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# Identification of genotypic variants and its proteomic mutations of Brazilian SARS-CoV-2 isolates

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## ABSTRACT

The second wave of COVID-19 caused by severe acute respiratory syndrome virus (SARS-CoV-2) is rapidly spreading over the world. Mechanisms behind the flee from current antivirals are still unclear due to the continuous occurrence of SARS-CoV-2 genetic variants. Brazil is the world's second-most COVID-19 affected country. In the present study, we identified the genomic and proteomic variants of Brazilian SARS-CoV-2 isolates. We identified 16 different genotypic variants were found among the 27 isolates. The genotypes of three isolates such as Bra/1236/2021 (G15), Bra/MASP2C844R2/2020 (G11), and Bra/RJ-DCVN5/2020 (G9) have a unique mutant in NSP4 (S184N), 2'O-Mutase (R216N), membrane protein (A2V) and Envelope protein (V5A). A mutation in RdRp of SARS-CoV-2, particularly the change of Pro-to Leu-at 323 resulted in the stabilization of the structure in BRA/CD1739-P4/2020. NSP4, NSP5 protein mutants are more virulent in genotype 15 and 16. A fast protein folding rate changes the structural stability and leads to escape for current antivirals. Thus, our findings help researchers to develop the best potent antivirals based on the new mutant of Brazilian isolates.

## 1. Introduction

### 1.1. SARS-CoV-2 genome

Severe acute respiratory syndrome coronavirus-2 (SARS-CoV-2) is a single strand positive RNA genome that causes coronavirus disease (COVID-19). World Health Organization (WHO) has declared the COVID-19 pandemic as a global public health emergency (Cucinotta and Vanelli, 2020). On 15th July 2021, WHO reported a total of 72,528 deaths out of 3.7 million new weekly affected cases. Brazil is the second-largest COVID-19 affected place, globally. So far 17, 296, 118 affected cases have been reported in Brazil, of which 4, 54, 710 new cases have been reported in the last seven days as of 6th June 2021. Overall 4, 84, 235 death cases have been reported so far (World Health Organization WHO, 2021), <https://www.who.int/publications/m/item/weekly-epidemiological-update-on-covid-19—20-july-2021>.

Several SARS-CoV-2 variants were found to be associated with

increased infectivity rate, pathogenesis and displayed a considerably faster transmission through droplets than the wild-type strain (Prathiviraj et al., 2020; Rajeev et al. 2020; Prathiviraj et al., 2021). A study realized with the primary human airway epithelial (hACE) cell culture reveals that enhanced transmission rate of SARS-CoV-2 is linked with the effective replication of the viral particle in the upper respiratory tract temperature of 33 °C when compared with the lower respiratory tract temperature of 37 °C (V'kovski et al., 2021). RNA-dependent RNA polymerase (RdRp), the enzyme in charge of viral genome replication and transcription (Posthuma et al., 2017) facilitates mutations at various locations in the genome. Errors in the replication and repair systems of the virus lead to the accumulation of additional mutations in the genome (Banoun, 2021; Pachetti et al., 2020). It must be noted that the mutations described at particular locations do not individually provide fitness to the virus, but a combination of favourable mutations at different locations in the genome, resulting in the improved or diminished functionalities have been classified as clades of mutant viruses harbouring certain moderations (Mercatelli and Giorgi, 2020).

**Abbreviation:** hACE2, Human-Angiotensin converting enzyme 2 receptor; SARS-CoV-2, Severe acute respiratory syndrome coronavirus 2; COVID-19, Coronavirus disease-2019; NSP, Non-structural protein; RdRp, RNA-dependent RNA polymerase.

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It is important to analyze and interpret the changes at both genotypic and phenotypic levels, as these changes help in understanding the structural changes, pathogenesis, replication and transmission of SARS-CoV-2 (Mousavizadeh and Ghasemi, 2021) in a different environmental niche. Genotyping approach employing single nucleotide polymorphism is essential to identify the genetic changes accumulating during the transmission of SARS-CoV-2 and helps to determine the changes in the property of viral transmission and virulence. Thus, helps to track genotypes associated with topography and epidemiology. The identification of genotypes associated with topographical and contagious groups helps in tracking and monitoring the single nucleotide polymorphism (Yin, 2020). Genotypic studies help in identifying the phenotypical changes caused due to the replacements of amino acids (Morais et al., 2020). The protein folding information plays a key role in the therapeutic intervention of many viral diseases (Broglia et al., 2005; Prathiviraj et al., 2021). The folding process of a protein determines the structural stability and drug binding specificity (Prisilla et al., 2016; Prathiviraj et al., 2016) thus it's most important to identify the folding rate of mutants (Murugan et al., 2019; Prathiviraj et al., 2021). Analysis of the genotype and phenotype is essential as it aids in understanding the missense, synonymous, and non-synonymous mutations in the virus. In the present study, we identify the number of genotypic variants and proteomic mutants that have recently evolved in Brazilian SARS-CoV-2 isolates. Thus the identified genotypic variants would be useful for developing a new antivirals depending upon the stability of mutants.

## 2. Methodology

### 2.1. Dataset preparation and sequence alignment

The complete genomic/nucleotide sequences of Brazilian SARS-CoV-2 isolates and reference Wuhan-Hu-1 SARS-CoV-2 genome were retrieved from the NCBI Virus data repository (<https://www.ncbi.nlm.nih.gov/sars-cov-2/>). Initial multiple sequence alignment was performed using the PartTree (Katoh and Toh, 2007) algorithm (systematic of large scale alignment) in the MAFFT online server (Katoh et al., 2019).

### 2.2. Analysis of evolutionary imprints

The aligned file was further imported in MEGA Version X (Kumar et al., 2018) to perform the molecular phylogenomic analysis using the neighbor-joining algorithm (Saitou and Nei, 1987). Kimura two-parameter (Nishimaki and Sato, 2019) evolutionary substitution model was carried out for estimation of genetic diversity. The phylogenomic tree was computed with 1000 bootstrapping iteration to identify replication among selected isolates. Finally, a huge scale phylogenomic supertree was drawn using the Interactive Tree of Life (iTOL) v4 (Letunic and Bork, 2019). Nodes of each isolate in the cluster are grouped, colored and classified according to its features.

### 2.3. Analysis of genomic and proteomic mutation

Each open reading frame (ORFs) of nucleotide and protein sequences of retrieved Brazilian isolates were separated, correspond to the genomic and proteomic positions of the reference genome (Wuhan-Hu-1). The alignment of each ORFs was carried out to identify the mutated residues (mismatch region) compared with Wuhan-Hu-1 genome using the BioEdit v7.2 software (Hall, 1999). Each mutation in the genomic bases of corresponding isolates were cross verified with the Virus pathogen database and analysis resource (ViPR) (Pickett et al., 2012).

### 2.4. Prediction of virulence mechanism and protein folding rate

Virulence mechanism of identified mutation of each ORFs was predicted using the VICMPred (Saha and Raghava, 2006) and VirulentPred

server (Garg and Gupta, 2008). Identified prediction score of each mutated residue was compared with the reference proteome (Wuhan-Hu-1), to identify its near-native state. Prediction score was computed based on the support vector machine learning algorithm (Rashid et al., 2007) and the final sub-cellular localization score was determined by its functional motifs. The protein folding rate among all the identified mutants was calculated using the FOLD-RATE server (Gromiha et al., 2006).

## 3. Results

### 3.1. Analysis of evolutionary footprint

We performed the phylogenomic discrepancy among the 27 full-length genomic sequences of Brazilian SARS-CoV-2 isolates, so far yet deposited in the NCBI-virus repository (Fig 1). Out of 27 genomic isolates 19 samples were collected from the year 2020 and 8 from 2021. Genomic features of the selected isolates are represented in Supplementary Table S1. Phylogenetic analysis demonstrated that the tree was separated into two major clades. First clade shows the origin and replication of Brazilian isolate from the reference Wuhan-Hu-1 genome (red color). Cluster in blue indicates Brazilian isolates of 2020, whereas the second clade represents isolates from 2021 (green).

### 3.2. Analysis of genotypic and proteomic variants

The selected isolates were aligned and compared with Wuhan-Hu-1 genome to identify the mutations. Each ORFs (including non-structural protein from ORF1ab) is pair-wisely aligned separately based on the genomic organization and its position of a reference genome (Table S2). Interestingly we found 16 genotypic variants among the 27 Brazilian isolates, based upon the mutation analysis.

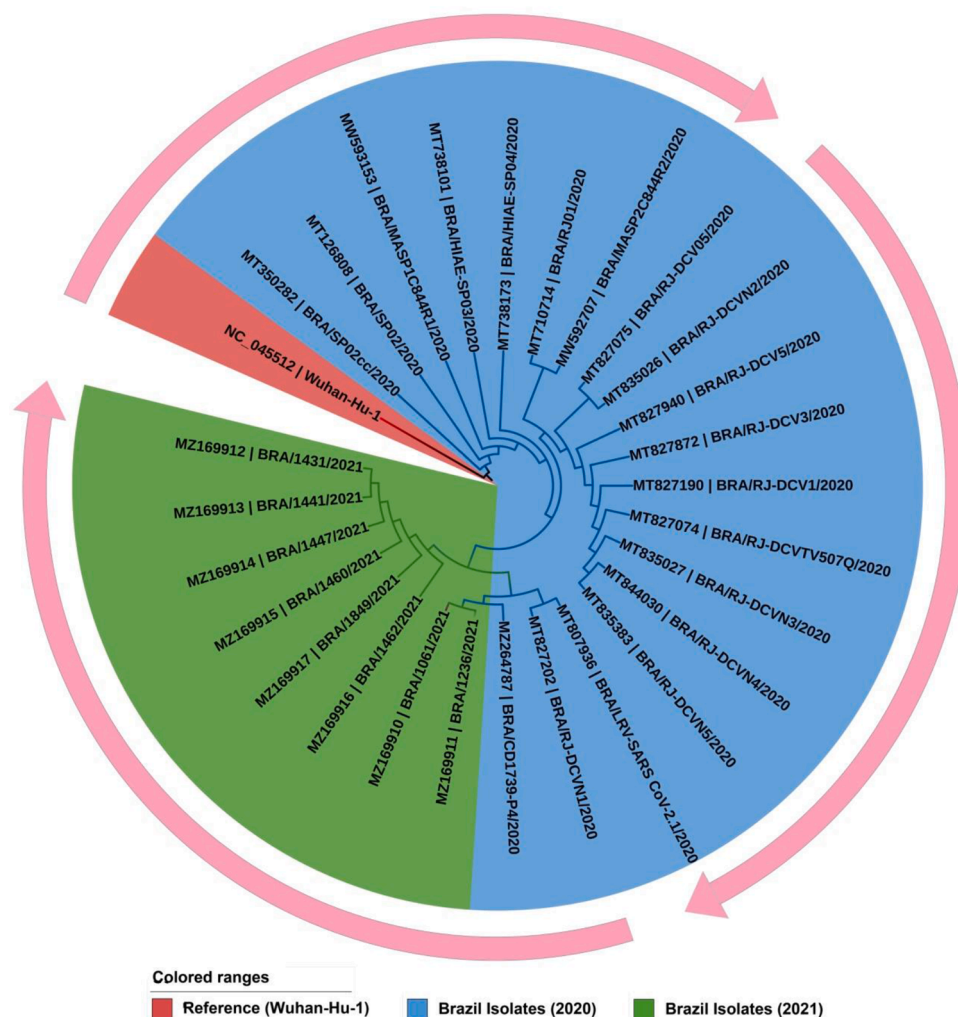
The first wave of SARS-CoV-2 genome of 2020 has 3–14 mutations, whereas the second wave SARS-CoV-2 genome has 15–20 mutants (Fig 2a; Table S3). 3 to 7 mutants were identified in the Nucleocapsid genes with all genotypes (G1 to G16). Numerous mutations were found in PLPro and spike genes of the isolates BRA/CD1739-P4/2020 (11 base change), Bra/1236/2021 (13 base change), and Bra/1061/2021 (12 base change).

Comparatively, the identified mutant in the proteomic regions (Fig 2b; Table 1) show a huge variation from isolates BRA/CD1739-P4/2020 (11 base changes), BRA/1236/2021, and BRA/1061/2021 (12 base changes) with a higher number of mutations in the spike genes are more virulent. Two to five mutations were identified in the nucleocapsid protein of genotypes G1 to G16. Gene NSP4 (S184N) and membrane protein (A2V) from the isolate BRA/1236/2021 (G15), envelop protein (V5A) from BRA/RJ-DCVN5/2020 (G9), 2'O-mutase (R216N) from BRA/MASP2C844R2/2020 (G11) had the single mutation. No mutations were observed in NSP1, NSP8-NSP10 genes of ORF4b. The second wave of Brazilian isolates especially spike protein of the genotypes G14-G16 had four times higher mutation than the first wave (Table 1). Out of the 27 isolates, eight isolates of 2021 second wave had five times higher mutation than the first wave of 2020 isolates (Fig 2C).

### 3.3. Analysis of virulence mechanism and protein folding state

The virulence mechanism of a protein can be resolute by its amino acid composition and the physicochemical properties of substituted amino acids. Our findings show that most of the proteins are involved in the cellular and metabolic process and none are identified from information molecules. The mutant (P323L) identified in RdRp of BRA/CD1739-P4/2020 (G14) transmits its biological function from cellular process to virulence mechanism (Table 2).

The protein folding rate is most important to determine the stability of structure and function. It is most imperative to predict the folding rate of protein to identify the various structural changes in the drug binding



**Fig. 1.** A circular view of the phylogenomic tree represents the evolutionary transmittance of Brazilian SARS-CoV-2 isolates along with the reference genome (Wuhan-Hu-1). Each color of nodes and clades are represented a corresponding genomic isolated year. The lengths of the branches are proportional to the evolutionary distances. The outer circular arrow indicated the evolutionary flow of Brazilian SARS-CoV-2 isolates.

region. We also predicted the folding mechanism based on its structural classes (Table 3). A fast-fold rate was identified in all mutated proteins exactly in  $\beta$ -classes. And slow fold rate was identified in other classes (All- $\alpha$ ,  $\alpha+\beta$ : $\alpha/\beta$  and unknown).

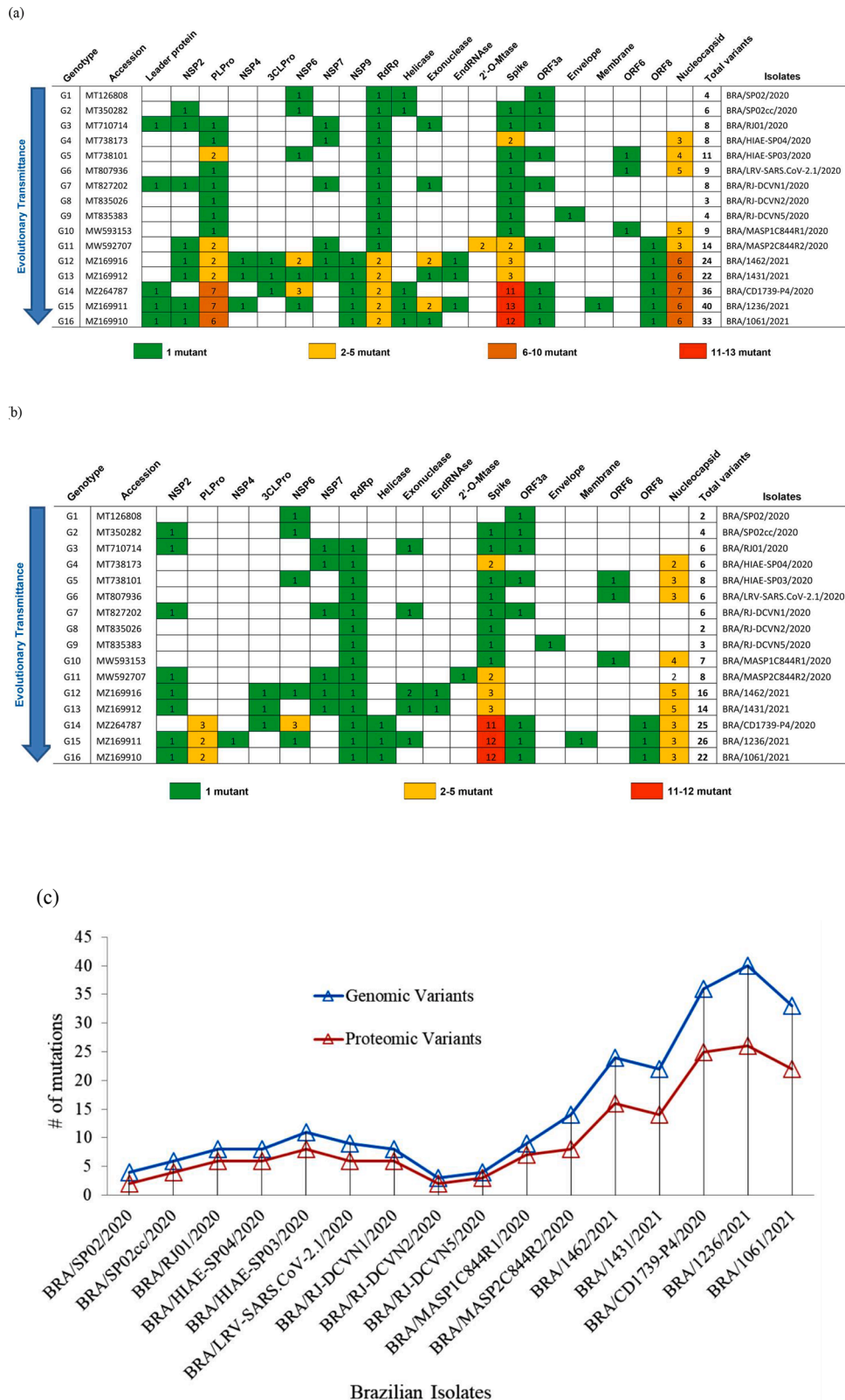
#### 4. Discussion

Our consequence from evolutionary footprints determines that the mean G + C content was found to be low (< 38%) while comparing to the A + T disparity index. It determines that some divergence may occur during the evolutionary transmittance of this genome. Compared with previous research (Prathiviraj et al., 2020), our findings indicate that some genomic variations may occur at the sequence level, and leads to the origination of a new clade within the isolates in a short time (short-term evolution). Host RNA editing systems, APOBEC and ADAR, responsible for C>U (accompanied by U>C mutations by the virus' defense system) and A>G mutations respectively, account for around 65% of the mutations recorded (Banoun, 2021; Klimczak et al., 2020; Wang et al., 2020). Deletion of one or a few nucleotides has been reported chiefly in the genes encoding proteins involved in host interaction and the spike protein (Banoun, 2021). Lin et al. (2021) designated the ~ 500–532 coding region of NSP as a deletion hotspot, and the mutants were found to be linked with reduced virulence (mild disease, low IFN- $\beta$  in serum) and lower viral load (p14). More deletion variants, primarily

of structural protein origin are under scrutiny (Banoun, 2021). The transition, C>U in the genome has altered the codons to encode amino acids of more hydrophobic nature, thereby producing intense effects on the properties of the protein. This deamination of cytosine-mediated conversion of C to U has helped the SARS-CoV-2 to prompt evolution and adaptation in the host (Matyášek and Kovaří, 2020).

By analyzing the genomic and proteomic variants of selected isolates we identified both synonymous and non-synonymous substitution mutation evenly occur in all isolates, which leads to drastic changes that may occur in their protein sequences (Chu and Wei, 2019). Several reports have been previously published, that an increased level of mutation in surface glycoprotein may escort a significant role in viral contamination and disease transmission (Yan et al., 2020; Prathiviraj et al., 2021). As same as the previous report, consequently, our study also correlates the mutational rate is moderately increased in 2020 (Zhao et al., 2004; van Dorp et al., 2020) isolate and drastically increased in 2021 isolates (Pachetti et al., 2020; Callaway, 2020; Vilar and Isom, 2021) for relapsing the antivirals against COVID-19.

The spike protein of SARS-CoV-2 is more prone to multiple point mutations (Guruprasad, 2021; Ogawa et al., 2020; Plante et al., 2021), most notable being D614G (Asp<sup>614</sup>-to-Gly) variant (globally dominant viral form) with increment in transmissibility, infectivity and neutralization susceptibility (Korber et al., 2020; Koyama et al., 2020; Zhang et al., 2020; Weissman et al., 2021). Several studies have implied that



**Fig. 2.** The heat map representation indicates the identified mutants in genomic (a) and proteomic (b) regions among the selected 16 genotypic variants from Brazilian SARS-CoV-2 isolates. (c) A graphical representation of mutation rates was predicted from the total hotspot of Brazilian isolates (c). *Note:* The number inside the cell indicated the total number of mutations.

**Table 1**

Identification of proteomic variants of Brazilian SARS-CoV-2 isolates. The variants were represented among each open reading frame (ORFs).

Genotype	Accession	Isolates	Genes	Mutation
G1	MT126808	BRA/SP02/2020	NSP6 ORF3a	L37F G251V
G2	MT350282	BRA/SP02cc/ 2020	NSP2 NSP6 Spike ORF3a	T528I L37F N74K G251V
G3	MT710714	BRA/RJ01/2020	NSP2 NSP7 RdRp Exonuclease Spike ORF3a	T85I S25L P323L A320V D614G Q57H
G4	MT738173	BRA/HIAE-SP04/ 2020	NSP7 RdRp Spike Nucleocapsid	L71F P323L D614G, V1176F R203K, G204R
G5	MT738101	BRA/HIAE-SP03/ 2020	NSP6 RdRp Spike ORF3a ORF6 Nucleocapsid	T17I P323L D614G S171L I33T R203K, G204R, I292T
G6	MT807936	BRA/LRV-SARS. CoV-2.1/2020	RdRp Spike ORF6 Nucleocapsid	P323L D614G I33T R203K, G204R, I292T
G7	MT827202	BRA/RJ-DCVN1/ 2020	NSP2 NSP7 RdRp Exonuclease Spike ORF3a	T85I S25L P323L A320V D614G Q57H
G8	MT835026	BRA/RJ-DCVN2/ 2020	RdRp Spike	P323L D614G
G9	MT835383	BRA/RJ-DCVN5/ 2020	RdRp Spike Envelope	P323L D614G V5A
G10	MW593153	BRA/ MASP1C844R1/ 2020	RdRp Spike ORF6 Nucleocapsid	P323L D614G I33T R203K, G204R, I292T, P383L
G11	MW592707	BRA/ MASP2C844R2/ 2020	NSP2 NSP7 RdRp Ribose Spike Nucleocapsid	V577F L71F P323L R216N D614G, V1176F R203K, G204R
G12	MZ169916	BRA/1462/2021	NSP2 3CLPro NSP6 NSP7 RdRp Exonuclease EndRNase Spike Nucleocapsid	G88E L205V L37F L71F P323L T16A, Y235N K110R E484K, D614G, V1176F A119S, S202T, R203K, G204R, M234I
G13	MZ169912	BRA/1431/2021	NSP2 3CLPro NSP7 RdRp Exonuclease EndRNase Spike Nucleocapsid	G88E L205V L71F P323L T16A K110R E484K, D614G, V1176F A119S, S202T, R203K, G204R, M234I

**Table 1 (continued)**

Genotype	Accession	Isolates	Genes	Mutation
G14	MZ264787	BRA/CD1739- P4/2020	PLPro 3CLPro NSP6 RdRp Helicase Spike	S375L, K982Q, S1740F P96H S106T, G107S, F108L P323L E341D L18F, T20N, P26S, D138Y, R190S, E484K, N501Y, D614G, H655Y, T1027I, V1176F
G15	MZ169911	BRA/1236/2021	NSP2 PLPro NSP4 NSP6 RdRp Helicase Exonuclease Spike	S138L S375L, K982Q S184N F108L P323L E341D N256S L18F, T20N, P26S, D138Y, R190S, K417T, E484K, N501Y, D614G, H655Y, T1027I, V1176F
G16	MZ169910	BRA/1061/2021	NSP2 PLPro RdRp Helicase Spike	S253P A2V E92K P80R, R203K, G204R K534N S375L, K982Q P323L E341D L18F, T20N, P26S, D138Y, R190S, K417T, E484K, N501Y, D614G, H655Y, T1027I, V1176F
			ORF3a Membrane ORF8 Nucleocapsid	S253P A2V E92K P80R, R203K, G204R
			NSP2 PLPro RdRp Helicase Spike	S375L, K982Q P323L E341D L18F, T20N, P26S, D138Y, R190S, K417T, E484K, N501Y, D614G, H655Y, T1027I, V1176F
			ORF3a ORF8 Nucleocapsid	S253P E92K P80R, R203K, G204R

the D614G mutation in the spike glycoprotein of SARS-CoV-2, being closely located in the receptor-binding domain. It has an open conformation by changing hydrogen-bond interactions in the spike trimer, consequentially, its binding ability to the hACE2 receptor is enhanced and increased infectivity (Mansbach et al., 2021; Omotuyi et al., 2020; Yurkovetskiy et al., 2020). Certain studies reported the increment in viral load in the upper respiratory tract (URT) of patients infected with the D614G mutant type virus (Korber et al., 2020). Further experimental studies correlated this spike protein mutation to enhanced viral replication *in vitro* (primary human URT cell lines, animal models), consistent with the URT viral load increment (; Zhou et al., 2021).

N501Y mutation in the spike protein of B.1.1.7 mutant viruses was experimentally showed to exert enhanced replication rates in cell lines and human respiratory cells (*ex-vivo* studies), which might occur through the same mechanism (Liu et al., 2021). It enhanced the rate of transmission and virulence probability due to its increased interaction with the human ACE2 receptor (Zhao et al., 2021). Accessory protein ORF3a was found to be four distinct types in India, namely, Type 1–4 and it changed the amino sequences in each type. Type 2 of ORF3a was raised from Type 1 by the change of amino acid mutation at the 171<sup>st</sup> position. Similarly, the change of amino acids from Asp-to Tyr-and

Table 2

. Comparative analysis of predicted virulence properties of protein mutants identified from Brazilian SARS-CoV-2 isolates. The virulence properties were predicted for both reference and variant proteins. The blue shadow specifies the same functional properties compared with reference ORFs. The orange shadow specifies the functional variants between the reference and mutated proteins.

Genotype	Isolates	Genes	Mutation	Cellular Process		Metabolism		Virulence factors	
				Ref.	Var.	Ref.	Var.	Ref.	Var.
G1	BRA/SP02/2020	NSP6	L37F	1.988548	1.988548	-0.29145	-0.29145	-0.91113	-0.91113
		ORF3a	G251V	1.59006	1.952861	0.0081	0.104934	-0.3168	-0.83548
G2	BRA/SP02cc/2020	NSP2	T528I	-2.72165	-2.72165	3.304706	3.304706	-3.39956	-3.39956
		NSP6	L37F	1.988548	1.988548	-0.29145	-0.29145	-0.91113	-0.91113
		Spike	N74K	1.507647	1.492872	-1.38428	-1.25244	-0.67481	-0.69898
		ORF3a	G251V	1.59006	1.952861	0.0081	0.104934	-0.3168	-0.83548
G3	BRA/RJ01/2020	NSP2	T85I	-2.72165	-2.72165	3.304706	3.304706	-3.39956	-3.39956
		NSP7	S25L	1.227022	1.227022	-2.16893	-2.16893	0.960535	0.960535
		RdRp	P323L	-0.54011	-0.54011	0.931417	0.931417	1.304757	1.304757
		Exonuclease	A320V	0.205947	0.703741	-1.91813	-0.93051	1.812892	0.810148
		Spike	D614G	1.507647	1.492872	-1.38428	-1.25244	-0.67481	-0.69898
		ORF3a	Q57H	1.59006	1.952861	0.0081	0.104934	-0.3168	-0.83548
		NSP7	L71F	1.521723	1.521723	0.519135	0.519135	-1.40537	-1.40537
G4	BRA/HIAE-SP04/2020	RdRp	P323L	1.227022	1.227022	-2.16893	-2.16893	0.960535	0.960535
		Spike	D614G, V1176F	1.507647	1.492872	-1.38428	-1.25244	-0.67481	-0.69898
		Nucleocapsid	R203K, G204R	0.623184	0.766355	0.766728	0.788503	-0.26696	-0.85619
G5	BRA/HIAE-SP03/2020	NSP6	T17I	1.988548	1.988548	-0.29145	-0.29145	-0.91113	-0.91113
		RdRp	P323L	1.227022	1.227022	-2.16893	-2.16893	0.960535	0.960535
		Spike	D614G	1.507647	1.492872	-1.38428	-1.25244	-0.67481	-0.69898
		ORF3a	S171L	1.59006	1.736869	0.0081	0.319992	-0.3168	-0.61861
		ORF6	I33T	1.347941	1.492923	0.091501	0.946338	-0.55216	-1.3189
		Nucleocapsid	R203K, G204R, I292T	0.623184	0.766355	0.766728	0.788503	-0.26696	-0.85619
G6	BRA/LRV-SARS-CoV-2.1/2020	RdRp	P323L	1.227022	1.227022	-2.16893	-2.16893	0.960535	0.960535
		Spike	D614G	1.507647	1.492872	-1.38428	-1.25244	-0.67481	-0.69898
		ORF6	I33T	1.347941	1.492923	0.091501	0.946338	-0.55216	-1.3189
		Nucleocapsid	R203K, G204R, I292T	0.623184	0.766355	0.766728	0.788503	-0.26696	-0.85619
G7	BRA/RJ-DCVN1/2020	RdRp	P323L	1.227022	1.227022	-2.16893	-2.16893	0.960535	0.960535
		Spike	D614G	1.507647	1.492872	-1.38428	-1.25244	-0.67481	-0.69898
		ORF6	I33T	1.347941	1.492923	0.091501	0.946338	-0.55216	-1.3189
		Nucleocapsid	R203K, G204R, I292T	0.623184	0.766355	0.766728	0.788503	-0.26696	-0.85619
		NSP2	T85I	-2.72165	-2.72165	3.304706	3.304706	-3.39956	-3.39956
G8	BRA/RJ-DCVN2/2020	NSP7	S25L	1.521723	1.521723	0.519135	0.519135	-1.40537	-1.40537
		RdRp	P323L	1.227022	1.227022	-2.16893	-2.16893	0.960535	0.960535
		Exonuclease	A320V	0.205947	0.703741	-1.91813	-0.93051	1.812892	0.810148
		Spike	D614G	1.507647	1.492872	-1.38428	-1.25244	-0.67481	-0.69898
		ORF3a	Q57H	1.59006	1.952861	0.0081	0.104934	-0.3168	-0.83548
		RdRp	P323L	1.227022	1.227022	-2.16893	-2.16893	0.960535	0.960535
G9	BRA/RJ-DCVN5/2020	Spike	D614G	1.507647	1.492872	-1.38428	-1.25244	-0.67481	-0.69898
		RdRp	P323L	1.227022	1.227022	-2.16893	-2.16893	0.960535	0.960535
G10	BRA/MASPI844R1/2020	Spike	D614G	1.507647	1.492872	-1.38428	-1.25244	-0.67481	-0.69898
		Envelope	V5A	0.736976	0.727204	0.567783	1.294167	-0.08788	-1.05017
		RdRp	P323L	1.227022	1.227022	-2.16893	-2.16893	0.960535	0.960535
		Spike	D614G	1.507647	1.492872	-1.38428	-1.25244	-0.67481	-0.69898
G11	BRA/MASP2C844R2/2020	ORF6	I33T	1.347941	1.492923	0.091501	0.946338	-0.55216	-1.3189
		Nucleocapsid	R203K, G204R, I292T, P383L	0.623184	1.516895	0.766728	-0.32718	-0.26696	-0.71549
		NSP2	V577F	-2.72165	-2.11747	3.304706	3.081117	-3.39956	-2.99917
G12	BRA/1462/2021	NSP7	L71F	1.521723	1.521723	0.519135	0.519135	-1.40537	-1.40537
		RdRp	P323L	1.227022	1.227022	-2.16893	-2.16893	0.960535	0.960535
		Ribose	R216N	1.464238	1.464238	-0.14461	-0.14461	-0.21774	-0.21774
		Spike	D614G, V1176F	1.507647	1.492872	-1.38428	-1.25244	-0.67481	-0.69898
		Nucleocapsid	R203K, G204R	0.623184	0.766355	0.766728	0.788503	-0.26696	-0.85619
		NSP2	G88E	-2.72165	-2.72165	3.304706	3.304706	-3.39956	-3.39956
		3CLPro	L205V	0.521603	0.521603	-0.90669	-0.90669	1.521026	1.521026
G13	BRA/1431/2021	NSP6	L37F	1.988548	1.988548	-0.29145	-0.29145	-0.91113	-0.91113
		NSP7	L71F	1.521723	1.521723	0.519135	0.519135	-1.40537	-1.40537
		RdRp	P323L	1.227022	1.227022	-2.16893	-2.16893	0.960535	0.960535
		Exonuclease	T16A, Y235N	0.205947	0.212552	-1.91813	-1.27247	1.812892	1.925776
		EndRNase	K110R	0.886765	0.975383	-0.18029	0.012195	0.194409	-0.08423
		Spike	E484K, D614G, V1176F	1.507647	1.492872	-1.38428	-1.25244	-0.67481	-0.69898
		Nucleocapsid	A119S, S202T, R203K, G204R, M234I	0.623184	0.379777	0.766728	1.258901	-0.26696	-0.97281
		NSP2	G88E	-2.72165	-2.72165	3.304706	3.304706	-3.39956	-3.39956
		3CLPro	L205V	0.521603	0.521603	-0.90669	-0.90669	1.521026	1.521026
		NSP7	L71F	1.521723	1.521723	0.519135	0.519135	-1.40537	-1.40537
RdRp	P323L	1.227022	1.227022	-2.16893	-2.16893	0.960535	0.960535		
Exonuclease	T16A	0.205947	0.212552	-1.91813	-1.27247	1.812892	1.925776		
EndRNase	K110R	0.886765	0.975383	-0.18029	0.012195	0.194409	-0.08423		
Spike	E484K, D614G, V1176F	1.507647	1.492872	-1.38428	-1.25244	-0.67481	-0.69898		

(continued on next page)

Table 2 (continued)

Genotype	Isolates	Genes	Mutation	Cellular Process		Metabolism		Virulence factors	
				Ref.	Var.	Ref.	Var.	Ref.	Var.
G14	BRA/CD1739-P4/2020	Nucleocapsid	A119S, S202T, R203K, G204R, M234I	0.623184	0.379777	0.766728	1.258901	-0.26696	-0.97281
		PLPro	S375L, K982Q, S1740F	-3.81331	-3.93465	-3.09635	-3.2623	0.135194	0.258528
		3CLPro	P96H	0.521603	0.521603	-0.90669	-0.90669	1.521026	1.521026
		NSP6	S106T, G107S, F108L	1.988548	2.186767	-0.29145	-0.68079	-0.91113	-1.26714
		RdRp	P323L	-0.54011	1.227022	0.931417	-2.16893	1.304757	0.960535
		Helicase	E341D	-0.54011	-0.54011	0.931417	0.931417	1.304757	1.304757
		Spike	L18F, T20N, P26S, D138Y, R190S, E484K, N501Y, D614G, H655Y, T1027I, V1176F	1.507647	1.75435	-1.38428	-1.31278	-0.67481	-1.06524
		ORF3a	S253P	1.59006	2.069778	0.0081	0.103932	-0.3168	-1.04161
		ORF8	E92K	0.70646	0.947547	-0.46479	0.551035	0.633644	-0.30211
		G15	BRA/1236/2021	Nucleocapsid	P80R, R203K, G204R	0.623184	0.766355	0.766728	0.788503
NSP2	S138L			-2.72165	-1.73529	3.304706	2.882199	-3.39956	-2.7792
PLPro	S375L, K982Q			-3.81331	-3.93465	-3.09635	-3.2623	0.135194	0.258528
NSP4	S184N			-0.47381	-0.47381	2.584585	2.584585	-1.82286	-1.82286
NSP6	F108L			1.988548	1.969257	-0.29145	-0.3559	-0.91113	-0.76909
RdRp	P323L			1.227022	1.227022	-2.16893	-2.16893	0.960535	0.960535
Helicase	E341D			-0.54011	-0.54011	0.931417	0.931417	1.304757	1.304757
Exonuclease	N256S			0.205947	0.205947	-1.91813	-1.91813	1.812892	1.812892
Spike	L18F, T20N, P26S, D138Y, R190S, K417T, E484K, N501Y, D614G, H655Y, T1027I, V1176F			1.507647	1.75435	-1.38428	-1.31278	-0.67481	-1.06524
G16	BRA/1061/2021			ORF3a	S253P	1.59006	2.069778	0.0081	0.103932
		Membrane	A2V	1.772062	1.758567	0.905378	1.061507	-1.40208	-2.02498
		ORF8	E92K	0.70646	0.947547	-0.46479	0.551035	0.633644	-0.30211
		Nucleocapsid	P80R, R203K, G204R	0.623184	0.766355	0.766728	0.788503	-0.26696	-0.85619
		NSP2	K534N	-2.72165	-2.72165	3.304706	3.304706	-3.39956	-3.39956
		PLPro	S375L, K982Q	-3.81331	-3.93465	-3.09635	-3.2623	0.135194	0.258528
		RdRp	P323L	1.227022	1.227022	-2.16893	-2.16893	0.960535	0.960535
		Helicase	E341D	-0.54011	-0.54011	0.931417	0.931417	1.304757	1.304757
		Spike	L18F, T20N, P26S, D138Y, R190S, K417T, E484K, N501Y, D614G, H655Y, T1027I, V1176F	1.507647	1.75435	-1.38428	-1.31278	-0.67481	-1.06524
				ORF3a	S253P	1.59006	2.069778	0.0081	0.103932
ORF8	E92K			0.70646	0.947547	-0.46479	0.551035	0.633644	-0.30211
		Nucleocapsid	P80R, R203K, G204R	0.623184	0.766355	0.766728	0.788503	-0.26696	-0.85619

Ser-to Leu-by mutation at 463<sup>rd</sup> position and 512<sup>th</sup> position led to the generation of Type 3 and Type 4, respectively from Type 2. These mutations possibly will affect the pathogenesis of the virus by activating the inflammasomes (Hassan et al., 2020a). Type 2 mutation (S171L) of ORF3a was found in BRA/HIAE-SP03/2020, none other type doesn't exist in Brazilian isolates. Non-synonymous mutations are reported in the envelope protein (E) of the novel coronavirus. The SARS-CoV-2 in comparison with SARS-CoV has the E protein with one deletion at 70th position and three-point mutations with Thr-to Ser, Val-to Phe, and Glu-to Arg-at 55th, 56th and 69th positions at its C terminal. These mutations might have effects on the propagation and multiplication of the virus (Hassan et al., 2020b).

BRA/CD1739-P4/2020 (G14) isolates can transmit their cellular and biological process into virulence mechanism by changing its amino acid from Pro-to Leu-at 323rd position in RdRp gene and resulted in the destabilization of its structure (Begum et al., 2020). Similarly, the change of Pro-to Leu-in helicase protein resulted in the enhanced flexibility of the secondary structure. Both of these mutations in SARS-CoV2 helped in the replication of the virus (Begum et al., 2020). A few mutants in NSP4, NSP5 are becoming more virulent in Genotype 15 and 16. All other mutants are remaining the same sub-cellular localization. The mutation is responsible for emerging low-frequency viral variants which are drug-resistant, resulting in therapy failure (Begum et al., 2020). Some mutational events may occur in nucleocapsid-associated proteins with a mid and severe clinical outcome (Nagy et al., 2021). A protein's biophysical properties, such as folding, stability, and functions, are drastically altered by amino acid substitution (Gromiha et al., 2009; Prathiviraj et al., 2016). Hence, the fluctuation in protein folding leads to replication and multiplication of SARS-CoV-2 in the new environmental niche (Prisilla et al., 2016; Prathiviraj et al., 2016, 2021).

It must be noted that the mutations described at particular locations do not individually provide fitness to the virus, but a combination of favourable mutations at different locations in the genome, resulting in the improved or diminished functionalities have been classified as a cluster of mutant viruses harbouring certain moderations (Mercatelli and Giorgi, 2020). For relapse contemporary antiviral treatments, it is crucial because of randomized genomic variants in SARS-CoV-2 isolates at a global level (Prathiviraj et al., 2021). Consequently, the predicted mutations in our study help researchers to determine new antiviralants based on their genomic variants. Thus, computational strategies to assess the side effects of therapeutic drugs are propitious to improve efficiency and accelerate the development of drug discovery (Wu et al., 2020; Murugesan et al., 2021). Recent technological and computational advances in genomics, systems biology and drug repurposing have offered possibilities for antiviral detection and identifying side effects or therapeutic agents by integrating disease proteins and drug-target interactions for the treatment of COVID-19 (Hashimoto 2021; Yousefi et al., 2021).

## 5. Conclusion

We performed a complete genomic and proteomic investigation of the Brazilian SARS-CoV-2 isolate to identify its genotypic variants. From our study, we identified 16 genotypic variants among 27 isolates. The genotype G14-G16 has a high mutation rate in spike protein. The mutations in NSP4 and NSP5 in genotype (G15 and G16) confer lower stability of the protein structures. Our study revealed the mutational effect on the folding rate of assembly and maturation proteins. The slow and fast folding rate of SARS-CoV-2 protein was observed concerning specific mutations. As a result, the current study will help researchers



**Table 3**

. A comparison of the folding rate and mechanism of protein mutants isolated from Brazilian SARS-CoV-2 isolates. The folding rate was predicted for both reference and variant proteins. The positive value represents fast-folding rate whereas negative indicates slow-folding rate.

Genotype	Isolates	Genes	Mutation	All- $\alpha$		All- $\beta$		$\alpha+\beta:\alpha/\beta$		Unknown	
				Ref..	Var.	Ref..	Var.	Ref..	Var.	Ref..	Var.
G1	BRA/SP02/2020	NSP6	L37F	6.6	7.5	63.8	66.9	-17.9	-3.54	0.996	-7.75
G2	BRA/SP02cc/2020	ORF3a	G251V	-6.16	-5.23	39.4	38.9	0.335	-2.42	-4.11	2.74
		NSP2	T528I	12.3	12.5	10.5	16.7	-12.7	-23.7	4.41	-2.22
G3	BRA/RJ01/2020	NSP6	L37F	6.6	7.5	63.8	66.9	-17.9	-3.54	0.996	-7.75
		Spike	N74K	3.4	4.1	21.5	17.7	-11	-9.97	6.12	2.25
		ORF3a	G251V	-6.16	-5.23	39.4	38.9	0.335	-2.42	-4.11	2.74
		NSP2	T85I	12.3	12.5	10.5	16.7	-12.7	-23.7	4.41	-2.22
		NSP7	S25L	21.2	22.5	37.2	34.2	22.3	-0.383	13.1	7.57
		RdRp	P323L	13.9	13.8	21.7	21.5	-13.3	-0.237	4.04	4.96
G4	BRA/HIAE-SP04/2020	Exonuclease	A320V	9.05	9.47	16.9	22.5	4.29	2.22	0.83	2.97
		Spike	D614G	3.4	4.65	21.5	24.9	-11	-0.137	6.12	5.89
		ORF3a	Q57H	-6.16	-5.22	39.4	39.2	0.335	-23.9	-4.11	4.27
		NSP7	L71F	21.2	20.5	37.2	33.7	22.3	-13.3	13.1	14.3
		RdRp	P323L	13.9	13.8	21.7	21.5	-13.3	-0.237	4.04	4.96
		Spike	D614G, V1176F	3.4	3.49	21.5	21.4	-11	9.7	6.12	11.7
G5	BRA/HIAE-SP03/2020	Nucleocapsid	R203K, G204R	-9.64	-10.2	-7.18	-6.32	28.6	17.2	5.53	6.23
		NSP6	T17I	6.6	6.89	63.8	66.6	-17.9	-26	0.996	-0.894
		RdRp	P323L	13.9	13.8	21.7	21.5	-13.3	-0.237	4.04	4.96
		Spike	D614G	3.4	4.65	21.5	24.9	-11	-0.137	6.12	5.89
		ORF3a	S171L	-6.16	-6.21	39.4	38.9	0.335	4.01	-4.11	-2.7
		ORF6	I33T	8.4	8.65	53.1	51	-32.1	-12.5	4.37	6.66
G6	BRA/LRV-SARS.CoV-2.1/2020	Nucleocapsid	R203K, G204R, I292T	-9.64	-9.41	-7.18	-6.69	28.6	29.5	5.53	-1.67
		RdRp	P323L	13.9	13.8	21.7	21.5	-13.3	-0.237	4.04	4.96
		Spike	D614G	3.4	4.65	21.5	24.9	-11	-0.137	6.12	5.89
		ORF6	I33T	8.4	8.65	53.1	51	-32.1	-12.5	4.37	6.66
		Nucleocapsid	R203K, G204R, I292T	-9.64	-9.41	-7.18	-6.69	28.6	29.5	5.53	-1.67
		NSP2	T85I	12.3	12.5	10.5	16.7	-12.7	-23.7	4.41	-2.22
G7	BRA/RJ-DCVN1/2020	NSP7	S25L	21.2	22.5	37.2	34.2	22.3	-0.383	13.1	7.57
		RdRp	P323L	13.9	13.8	21.7	21.5	-13.3	-0.237	4.04	4.96
		Exonuclease	A320V	9.05	9.47	16.9	22.5	4.29	2.22	0.83	2.97
		Spike	D614G	3.4	4.65	21.5	24.9	-11	-0.137	6.12	5.89
		ORF3a	Q57H	-6.16	-5.22	39.4	39.2	0.335	-23.9	-4.11	4.27
		RdRp	P323L	13.9	13.8	21.7	21.5	-13.3	-0.237	4.04	4.96
G8	BRA/RJ-DCVN2/2020	Spike	D614G	3.4	4.65	21.5	24.9	-11	-0.137	6.12	5.89
		RdRp	P323L	13.9	13.8	21.7	21.5	-13.3	-0.237	4.04	4.96
		Spike	D614G	3.4	4.65	21.5	24.9	-11	-0.137	6.12	5.89
G9	BRA/RJ-DCVN5/2020	RdRp	P323L	13.9	13.8	21.7	21.5	-13.3	-0.237	4.04	4.96
		Spike	D614G	3.4	4.65	21.5	24.9	-11	-0.137	6.12	5.89
G10	BRA/MASP1C844R1/2020	Envelope	V5A	7.51	7.54	55	53.9	3.46	-13.5	7.44	7.83
		RdRp	P323L	13.9	13.8	21.7	21.5	-13.3	-0.237	4.04	4.96
		Spike	D614G	3.4	4.65	21.5	24.9	-11	-0.137	6.12	5.89
		ORF6	I33T	8.4	8.65	53.1	51	-32.1	-12.5	4.37	6.66
G11	BRA/MASP2C844R2/2020	Nucleocapsid	R203K, G204R, I292T, P383L	-9.64	-9.3	-7.18	-3.3	28.6	27.1	5.53	7.81
		NSP2	V577F	12.3	12.8	10.5	14.2	-12.7	-16	4.41	6.91
		NSP7	L71F	21.2	20.5	37.2	33.7	22.3	-13.3	13.1	14.3
		RdRp	P323L	13.9	13.8	21.7	21.5	-13.3	-0.237	4.04	4.96
		Ribose	R216N	6.18	6.39	25.6	22.9	10.9	-0.719	0.743	5.63
		Spike	D614G, V1176F	3.4	3.49	21.5	21.4	-11	9.7	6.12	11.7
G12	BRA/1462/2021	Nucleocapsid	R203K, G204R	-9.64	-10.2	-7.18	-6.32	28.6	17.2	5.53	6.23
		NSP2	G88E	12.3	13.7	10.5	16.6	-12.7	-8.3	4.41	10.9
		3CLPro	L205V	15.5	15.8	19.2	22.3	-2.24	-17.1	5.22	2.44
		NSP6	L37F	6.6	7.5	63.8	66.9	-17.9	-3.54	0.996	-7.75
		NSP7	L71F	21.2	20.5	37.2	33.7	22.3	-13.3	13.1	14.3
		RdRp	P323L	13.9	13.8	21.7	21.5	-13.3	-0.237	4.04	4.96
		Exonuclease	T16A, Y235N	9.05	8.82	16.9	17.1	4.29	-8.21	0.83	-0.196
		EndRNase	K110R	6.52	7.37	18.4	22.7	27.4	2.66	5.26	5.64
		Spike	E484K, D614G, V1176F	3.4	4.02	21.5	22	-11	-8.88	6.12	10.8
		Nucleocapsid	A119S, S202T, R203K, G204R, M234I	-9.64	-10	-7.18	-3.17	28.6	22.4	5.53	9.46
G13	BRA/1431/2021	NSP2	G88E	12.3	13.7	10.5	16.6	-12.7	-8.3	4.41	10.9
		3CLPro	L205V	15.5	15.8	19.2	22.3	-2.24	-17.1	5.22	2.44
		NSP7	L71F	21.2	20.5	37.2	33.7	22.3	-13.3	13.1	14.3
		RdRp	P323L	13.9	13.8	21.7	21.5	-13.3	-0.237	4.04	4.96
		Exonuclease	T16A	9.05	8.22	16.9	21.5	4.29	-3.75	0.83	0.684
		EndRNase	K110R	6.52	7.37	18.4	22.7	27.4	2.66	5.26	5.64
		Spike	E484K, D614G, V1176F	3.4	4.02	21.5	22	-11	-8.88	6.12	10.8
		Nucleocapsid	A119S, S202T, R203K, G204R, M234I	-9.64	-10	-7.18	-3.17	28.6	22.4	5.53	9.46
		PLPro	S375L, K982Q, S1740F	5.63	6.62	16.6	13.1	-3.42	-5	0.533	1.81
		3CLPro	P96H	15.5	16.6	19.2	18.8	-2.24	-8.53	5.22	2.78
G14	BRA/CD1739-P4/2020	NSP6	S106T, G107S, F108L	6.6	6.84	63.8	61.7	-17.9	-22	0.996	1.26
		RdRp	P323L	13.9	13.8	21.7	21.5	-13.3	-0.237	4.04	4.96
		Helicase	E341D	3.56	3.24	17.8	16.9	-3.33	-19.7	11.2	7.38
		Spike	L18F, T20N, P26S, D138Y, R190S, E484K, N501Y, D614G, H655Y, T1027I, V1176F	3.4	4.05	21.5	24.6	-11	-1.44	6.12	13.3

(continued on next page)

Table 3 (continued)

Genotype	Isolates	Genes	Mutation	All- $\alpha$		All- $\beta$		$\alpha+\beta:\alpha/\beta$		Unknown	
				Ref..	Var.	Ref..	Var.	Ref..	Var.	Ref..	Var.
G15	BRA/1236/2021	ORF3a	S253P	-6.16	-6.18	39.4	37	0.335	-18	-4.11	0.908
		ORF8	E92K	0.827	0.363	42.2	45.3	-44.6	-25.5	5.47	1.2
		Nucleocapsid	P80R, R203K, G204R	-9.64	-8.92	-7.18	-5.31	28.6	27.1	5.53	7.52
		NSP2	S138L	12.3	13	10.5	15.6	-12.7	-20.4	4.41	0.365
		PLPro	S375L, K982Q	5.63	5.51	16.6	10.5	-3.42	-17.3	0.533	3.93
		NSP4	S184N	-0.339	0.632	41.8	38.5	-10.2	-28.3	4.57	-2.79
		NSP6	F108L	6.6	6.66	63.8	64.3	-17.9	-26.2	0.996	3.07
		RdRp	P323L	13.9	13.8	21.7	21.5	-13.3	-0.237	4.04	4.96
		Helicase	E341D	3.56	3.24	17.8	16.9	-3.33	-19.7	11.2	7.38
		Exonuclease	N256S	9.05	7.59	16.9	22.4	4.29	-0.968	0.83	-2.08
		Spike	L18F, T20N, P26S, D138Y, R190S, K417T, E484K, N501Y, D614G, H655Y, T1027I, V1176F	3.4	4.28	21.5	25.6	-11	-3.9	6.12	8.9
		ORF3a	S253P	-6.16	-6.18	39.4	37	0.335	-18	-4.11	0.908
		Membrane	A2V	0.355	0.722	43.8	45.6	-19.3	-14.7	-0.2	-3.23
ORF8	E92K	0.827	0.363	42.2	45.3	-44.6	-25.5	5.47	1.2		
G16	BRA/1061/2021	Nucleocapsid	P80R, R203K, G204R	-9.64	-8.92	-7.18	-5.31	28.6	27.1	5.53	7.52
		NSP2	K534N	12.3	11.7	10.5	15.3	-12.7	-8.76	4.41	2.65
		PLPro	S375L, K982Q	5.63	5.51	16.6	10.5	-3.42	-17.3	0.533	3.93
		RdRp	P323L	13.9	13.8	21.7	21.5	-13.3	-0.237	4.04	4.96
		Helicase	E341D	3.56	3.24	17.8	16.9	-3.33	-19.7	11.2	7.38
		Spike	L18F, T20N, P26S, D138Y, R190S, K417T, E484K, N501Y, D614G, H655Y, T1027I, V1176F	3.4	4.28	21.5	25.6	-11	-3.9	6.12	8.9
		ORF3a	S253P	-6.16	-6.18	39.4	37	0.335	-18	-4.11	0.908
		ORF8	E92K	0.827	0.363	42.2	45.3	-44.6	-25.5	5.47	1.2
		Nucleocapsid	P80R, R203K, G204R	-9.64	-8.92	-7.18	-5.31	28.6	27.1	5.53	7.52

understand the mutation rate among Brazilian isolates and will aid in the discovery of new drugs.

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## CRedit authorship contribution statement

**Ragothaman Prathiviraj:** Data curation, Formal analysis, Investigation, Methodology, Writing – original draft. **Paulchamy Chella-pandi:** Conceptualization, Validation, Supervision, Writing – review & editing. **Ajima Begum:** Visualization, Writing – review & editing. **George Seghal Kiran:** Visualization, Writing – review & editing. **Joseph Selvin:** Conceptualization, Validation, Supervision, Writing – review & editing.

## Declaration of Competing Interest

The authors declare that they have no conflict of interest.

## Supplementary materials

Supplementary material associated with this article can be found, in the online version, at [doi:10.1016/j.virusres.2021.198618](https://doi.org/10.1016/j.virusres.2021.198618).

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