799	SARS-CoV2/human/USA/NY-CDC-SURV0444NYC/2020	S	Homo sapiens	USA: NY	06-05-20
13	MT434785	SARS-CoV2/human/USA/NY-CDC-SURV039NYC/2020	S	Homo sapiens	USA: NY	06-05-20
14	MT472622	SARS-CoV2/human/USA/MD-CDC-0025/2020	S	Homo sapiens	USA:MD	15-05-20
15	MT434817	SARS-CoV2/human/USA/NY-CDC-SURV0985NYC/2020	S	Homo sapiens	USA: NY	06-05-20
16	MT434816	SARS-CoV2/human/USA/NY-CDC-SURV0592NYC/2020	S	Homo sapiens	USA: NY	06-05-20
17	MT434814	SARS-CoV2/human/USA/NY-CDC-SURV0168NYC/2020	S	Homo sapiens	USA: NY	06-05-20
18	MT434813	SARS-CoV2/human/USA/NY-CDC-SURV0144NYC/2020	S	Homo sapiens	USA: NY	06-05-20
19	MT434811	SARS-CoV2/human/USA/NY-CDC-SURV0996NYC/2020	S	Homo sapiens	USA: NY	06-05-20
20	MT434809	SARS-CoV2/human/USA/NY-CDC-SURV0983NYC/2020	S	Homo sapiens	USA: NY	06-05-20
21	MT434808	SARS-CoV2/human/USA/NY-CDC-SURV0982NYC/2020	S	Homo sapiens	USA: NY	06-05-20
22	MT434807	SARS-CoV2/human/USA/NY-CDC-SURV0874NYC/2020	S	Homo sapiens	USA: NY	06-05-20
23	MT434805	SARS-CoV2/human/USA/NY-CDC-SURV0862NYC/202	S	Homo sapiens	USA: NY	06-05-20
24	MT434804	SARS-CoV2/human/USA/NY-CDC-SURV0710NYC/2020	S	Homo sapiens	USA: NY	06-05-20
25	MT434803	SARS-CoV2/human/USA/NY-CDC-SURV0513NYC/2020	S	Homo sapiens	USA: NY	06-05-20
26	MT434801	SARS-CoV2/human/USA/NY-CDC-SURV	S	Homo sapiens	USA: NY	06-05-20
27	MT370836	SARS-CoV-2/human/USA/NY-PV08436/2020	S	Homo sapiens	USA: NY	06-08-20
28	MT370831	SARS-CoV-2/human/USA/NY-PV08464/2020	S	Homo sapiens	USA: NY	06-08-20
29	MT371049	SARS-CoV-2/human/LKA/COV91/2020	S	Homo sapiens	Sri Lanka	23-04-20
30	MT325597	SARS-CoV-2/human/USA/NV-CDC-0052/2020	S	Homo sapiens	USA: NV	29-07-20
31	MT630432	SARS-CoV-2/human/SAU/85791C/2020	S	Homo sapiens	Saudi Arabia: Jeddah	17-06-20
32	MT630431	SARS-CoV-2/human/SAU/85790C/2020	S	Homo sapiens	Saudi Arabia: Jeddah	17-06-20
33	MT630430	SARS-CoV-2/human/SAU/832279/2020	S	Homo sapiens	Saudi Arabia: Jeddah	17-06-20
34	MT630429	SARS-CoV-2/human/SAU/86650/2020	S	Homo sapiens	Saudi Arabia: Jeddah	17-06-20
35	MT630428	SARS-CoV-2/human/SAU/86327/2020	S	Homo sapiens	Saudi Arabia: Jeddah	17-06-20
36	MT630427	SARS-CoV-2/human/SAU/86267/2020	S	Homo sapiens	Saudi Arabia: Jeddah	17-06-20
37	MT630425	SARS-CoV-2/human/SAU/85790/2020	S	Homo sapiens	Saudi Arabia: Jeddah	17-06-20
38	MT630424	SARS-CoV-2/human/SAU/85715/2020	S	Homo sapiens	Saudi Arabia: Jeddah	17-06-20
39	MT630423	SARS-CoV-2/human/SAU/85613/2020	S	Homo sapiens	Saudi Arabia: Jeddah	17-06-20
40	MT630422	SARS-CoV-2/human/SAU/42952/2020	S	Homo sapiens	Saudi Arabia: Jeddah	17-06-20
41	MT630421	SARS-CoV-2/human/SAU/4637/2020	S	Homo sapiens	Saudi Arabia: Jeddah	17-06-20
42	MT582498	SARS-CoV-2/human/DEU/NRW-02.1/2020	S	Homo sapiens	Germany: Heinsberg	09-06-20
43	MT607608	SARS-CoV-2/human/IND/GBRC182a/2020	S	Homo sapiens	India: Ahmedabad	15-06-20
44	MT582494	SARS-CoV-2/human/DEU/NRW-06/2020	S	Homo sapiens	Germany: Heinsberg	09-06-20
45	MT370960	SARS-CoV-2/human/USA/NY-PV09097/2020	S	Homo sapiens	USA: NY	23-04-20
46	MT370967	SARS-CoV-2/human/USA/NY-PV09303/2020	S	Homo sapiens	USA: NY	23-04-20
47	MT675954	SARS-CoV-2/human/IND/GBRC231a/2020	S	Homo sapiens	India: Surat	29-06-20
48	MT675952	SARS-CoV-2/human/IND/GBRC229b/2020	S	Homo sapiens	India: Palanpur	29-06-20

Table 1. CONT.

Sr. No	Accession No.	Isolate Name/number	Name of gene	Host	Country: State: District	Date
49	MT675950	SARS-CoV-2/human/IND/GBRC229a/2020	S	Homo sapiens	India: Palanpur	29-06-20
50	MT675951	SARS-CoV-2/human/IND/GBRC230/2020	S	Homo sapiens	India: Surat	29-06-20
51	MT675945	SARS-CoV-2/human/IND/GBRC228b/2020	S	Homo sapiens	India: Ahmedabad	29-06-20
52	MT675944	SARS-CoV-2/human/IND/GBRC228a/2020	S	Homo sapiens	India: Ahmedabad	29-06-20
53	MT675943	SARS-CoV-2/human/IND/GBRC225a/2020	S	Homo sapiens	India: Savali	29-06-20
54	MT675942	SARS-CoV-2/human/IND/GBRC225b/2020	S	Homo sapiens	India: Savali	29-06-20
55	MT675940	SARS-CoV-2/human/IND/GBRC223b/2020	S	Homo sapiens	India: Vadodara	29-06-20
56	MT675941	SARS-CoV-2/human/IND/GBRC224b/2020	S	Homo sapiens	India: Vadodara	29-06-20
57	MT675939	SARS-CoV-2/human/IND/GBRC222b/2020	S	Homo sapiens	India: Vadodara	29-06-20
58	MT675938	SARS-CoV-2/human/IND/GBRC224a/2020	S	Homo sapiens	India: Vadodara	29-06-20
59	MT675937	SARS-CoV-2/human/IND/GBRC223a/2020	S	Homo sapiens	India: Vadodara	29-06-20
60	MT675933	SARS-CoV-2/human/IND/GBRC222a/2020	S	Homo sapiens	India: Vadodara	29-06-20
61	MT669322	SARS-CoV-2/human/IND/GBRC203b/2020	S	Homo sapiens	India: Palanpur	26-06-20
62	MT669321	SARS-CoV-2/human/IND/GBRC203a/2020	S	Homo sapiens	India: Palanpur	26-06-20
63	MT666042	SARS-CoV-2/human/IND/GBRC221/2020	S	Homo sapiens	India: Bhuj	25-06-20
64	MT665974	SARS-CoV-2/human/IND/GBRC220/2020	S	Homo sapiens	India: Mandvi	25-06-20
65	MT665972	SARS-CoV-2/human/IND/GBRC219b/2020	S	Homo sapiens	India: Kheda	25-06-20
66	MT665970	SARS-CoV-2/human/IND/GBRC219a/2020	S	Homo sapiens	India: Kheda	25-06-20
67	MT665028	SARS-CoV-2/human/IND/GBRC218b/2020	S	Homo sapiens	India: Vadodara	25-06-20
68	MT665006	SARS-CoV-2/human/IND/GBRC218a/2020	S	Homo sapiens	India: Vadodara	25-06-20
69	MT664990	SARS-CoV-2/human/IND/GBRC217b/2020	S	Homo sapiens	India: Vadodara	25-06-20
70	MT664986	SARS-CoV-2/human/IND/GBRC217a/2020	S	Homo sapiens	India: Vadodara	25-06-20
71	MT664822	SARS-CoV-2/human/IND/GBRC216b/2020	S	Homo sapiens	India: Vadodara	25-06-20
72	MT664808	SARS-CoV-2/human/IND/GBRC194b/2020	S	Homo sapiens	India: Savli	25-06-20
73	MT664807	SARS-CoV-2/human/IND/GBRC194a/2020	S	Homo sapiens	India: Savli	25-06-20
74	MT664796	SARS-CoV-2/human/IND/GBRC216a/2020	S	Homo sapiens	India: Vadodara	25-06-20
75	MT607608	SARS-CoV-2/human/IND/GBRC182a/2020	S	Homo sapiens	India: Ahmedabad	15-06-20
76	MT607611	SARS-CoV-2/human/IND/GBRC183a/2020	S	Homo sapiens	India: Ahmedabad	15-06-20
77	MT664774	SARS-CoV-2/human/IND/GBRC215/2020	S	Homo sapiens	India: Bharuch	25-06-20
78	MT664729	SARS-CoV-2/human/IND/GBRC214b/2020	S	Homo sapiens	India: Surat	25-06-20
79	MT664727	SARS-CoV-2/human/IND/GBRC214a/2020	S	Homo sapiens	India: Surat	25-06-20
80	MT664209	SARS-CoV-2/human/IND/GBRC210b/2020	S	Homo sapiens	India: Surat	25-06-20
81	MT664205	SARS-CoV-2/human/IND/GBRC210a/2020	S	Homo sapiens	India: Surat	25-06-20
82	MT664203	SARS-CoV-2/human/IND/GBRC209b/2020	S	Homo sapiens	India: Surat	25-06-20
83	MT664202	SARS-CoV-2/human/IND/GBRC209a/2020	S	Homo sapiens	India: Surat	25-06-20
84	MT664201	SARS-CoV-2/human/IND/GBRC208b/2020	S	Homo sapiens	India: Surat	25-06-20
85	MT664197	SARS-CoV-2/human/IND/GBRC208a/2020	S	Homo sapiens	India: Surat	25-06-20
86	MT664172	SARS-CoV-2/human/IND/GBRC207b/2020	S	Homo sapiens	India: Surat	25-06-20
87	MT664170	SARS-CoV-2/human/IND/GBRC207a/2020	S	Homo sapiens	India: Surat	25-06-20
88	MT664169	SARS-CoV-2/human/IND/GBRC206/2020	S	Homo sapiens	India: Surat	25-06-20
89	MT664161	SARS-CoV-2/human/IND/GBRC205b/2020	S	Homo sapiens	India: Surat	25-06-20
90	MT664143	SARS-CoV-2/human/IND/GBRC205a/2020	S	Homo sapiens	India: Surat	25-06-20
91	MT664118	SARS-CoV-2/human/IND/GBRC204b/2020	S	Homo sapiens	India: Palanpur	25-06-20
92	MT664117	SARS-CoV-2/human/IND/GBRC204a/2020	S	Homo sapiens	India: Palanpur	25-06-20
93	MT635858	SARS-CoV-2/human/IND/GBRC199/2020	S	Homo sapiens	India: Gujarat, Nadiad	18-06-20
94	MT635856	SARS-CoV-2/human/IND/GBRC202/2020	S	Homo sapiens	India: Gujarat, Dhanera	18-06-20
95	MT635857	SARS-CoV-2/human/IND/GBRC201b/2020	S	Homo sapiens	India: Gujarat, Mahemdavad	18-06-20
96	MT635855	SARS-CoV-2/human/IND/GBRC201a/2020	S	Homo sapiens	India: Gujarat, Mahemdavad	18-06-20
97	MT635410	SARS-CoV-2/human/IND/GBRC196/2020	S	Homo sapiens	India: Gujarat, Vadodara	18-06-20
98	MT635409	SARS-CoV-2/human/IND/GBRC198/2020	S	Homo sapiens	India: Gujarat, Nadiad	18-06-20
99	MT635408	SARS-CoV-2/human/IND/GBRC190/2020	S	Homo sapiens	India: Gujarat, Vadodara	18-06-20
100	MT635407	SARS-CoV-2/human/IND/GBRC195b/2020	S	Homo sapiens	India: Gujarat, Vadodara	18-06-20

Table 1. CONT.

Sr. No	Accession No.	Isolate Name/number	Name of gene	Host	Country: State: District	Date
101	MT635406	SARS-CoV-2/human/IND/GBRC191a/2020	S	Homo sapiens	India: Gujarat, Vadodara	18-06-20
102	MT635404	SARS-CoV-2/human/IND/GBRC197/2020	S	Homo sapiens	India: Gujarat, Vadodara	18-06-20
103	MT635405	SARS-CoV-2/human/IND/GBRC195a/2020	S	Homo sapiens	India: Gujarat, Vadodara	18-06-20
104	MT635403	SARS-CoV-2/human/IND/GBRC188/2020	S	Homo sapiens	India: Gujarat, Vadodara	18-06-20
105	MT635397	SARS-CoV-2/human/IND/GBRC187b/2020	S	Homo sapiens	India: Gujarat, Vadodara	18-06-20
106	MT635393	SARS-CoV-2/human/IND/GBRC193a/2020	S	Homo sapiens	India: Gujarat, Vadodara	18-06-20
107	MT635392	SARS-CoV-2/human/IND/GBRC193b/2020	S	Homo sapiens	India: Gujarat, Vadodara	18-06-20
108	MT635391	SARS-CoV-2/human/IND/GBRC191b/2020	S	Homo sapiens	India: Gujarat, Vadodara	18-06-20
109	MT635339	SARS-CoV-2/human/IND/GBRC192/2020	S	Homo sapiens	India: Gujarat, Vadodara	18-06-20
110	MT635328	SARS-CoV-2/human/IND/GBRC185a/2020	S	Homo sapiens	India: Gujarat, Ahmedabad	18-06-20
111	MT635272	SARS-CoV-2/human/IND/GBRC186b/2020	S	Homo sapiens	India: Gujarat, Ahmedabad	18-06-20
112	MT635271	SARS-CoV-2/human/IND/GBRC185b/2020	S	Homo sapiens	India: Gujarat, Ahmedabad	18-06-20
113	MT635269	SARS-CoV-2/human/IND/GBRC186a/2020	S	Homo sapiens	India: Gujarat, Ahmedabad	18-06-20
114	MT635270	SARS-CoV-2/human/IND/GBRC184/2020	S	Homo sapiens	India: Gujarat, Ahmedabad	18-06-20
115	MT608648	SARS-CoV-2/human/IND/GBRC183b/2020	S	Homo sapiens	India: Ahmedabad	15-06-20
116	MT607618	SARS-CoV-2/human/IND/GBRC176/2020	S	Homo sapiens	India: Vadodara	15-06-20
117	MT607621	SARS-CoV-2/human/IND/GBRC178a/2020	S	Homo sapiens	India: Ahmedabad	15-06-20
118	MT607619	SARS-CoV-2/human/IND/GBRC182b/2020	S	Homo sapiens	India: Ahmedabad	15-06-20
119	MT607620	SARS-CoV-2/human/IND/GBRC180b/2020	S	Homo sapiens	India: Ahmedabad	15-06-20
120	MT607617	SARS-CoV-2/human/IND/GBRC181a/2020	S	Homo sapiens	India: Ahmedabad	15-06-20
121	MT607615	SARS-CoV-2/human/IND/GBRC175/2020	S	Homo sapiens	India: Vadodara	15-06-20
122	MT607616	SARS-CoV-2/human/IND/GBRC173b/2020	S	Homo sapiens	India: Kalol	15-06-20
123	MT607613	SARS-CoV-2/human/IND/GBRC173a/2020	S	Homo sapiens	India: Kalol	15-06-20
124	MT607614	SARS-CoV-2/human/IND/GBRC179a/2020	S	Homo sapiens	India: Ahmedabad	15-06-20
125	MT607612	SARS-CoV-2/human/IND/GBRC171/2020	S	Homo sapiens	India: Gandhinagar	15-06-20
126	MT607609	SARS-CoV-2/human/IND/GBRC177a/2020	S	Homo sapiens	India: Ahmedabad	15-06-20
127	LC547528	hCoV-19/Japan/P4-6/2020	S	Homo sapiens	Japan: Saitama	16-05-20
128	LC547532	hCoV-19/Japan/P5-2/2020	S	Homo sapiens	Japan: Chiba	16-05-20
129	LC547531	hCoV-19/Japan/P5-1/2020	S	Homo sapiens	Japan: Chiba	16-05-20
130	LC547530	hCoV-19/Japan/P4-8/2020	S	Homo sapiens	Japan: Saitama	16-05-20
131	LC547529	hCoV-19/Japan/P4-7/2020	S	Homo sapiens	Japan: Saitama	16-05-20
132	LC547527	hCoV-19/Japan/P4-5/2020	S	Homo sapiens	Japan: Saitama	16-05-20
133	LC547526	hCoV-19/Japan/P4-4/2020	S	Homo sapiens	Japan: Saitama	16-05-20
134	LC547525	hCoV-19/Japan/P4-3/2020	S	Homo sapiens	Japan: Saitama	16-05-20
135	LC547524	hCoV-19/Japan/P4-2/2020	S	Homo sapiens	Japan: Saitama	16-05-20
136	LC547523	hCoV-19/Japan/P4-1/2020	S	Homo sapiens	Japan: Saitama	16-05-20
137	LC547522	hCoV-19/Japan/P3-2/2020	S	Homo sapiens	Japan: Ishikawa	16-05-20
138	LC547521	hCoV-19/Japan/P3-1/2020	S	Homo sapiens	Japan: Ishikawa	16-05-20
139	LC547520	hCoV-19/Japan/P2-2/2020	S	Homo sapiens	Japan: Chiba	16-05-20
140	LC547519	hCoV-19/Japan/P2-1/2020	S	Homo sapiens	Japan:Chiba	16-05-20
141	LC547518	hCoV-19/Japan/P1/2020	S	Homo sapiens	Japan: Kochi	16-05-20
142	MT416726	SARS-CoV-2/human/IND/8004/2020	S	Homo sapiens	India: Pune	01-06-20
143	MT416725	SARS-CoV-2/human/IND/8003/2020	S	Homo sapiens	India: Pune	01-06-20
144	MT435082	SARS-CoV-2/human/IND/GBRC5/2020	S	Homo sapiens	India: Ahmedabad	06-05-20
145	MT435086	SARS-CoV-2/human/IND/GBRC9/2020	S	Homo sapiens	India: Mansa	06-05-20
146	MT435085	SARS-CoV-2/human/IND/GBRC8/2020	S	Homo sapiens	India: Gandhinagar	06-05-20
147	MT435084	SARS-CoV-2/human/IND/GBRC7/2020	S	Homo sapiens	India: Ahmedabad	06-05-20
148	MT435083	SARS-CoV-2/human/IND/GBRC6/2020	S	Homo sapiens	India: Ahmedabad	06-05-20
149	MT435081	SARS-CoV-2/human/IND/GBRC4/2020	S	Homo sapiens	India: Ahmedabad	06-05-20
150	MT435080	SARS-CoV-2/human/IND/GBRC3/2020	S	Homo sapiens	India: Ahmedabad	06-05-20
151	MT483560	SARS-CoV-2/human/IND/GBRC81b/2020	S	Homo sapiens	India: Modasa	19-05-20

Table 2. Table indicating nucleotide variations in the selected 150 viral spike gene sequences along with the reference SARS-COV-2 (Wuhan Hu 1) sequence.

Sr. No	Isolate name	Accession number	Variations in nucleotide position of spike gene.		
1	Wuhan-Hu-1	NC_045512	882	1841	
2	SARS-CoV2/human/USA/PR-CDC-S11/2020	MT419820	882		
3	SARS-CoV2/human/USA/PR-CDC-S9/2020	MT419818	882	716	
4	SARS-CoV2/human/USA/PR-CDC-S6/2020	MT419815	882		
5	SARS-CoV2/human/USA/PR-CDC-S5/2020	MT419814	882		
6	SARS-CoV2/human/USA/PR-CDC-S3/2020	MT419812	882	1841	
7	SARS-CoV2/mink/NLD/1/2020	MT396266	882	3300	
8	SARS-CoV2/human/SRB/Novi Pazar-363/2020	MT459979	882		
9	SARS-CoV2/human/SRB/KV26/2020	MT450872	882		
10	SARS-CoV2/human/USA/FL-CDC-7619/2020	MT472624	882		
11	SARS-CoV2/human/USA/IA-CDC-8200/2020	MT472626	882	773	
12	SARS-CoV2/human/USA/NY-CDC SURV0444NYC/2020	MT434799	882	284	
13	SARS-CoV2/human/USA/NY-CDC-SURV039NYC/2020	MT434785	882	1841	2533
14	SARS-CoV2/human/USA/MD-CDC-0025/2020	MT472622	882		
15	SARS-CoV2/human/USA/NY-CDC-SURV0985NYC/2020	MT434817	882		
16	SARS-CoV2/human/USA/NY-CDC-SURV0592NYC/2020	MT434816	882		
17	SARS-CoV2/human/USA/NY-CDC-SURV0168NYC/2020	MT434814	882	1841	
18	SARS-CoV2/human/USA/NY-CDC-SURV0144NYC/2020	MT434813	882	1841	
19	SARS-CoV2/human/USA/NY-CDC-SURV0996NYC/2020	MT434811	882		
20	SARS-CoV2/human/USA/NY-CDC-SURV0983NYC/2020	MT434809	882		
21	SARS-CoV2/human/USA/NY-CDC-SURV0982NYC/2020	MT434808	882		
22	SARS-CoV2/human/USA/NY-CDC-SURV0874NYC/2020	MT434807	882	1841	
23	SARS-CoV2/human/USA/NY-CDC-SURV0862NYC/202	MT434805	882		
24	SARS-CoV2/human/USA/NY-CDC-SURV0710NYC/2020	MT434804	882		
25	SARS-CoV2/human/USA/NY-CDC-SURV0513NYC/2020	MT434803	882		
26	SARS-CoV2/human/USA/NY-CDC-SURV0475NYC/2020	MT434801	882		
27	hCoV-19/Japan/P4-6/2020	LC547528	882	1686	
28	hCoV-19/Japan/P5-2/2020	LC547532	882		
29	hCoV-19/Japan/P5-1/2020	LC547531	882		
30	hCoV-19/Japan/P4-8/2020	LC547530	882	1686	
31	hCoV-19/Japan/P4-7/2020	LC547529	882	1686	
32	hCoV-19/Japan/P4-5/2020	LC547527	882		
33	hCoV-19/Japan/P4-4/2020	LC547526	882		
34	hCoV-19/Japan/P4-3/2020	LC547525	882		
35	hCoV-19/Japan/P4-2/2020	LC547524	882		
36	hCoV-19/Japan/P4-1/2020	LC547523	882		
37	hCoV-19/Japan/P3-2/2020	LC547522	882		
38	hCoV-19/Japan/P3-1/2020	LC547521	882		
39	hCoV-19/Japan/P2-2/2020	LC547520	882		
40	hCoV-19/Japan/P2-1/2020	LC547519	882		
41	hCoV-19/Japan/P1/2020	LC547518	882		
42	SARS-CoV-2/human/IND/8004/2020	MT416726	882	2031	
43	SARS-CoV-2/human/IND/8003/2020	MT416725	882	2031	
44	SARS-CoV-2/human/IND/GBRC5/2020	MT435082	882		
45	SARS-CoV-2/human/IND/GBRC9/2020	MT435086	882		
46	SARS-CoV-2/human/IND/GBRC8/2020	MT435085	882		
47	SARS-CoV-2/human/IND/GBRC7/2020	MT435084	882		
48	SARS-CoV-2/human/IND/GBRC6/2020	MT435083	882	2076	

Table 2. CONT.

Sr. No	Isolate name	Accession number	Variations in nucleotide position of spike gene.		
49	SARS-CoV-2/human/IND/GBRC4/2020	MT435081	882		
50	SARS-CoV-2/human/IND/GBRC3/2020	MT435080	882		
51	SARS-CoV-2/human/IND/GBRC81b/2020	MT483560	882	233	
52	SARS-CoV-2/human/USA/NY-PV08436/2020	MT370836	882		
53	SARS-CoV-2/human/LKA/COV91/2020	MT371049	882		
54	SARS-CoV-2/human/USA/NV-CDC-0052/2020	MT325597	882	1841	13
55	SARS-CoV-2/human/USA/NY-PV08464/2020	MT370831	882	268	906
56	SARS-CoV-2/human/SAU/85791C/2020	MT630432	882		
57	SARS-CoV-2/human/SAU/85790C/2020	MT630431	882		
58	SARS-CoV-2/human/SAU/832279/2020	MT630430	882		
59	SARS-CoV-2/human/SAU/86650/2020	MT630429	882		
60	SARS-CoV-2/human/SAU/86327/2020	MT630428	882		
61	SARS-CoV-2/human/SAU/86267/2020	MT630427	2051		
62	SARS-CoV-2/human/SAU/85790/2020	MT630425	882		
63	SARS-CoV-2/human/SAU/85715/2020	MT630424	882		
64	SARS-CoV-2/human/SAU/85613/2020	MT630423	882	906	1841
65	SARS-CoV-2/human/SAU/42952/2020	MT630422	882		
66	SARS-CoV-2/human/SAU/4637/2020	MT630421	882		
67	SARS-CoV-2/human/DEU/NRW-02.1/2020	MT582498	882	1841	
68	SARS-CoV-2/human/IND/GBRC182a/2020	MT607608	882		
69	SARS-CoV-2/human/DEU/NRW-06/2020	MT582494	882	1841	
70	SARS-CoV-2/human/USA/NY-PV09097/2020	MT370960	882		
71	SARS-CoV-2/human/USA/NY-PV09303/2020	MT370967	882		
72	SARS-CoV-2/human/IND/GBRC231a/2020	MT675954	882	162	
73	SARS-CoV-2/human/IND/GBRC229b/2020	MT675952	882	162	
74	SARS-CoV-2/human/IND/GBRC229a/2020	MT675950	882	141	
75	SARS-CoV-2/human/IND/GBRC230/2020	MT675951	882		
76	SARS-CoV-2/human/IND/GBRC228b/2020	MT675945	882		
77	SARS-CoV-2/human/IND/GBRC228a/2020	MT675944	882		
78	SARS-CoV-2/human/IND/GBRC225a/2020	MT675943	882	162	
79	SARS-CoV-2/human/IND/GBRC225b/2020	MT675942	882	162	
80	SARS-CoV-2/human/IND/GBRC223b/2020	MT675940	882	162	
81	SARS-CoV-2/human/IND/GBRC224b/2020	MT675941	882	162	
82	SARS-CoV-2/human/IND/GBRC222b/2020	MT675939	882	162	
83	SARS-CoV-2/human/IND/GBRC224a/2020	MT675938	882	162	
84	SARS-CoV-2/human/IND/GBRC223a/2020	MT675937	882	162	
85	SARS-CoV-2/human/IND/GBRC222a/2020	MT675933	882	162	
86	SARS-CoV-2/human/IND/GBRC203b/2020	MT669322	882	162	
87	SARS-CoV-2/human/IND/GBRC203a/2020	MT669321	882		
88	SARS-CoV-2/human/IND/GBRC221/2020	MT666042	882	328	2383
89	SARS-CoV-2/human/IND/GBRC220/2020	MT665974	882		
90	SARS-CoV-2/human/IND/GBRC219b/2020	MT665972	882	162	
91	SARS-CoV-2/human/IND/GBRC219a/2020	MT665970	882	162	
92	SARS-CoV-2/human/IND/GBRC218b/2020	MT665028	882	162	
93	SARS-CoV-2/human/IND/GBRC218a/2020	MT665006	882	162	
94	SARS-CoV-2/human/IND/GBRC217b/2020	MT664990	882	162	
95	SARS-CoV-2/human/IND/GBRC217a/2020	MT664986	882	162	
96	SARS-CoV-2/human/IND/GBRC216b/2020	MT664822	882	162	
97	SARS-CoV-2/human/IND/GBRC194b/2020	MT664808	882	162	

Table 2. CONT.

Sr. No	Isolate name	Accession number	Variations in nucleotide position of spike gene.		
98	SARS-CoV-2/human/IND/GBRC194a/2020	MT664807	882	162	
99	SARS-CoV-2/human/IND/GBRC216a/2020	MT664796	882	162	
100	SARS-CoV-2/human/IND/GBRC182a/2020	MT607608	882		
101	SARS-CoV-2/human/IND/GBRC183a/2020	MT607611	882		
102	SARS-CoV-2/human/IND/GBRC215/2020	MT664774	882		
103	SARS-CoV-2/human/IND/GBRC214b/2020	MT664729	882	162	
104	SARS-CoV-2/human/IND/GBRC214a/2020	MT664727	882	784	
105	SARS-CoV-2/human/IND/GBRC210b/2020	MT664209	882		
106	SARS-CoV-2/human/IND/GBRC210a/2020	MT664205	882	162	
107	SARS-CoV-2/human/IND/GBRC209b/2020	MT664203	882		
108	SARS-CoV-2/human/IND/GBRC209a/2020	MT664202	882	162	
109	SARS-CoV-2/human/IND/GBRC208b/2020	MT664201	882	1841	
110	SARS-CoV-2/human/IND/GBRC208a/2020	MT664197	882	906	
111	SARS-CoV-2/human/IND/GBRC207b/2020	MT664172	882	162	
112	SARS-CoV-2/human/IND/GBRC207a/2020	MT664170	882	162	
113	SARS-CoV-2/human/IND/GBRC206/2020	MT664169	882		
114	SARS-CoV-2/human/IND/GBRC205b/2020	MT664161	882	162	
115	SARS-CoV-2/human/IND/GBRC205a/2020	MT664143	882	162	
116	SARS-CoV-2/human/IND/GBRC204b/2020	MT664118	882	162	
117	SARS-CoV-2/human/IND/GBRC204a/2020	MT664117	882		
118	SARS-CoV-2/human/IND/GBRC199/2020	MT635858	882	455	
119	SARS-CoV-2/human/IND/GBRC202/2020	MT635856	882		
120	SARS-CoV-2/human/IND/GBRC201b/2020	MT635857	882	162	
121	SARS-CoV-2/human/IND/GBRC201a/2020	MT635855	882	455	
122	SARS-CoV-2/human/IND/GBRC196/2020	MT635410	882		
123	SARS-CoV-2/human/IND/GBRC198/2020	MT635409	882	906	1841
124	SARS-CoV-2/human/IND/GBRC190/2020	MT635408	882		
125	SARS-CoV-2/human/IND/GBRC195b/2020	MT635407	882		
126	SARS-CoV-2/human/IND/GBRC191a/2020	MT635406	882		
127	SARS-CoV-2/human/IND/GBRC197/2020	MT635404	882		
128	SARS-CoV-2/human/IND/GBRC195a/2020	MT635405	882	2520	
129	SARS-CoV-2/human/IND/GBRC188/2020	MT635403	882		
130	SARS-CoV-2/human/IND/GBRC187b/2020	MT635397	882		
131	SARS-CoV-2/human/IND/GBRC193a/2020	MT635393	882		
132	SARS-CoV-2/human/IND/GBRC193b/2020	MT635392	882	162	
133	SARS-CoV-2/human/IND/GBRC191b/2020	MT635391	882		
134	SARS-CoV-2/human/IND/GBRC192/2020	MT635339	882		
135	SARS-CoV-2/human/IND/GBRC185a/2020	MT635328	882		
136	SARS-CoV-2/human/IND/GBRC186b/2020	MT635272	882	1749	
137	SARS-CoV-2/human/IND/GBRC185b/2020	MT635271	882		
138	SARS-CoV-2/human/IND/GBRC186a/2020	MT635269	882		
139	SARS-CoV-2/human/IND/GBRC184/2020	MT635270	882		
140	SARS-CoV-2/human/IND/GBRC183b/2020	MT608648	882		
141	SARS-CoV-2/human/IND/GBRC176/2020	MT607618	882		
142	SARS-CoV-2/human/IND/GBRC178a/2020	MT607621	882		
143	SARS-CoV-2/human/IND/GBRC182b/2020	MT607619	882		
144	SARS-CoV-2/human/IND/GBRC180b/2020	MT607620	882		
145	SARS-CoV-2/human/IND/GBRC181a/2020	MT607617	882	1749	
146	SARS-CoV-2/human/IND/GBRC175/2020	MT607615	882		

Table 2. CONT.

Sr. No	Isolate name	Accession number	Variations in nucleotide position of spike gene.	
147	SARS-CoV-2/human/IND/GBRC173b/2020	MT607616	882	
148	SARS-CoV-2/human/IND/GBRC173a/2020	MT607613	882	
149	SARS-CoV-2/human/IND/GBRC179a/2020	MT607614	882	1715
150	SARS-CoV-2/human/IND/GBRC171/2020	MT607612	882	459
151	SARS-CoV-2/human/IND/GBRC177a/2020	MT607609	882	1715

Table 3. Table depicting the positions of the corresponding amino acid variations from the selected 150 spike gene sequences along with the reference (Wuhan-Hu-1) sequence at the top

Sr. No	Isolate Name	Accession Number	A.A. Variations
1	Wuhan-Hu-1	NC_045512	614
2	SARS-CoV2/human/USA/PR-CDC- S11/2020	MT419820	
3	SARS-CoV2/human/USA/PR-CDC- S9/2020	MT419818	239
4	SARS-CoV2/human/USA/PR-CDC- S6/2020	MT419815	
5	SARS-CoV2/human/USA/PR-CDC- S5/2020	MT419814	
6	SARS-CoV2/human/USA/PR-CDC- S3/2020	MT419812	614
7	SARS-CoV2/mink/NLD/1/2020	MT396266	
8	SARS-CoV2/human/SRB/Novi Pazar- 363/2020	MT459979	
9	SARS-CoV2/human/SRB/KV26/2020	MT450872	
10	SARS-CoV2/human/USA/FL-CDC- 7619/2020	MT472624	
11	SARS-CoV2/human/USA/IA-CDC- 8200/2020	MT472626	258
12	SARS-CoV2/human/USA/NY-CDC SURV0444NYC/2020	MT434799	95
13	SARS-CoV2/human/USA/NY-CDC- SURV039NYC/2020	MT434785	614, 845
14	SARS-CoV2/human/USA/MD-CDC-0025/2020	MT472622	
15	SARS-CoV2/human/USA/NY-CDC- SURV0985NYC/2020	MT434817	
16	SARS-CoV2/human/USA/NY-CDC- SURV0592NYC/2020	MT434816	
17	SARS-CoV2/human/USA/NY-CDC- SURV0168NYC/2020	MT434814	614
18	SARS-CoV2/human/USA/NY-CDC- SURV0144NYC/2020	MT434813	614
19	SARS-CoV2/human/USA/NY-CDC- SURV0996NYC/2020	MT434811	
20	SARS-CoV2/human/USA/NY-CDC-SURV0983NYC/2020	MT434809	
21	SARS-CoV2/human/USA/NY-CDC- SURV0982NYC/2020	MT434808	
22	SARS-CoV2/human/USA/NY-CDC- SURV0874NYC/2020	MT434807	614
23	SARS-CoV2/human/USA/NY-CDC- SURV0862NYC/202	MT434805	
24	SARS-CoV2/human/USA/NY-CDC- SURV0710NYC/2020	MT434804	
25	SARS-CoV2/human/USA/NY-CDC- SURV0513NYC/2020	MT434803	
26	SARS-CoV2/human/USA/NY-CDC- SURV	MT434801	
27	hCoV-19/Japan/P4-6/2020	LC547528	
28	hCoV-19/Japan/P5-2/2020	LC547532	
29	hCoV-19/Japan/P5-1/2020	LC547531	
30	hCoV-19/Japan/P4-8/2020	LC547530	
31	hCoV-19/Japan/P4-7/2020	LC547529	
32	hCoV-19/Japan/P4-5/2020	LC547527	
33	hCoV-19/Japan/P4-4/2020	LC547526	
34	hCoV-19/Japan/P4-3/2020	LC547525	
35	hCoV-19/Japan/P4-2/2020	LC547524	
36	hCoV-19/Japan/P4-1/2020	LC547523	
37	hCoV-19/Japan/P3-2/2020	LC547522	
38	hCoV-19/Japan/P3-1/2020	LC547521	
39	hCoV-19/Japan/P2-2/2020	LC547520	
40	hCoV-19/Japan/P2-1/2020	LC547519	
32	hCoV-19/Japan/P1/2020	LC547518	
33	hCoV-19/Japan/P4-4/2020	LC547526	
34	hCoV-19/Japan/P4-3/2020	LC547525	
35	hCoV-19/Japan/P4-2/2020	LC547524	
36	hCoV-19/Japan/P4-1/2020	LC547523	
37	hCoV-19/Japan/P3-2/2020	LC547522	
38	hCoV-19/Japan/P3-1/2020	LC547521	
39	hCoV-19/Japan/P2-2/2020	LC547520	
40	hCoV-19/Japan/P2-1/2020	LC547519	

Table 3. CONT.

Sr. No	Isolate Name	Accession Number	A.A. Variations
41	hCoV-19/Japan/P1/2020	LC547518	
42	SARS-CoV-2/human/IND/8004/2020	MT416726	677
43	SARS-CoV-2/human/IND/8003/2020	MT416725	677
44	SARS-CoV-2/human/IND/GBRC5/2020	MT435082	
45	SARS-CoV-2/human/IND/GBRC9/2020	MT435086	
46	SARS-CoV-2/human/IND/GBRC8/2020	MT435085	
47	SARS-CoV-2/human/IND/GBRC7/2020	MT435084	
48	SARS-CoV-2/human/IND/GBRC6/2020	MT435083	
49	SARS-CoV-2/human/IND/GBRC4/2020	MT435081	
50	SARS-CoV-2/human/IND/GBRC3/2020	MT435080	
51	SARS-CoV-2/human/IND/GBRC81b/2020	MT483560	78
52	SARS-CoV-2/human/USA/NY- PV08436/2020	MT370836	
53	SARS-CoV-2/human/LKA/COV91/2020	MT371049	
54	SARS-CoV-2/human/USA/NV-CDC- 0052/2020	MT325597	5, 614
56	SARS-CoV-2/human/USA/NY- PV08464/2020	MT370831	90
57	SARS-CoV- 2/human/SAU/85790C/2020	MT630431	
58	SARS-CoV- 2/human/SAU/832279/2020	MT630430	
59	SARS-CoV2/human/SAU/86650/2020	MT630429	
60	SARS-CoV- 2/human/SAU/86327/2020	MT630428	
61	SARS-CoV- 2/human/SAU/86267/2020	MT630427	684
62	SARS-CoV- 2/human/SAU/85790/2020	MT630425	
63	SARS-CoV- 2/human/SAU/85715/2020	MT630424	
64	SARS-CoV- 2/human/SAU/85613/2020	MT630423	614
65	SARS-CoV- 2/human/SAU/42952/2020	MT630422	
66	SARS-CoV-2/human/SAU/637/2020	MT630421	
67	SARS-CoV-2/human/DEU/NRW- 02.1/2020	MT582498	614
68	SARS-CoV- 2/human/IND/GBRC182a/2020	MT607608	
69	SARS-CoV-2/human/DEU/NRW- 06/2020	MT582494	614
70	SARS-CoV-2/human/USA/NY- PV09097/2020	MT370960	
71	SARS-CoV-2/human/USA/NY- PV09303/2020	MT370967	
72	SARS-CoV- 2/human/IND/GBRC231a/2020	MT675954	54
73	SARS-CoV-2/human/IND/GBRC229b/2020	MT675952	54
74	SARS-CoV-2/human/IND/GBRC229a/2020	MT675950	
75	SARS-CoV-2/human/IND/GBRC230/2020	MT675951	
76	SARS-CoV-2/human/IND/GBRC228b/2020	MT675945	
77	SARS-CoV- 2/human/IND/GBRC228a/2020	MT675944	
78	SARS-CoV- 2/human/IND/GBRC225a/2020	MT675943	54
79	SARS-CoV-2/human/IND/GBRC225b/2020	MT675942	54
80	SARS-CoV- 2/human/IND/GBRC223b/2020	MT675940	54
81	SARS-CoV-2/human/IND/GBRC224b/2020	MT675941	54
82	SARS-CoV-2/human/IND/GBRC222b/2020	MT675939	54
83	SARS-CoV-2/human/IND/GBRC224a/2020	MT675938	54
84	SARS-CoV-2/human/IND/GBRC223a/2020	MT675937	54
85	SARS-CoV-2/human/IND/GBRC222a/2020	MT675933	54
86	SARS-CoV-2/human/IND/GBRC203b/2020	MT669322	54
87	SARS-CoV-2/human/IND/GBRC203a/2020	MT669321	
88	SARS-CoV-2/human/IND/GBRC221/2020	MT666042	795
89	SARS-CoV-2/human/IND/GBRC220/2020	MT665974	
90	SARS-CoV-2/human/IND/GBRC219b/2020	MT665972	54
91	SARS-CoV- 2/human/IND/GBRC219a/2020	MT665970	54

Table 3. CONT.

Sr. No	Isolate Name	Accession Number	A.A. Variations
92	SARS-CoV-2/human/IND/GBRC218b/2020	MT665028	54
93	SARS-CoV-2/human/IND/GBRC218a/2020	MT665006	54
94	SARS-CoV-2/human/IND/GBRC217b/2020	MT664990	54
95	SARS-CoV-2/human/IND/BRC217a/2020	MT664986	54
96	SARS-CoV-2/human/IND/GBRC216b/2020	MT664822	54
97	SARS-CoV-2/human/IND/GBRC194b/2020	MT664808	54
98	SARS-CoV-2/human/IND/GBRC194a/2020	MT664807	54
99	SARS-CoV-2/human/IND/GBRC216a/2020	MT664796	54
100	SARS-CoV-2/human/IND/GBRC182a/2020	MT607608	
101	SARS-CoV-2/human/IND/GBRC183a/2020	MT607611	
102	SARS-CoV-2/human/IND/GBRC215/2020	MT664774	
103	SARS-CoV-2/human/IND/GBRC214b/2020	MT664729	54
104	SARS-CoV-2/human/IND/GBRC214a/2020	MT664727	262
105	SARS-CoV-2/human/IND/GBRC210b/2020	MT664209	
106	SARS-CoV-2/human/IND/GBRC210a/2020	MT664205	54
107	SARS-CoV-2/human/IND/GBRC209b/2020	MT664203	
108	SARS-CoV-2/human/IND/GBRC209a/2020	MT664202	54
109	SARS-CoV-2/human/IND/GBRC208b/2020	MT664201	614
110	SARS-CoV-2/human/IND/GBRC208a/2020	MT664197	
111	SARS-CoV-2/human/IND/GBRC207b/2020	MT664172	54
112	SARS-CoV-2/human/IND/GBRC207a/2020	MT664170	54
113	SARS-CoV-2/human/IND/GBRC206/2020	MT664169	
114	SARS-CoV-2/human/IND/GBRC205b/2020	MT664161	54
115	SARS-CoV-2/human/IND/GBRC205a/2020	MT664143	54
116	SARS-CoV-2/human/IND/GBRC204b/2020	MT664118	54
117	SARS-CoV-2/human/IND/GBRC204a/2020	MT664117	
118	SARS-CoV-2/human/IND/GBRC199/2020	MT635858	152
119	SARS-CoV-2/human/IND/GBRC202/2020	MT635856	
120	SARS-CoV-2/human/IND/GBRC201b/2020	MT635857	54
121	SARS-CoV-2/human/IND/GBRC201a/2020	MT635855	152
122	SARS-CoV-2/human/IND/GBRC196/2020	MT635410	
123	SARS-CoV-2/human/IND/GBRC198/2020	MT635409	614
124	SARS-CoV-2/human/IND/GBRC190/2020	MT635408	
125	SARS-CoV-2/human/IND/GBRC195b/2020	MT635407	
126	SARS-CoV-2/human/IND/GBRC191a/2020	MT635406	
127	SARS-CoV-2/human/IND/GBRC197/2020	MT635404	
128	SARS-CoV-2/human/IND/GBRC195a/2020	MT635405	
129	SARS-CoV-2/human/IND/GBRC188/2020	MT635403	
130	SARS-CoV-2/human/IND/GBRC187b/2020	MT635397	
131	SARS-CoV-2/human/IND/GBRC193a/2020	MT635393	
132	SARS-CoV-2/human/IND/GBRC193b/2020	MT635392	54
133	SARS-CoV-2/human/IND/GBRC191b/2020	MT635391	
134	SARS-CoV-2/human/IND/GBRC192/2020	MT635339	
135	SARS-CoV-2/human/IND/GBRC185a/2020	MT635328	
136	SARS-CoV-2/human/IND/GBRC186b/2020	MT635272	583
137	SARS-CoV-2/human/IND/GBRC185b/2020	MT635271	
138	SARS-CoV-2/human/IND/GBRC186a/2020	MT635269	
139	SARS-CoV-2/human/IND/GBRC184/2020	MT635270	
140	SARS-CoV-2/human/IND/GBRC183b/2020	MT608648	
129	SARS-CoV-2/human/IND/GBRC176/2020	MT607618	

Table 3. CONT.

Sr. No	Isolate Name	Accession Number	A.A. Variations
130	SARS-CoV-2/human/IND/GBRC187b/2020	MT635397	
141	SARS-CoV-2/human/IND/GBRC193a/2020	MT635393	
142	SARS-CoV-2/human/IND/GBRC178a/2020	MT607621	
143	SARS-CoV-2/human/IND/GBRC182b/2020	MT607619	
144	SARS-CoV-2/human/IND/GBRC180b/2020	MT607620	
145	SARS-CoV-2/human/IND/GBRC181a/2020	MT607617	583
146	SARS-CoV-2/human/IND/GBRC175/2020	MT607615	
147	SARS-CoV-2/human/IND/GBRC173b/2020	MT607616	
148	SARS-CoV-2/human/IND/GBRC173a/2020	MT607613	
149	SARS-CoV-2/human/IND/GBRC179a/2020	MT607614	572
150	SARS-CoV-2/human/IND/GBRC171/2020	MT607612	153
151	SARS-CoV-2/human/IND/GBRC177a/2020	MT607609	572