

Meta-Analysis of the Dynamics of the Emergence of Mutations and Variants of SARS-CoV-2

Nicolas Castonguay¹, Wandong Zhang^{2,3} and Marc-Andre Langlois^{1,4*}

¹Department of Biochemistry, Microbiology & Immunology, Faculty of Medicine, University of Ottawa, Ontario, Canada K1H 8M5.

²Department of Cellular & Molecular Medicine, Faculty of Medicine, University of Ottawa, Ontario, Canada K1H 8M5.

³Human Health Therapeutics Research Centre, National Research Council Canada, Ottawa, Canada K1A 0R6

⁴uOttawa Center for Infection, Immunity and Inflammation (CI3).

Running title: **Genome Evolution of SARS-CoV-2.**

*Correspondence should be addressed to: langlois@uottawa.ca

ABSTRACT

The novel Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2) emerged in late December 2019 in Wuhan, China, and is the causative agent for the worldwide COVID-19 pandemic. SARS-CoV-2 is a 29,811 nucleotides positive-sense single-stranded RNA virus belonging to the betacoronavirus genus. Due to inefficient proofreading ability of the viral RNA-dependent polymerase complex, coronaviruses are known to acquire new mutations following replication, which constitutes one of the main factors driving the evolution of its genome and the emergence of new genetic variants. In the last few months, the identification of new B.1.1.7 (UK), B.1.351 (South Africa) and P.1 (Brazil) variants of concern (VOC) highlighted the importance of tracking the emergence of mutations in the SARS-CoV-2 genome and their impact on transmissibility, infectivity, and neutralizing antibody escape capabilities. These VOC demonstrate increased transmissibility and antibody escape, and reduce current vaccine efficacy. Here we analyzed the appearance and prevalence trajectory of mutations that appeared in all SARS-CoV-2 genes from December 2019 to January 2021. Our goals were to identify which modifications are the most frequent, study the dynamics of their spread, their incorporation into the consensus sequence, and their impact on virus biology. We also analyzed the structural properties of the spike glycoprotein of the B.1.1.7, B.1.351 and P.1 variants. This study offers an integrative view of the emergence, disappearance, and consensus sequence integration of successful mutations that constitute new SARS-CoV-2 variants and their impact on neutralizing antibody therapeutics and vaccines.

43 **IMPORTANCE**

44 SARS-CoV-2 is the etiological agent of COVID-19, which has caused > 2 million deaths worldwide as of
 45 January, 2021. Mutations occur in the genome of SARS-CoV-2 during viral replication and affect viral
 46 infectivity, transmissibility and virulence. In early March 2020, the D614G mutation in the spike protein
 47 emerged, which increased the viral transmissibility and is now found in >90% of all SARS-CoV-2
 48 genomic sequences in GISAID database. Between October and December 2020, B.1.1.7 (UK), B.1.351
 49 (South Africa) and P.1 (Brazil) variants of concern (VOCs) emerged, which have increased neutralizing
 50 antibody escape capabilities because of mutations in the receptor binding domain of the spike protein.
 51 Characterizing mutations in these variants is crucial because of their effect on adaptive immune response,
 52 neutralizing antibody therapy, and their impact on vaccine efficacy. Here we tracked and analyzed
 53 mutations in SARS-CoV-2 genes over a twelve-month period and investigated functional alterations in
 54 the spike of VOCs.

55

INTRODUCTION

In late December 2019, a new betacoronavirus known as Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2) emerged in Wuhan, the province of Hubei, China (1). SARS-CoV-2 is the etiological agent for the worldwide COVID-19 pandemic resulting in more than 90 million infected and 2 million death worldwide as of Dec. 2020 (2,3). SARS-CoV-2 is an enveloped, positive-sense single-stranded RNA (+ssRNA) virus with a genome length of 26,000 to 32,000 bps (4). The mutation rates of RNA viruses are generally higher than that of DNA viruses because of the low fidelity of their viral RNA polymerases (5,6). Mutations occur when viral replication enzymes introduce errors in the viral genome resulting in the creation of premature termination codons, deletions and insertions of nucleotides that can change open reading frames, and changes in the nucleotide sequence that can result in amino acid substitutions in the viral proteins. These mutations combined with the selective pressure of the human immune system leads to the selection and evolution of viral genomes (6,7). However, coronaviruses are one of the few members of the RNA virus family that possess limited but measurable proofreading ability via the 3' to 5' exoribonuclease activity of the non-structural viral protein 14 (nsp14) (8,9). Coronaviruses are therefore expected to evolve through genetic drift much slower than other RNA viruses that do not have this ability, such as influenza and HIV (8,10). Additionally, SARS-CoV-2 and other coronaviruses have low known occurrences of recombination between family members (i.e., genetic shift), and therefore are mostly susceptible to genetic drift (11).

SARS-CoV-2 has reached pandemic status due to its presence on every continent and has since maintained a high level of transmissibility across hosts of varied ethnical and genetic backgrounds (2, 12). Moreover, SARS-CoV-2 infections have been reported to naturally infect minks, ferrets, cats, and dogs, which allows the virus to replicate in completely new hosts and mutate to produce new variants and possibly new strains (13,14). In March 2020, the now dominant D614G mutation first emerged in the spike protein (S) of SARS-CoV-2. The S protein is present as a trimer at the surface of the viral envelope and is responsible for attachment of the virus to the human angiotensin converting enzyme 2 (hACE2), the entry receptor for SARS-CoV-2 (15). Published evidence has now shown that D614G increases viral fitness, transmissibility and viral load but does not directly affect COVID-19 pathogenicity (16,17,18,19). Additionally, emerging evidence indicates that D614G may have epistatic interactions and exacerbate the impact of several other independent mutations (19). Mutations in the S protein, and particularly in receptor binding domain (RBD), are of very high concern given that they can directly influence viral infectivity, transmissibility and resistance to neutralizing antibodies and T cell responses.

New mutations are frequently and regularly detected in the genome of SARS-CoV-2 through whole genome sequencing, however very few of these mutations make it into the viral consensus sequence. The

consensus sequence or reference strain is generally regarded as the dominant transmitted strain at a given time. This sequence is determined by aligning large numbers of recently sequenced genomes and establishing a consensus of the highest frequency nucleotide for each position in the viral genome. A genetic variant is a version of the reference strain that has acquire one or several mutations and acts as the founder for further genetic diversification. Mutations arise regularly in the reference strain, but few are longitudinally conserved. Genetic variants are therefore successful offshoots of the reference strain.

Some variants rise rapidly in frequency and then collapse and disappear, others will rise and overtake the frequency of the reference strain and become the new reference, while others acquire additional mutations and continue their upward prevalence trajectory and evolution. There are three main genetic variants that have emerged in the past few months with a sustained upward frequency trajectory. The UK and South African variants have been reported in September and October 2020, respectively (20,21,22), while the P.1/501Y.N3 (P.1) variant is a branch off of the B.1.1.28 lineage, was first detected in travelers from Brazil that landed in Japan in January 2021 (23). These variants are associated with increased resistance to neutralizing antibodies (Nabs) (24). The UK variant known as B.1.1.7/501Y.V1 (B.1.1.7) is present worldwide with local transmission in Europe, China, Oceania, North and South America (20,21). The South African variant also known as B.1.352/501Y.V2 (B.1.351) has reported local transmission in the South African region, Europe, and North America (21,22). The P.1. variant has since been identified in 42% of specimens in the Amazonian city of Manaus and was detected for the first time in the U.S. at the end of January 2021 (landed in Japan in January 2021) (23). All these variants possess the N501Y mutation, which is a mutation in the RBD that is critical for the spike to interact with hACE2 (25). This mutation is reported to cause increased resistance to Nabs, increased infectivity, and virulence in animal models (26). In addition to the N501Y mutation, both the South African and Brazil variants possesses RBD mutations K417N and E484K, which are also associated with increased Nabs escape capabilities (24).

Here we present a retrospective metadata analysis study of mutations reaching higher than 1% global frequency occurring throughout the SARS-CoV-2 genome over the past year and we specifically investigate their frequency trajectory over time and their fixation into the reference sequencing using the Global Initiative on Sharing Avian Influenza Data (GISAID) (27). Additionally, we analyzed mutations in the S protein of the B.1.1.7, B.1.351 and P.1 variants and illustrated their impact on molecular interactions between the S protein and hACE2 and their potential impact on Nabs.

MATERIALS AND METHODS

Data collection and mutational analysis

Genomes uploaded to the GISAID EpiCoV™ server database were analyzed from December 1st, 2019, to December 31st, 2020, with collection of viral sequences from December 1st, 2019 to January 6, 2021. The mutations were selected by being in more than 500 reported genomes in August 2020, and another selection was made in January 2021 to capture mutations with more than 4000 reported genomes. Thus, allowing us to study mutations reaching at least 1% in global frequency. We filtered through 309,962 genomes for the analysis of selected mutations. The variants hCoV-19/Wuhan, hCoV-19/D614G, B.1.1.7, B.1.351, and P.1 were analyzed from December 1st, 2019 to February 17th, 2021. The collection dates were the same dates as the analysis. For the analysis of the mutations in B.1.1.7, B.1.351, and P.1 variants, we used the GISAID EpiCoV™ server database and analyzed the mutations in the variants from December 1st, 2019, to February 17th, 2021. The same timeline was used for the collection dates of the viral sequences. We filtered through 429,514 genomes for the analysis of the variants. Only complete SARS-CoV-2 genomes (28 to 30 Kbps) isolated from human hosts were analyzed. MUSCLE alignment tool on UGene, and SnapGene was used to determine the nucleotide mutations and codon changes of the non-synonymous and synonymous mutations by sequence alignments with NCBI SARS-CoV-2 reference genome (NC_045512). We acknowledge all genomes uploaded to the GISAID database, and aligned genomes used for **Tables 1, 2, 3 and 4** are presented in supplementary material 1. Graphs of mutations and variants were performed on RStudio with timelines, and genomes illustrations were produced in Biorender.

Structural modeling

Mutations in the spike protein in complex with hACE2 were analyzed using a mutagenesis tool on PyMOL (PDB: 7A94). Visualization of mutations in the B.1.1.7, B.1.351 and P.1 variants was produced using the spike protein closed conformation (PDB: 6ZGE), interaction with hACE2 (PDB: 7A94), interaction with C102 Nab (PDB: 7K8M), and interaction with C121 Nab (PDB: 7K8X). Figures and rendering were prepared with PyMOL.

RESULTS

Identification of emerging mutations in various SARS-CoV-2 genes. Emerging mutations in the SARS-CoV-2 genome were investigated to determine the fluctuations of these mutations during a period of twelve months. We compiled mutations reported in 500+ genomes in August, and 4000+ genomes in early January from the GISAID database, and followed their appearance in reported SARS-CoV-2 genomes until December 31st, 2020. Genes NSP8, NSP10, NS6, NS7a, and E are not illustrated in **Figure 1** given that did not display mutation frequencies sufficiently high to meet our inclusion conditions during the periods of data collection of our study. This is also indicative that these appear to be the most conserved sequences of the SARS-CoV-2 genome. Our analysis focused on the emergence, fixation, and fading of the mutations analyzed that met our inclusion criteria. Our analysis highlights the fixation of the D614G mutation in the S protein and the P323L in the RdRp (**Fig.1**). Both are the only mutations to have been successful to reach reference strain status until now. They appeared to have emerged simultaneously in January, 2020 and became present in >90% of all sequenced genomes by June, 2020. However, some other mutations emerged rapidly and then stabilized or faded out. For example, Q57H (NS3), R203K (N), G204R (N) are mutations that emerged rapidly and appeared to have stabilized at a frequency of 15% to 40%. Others like I120F (NSP2), L37F (NSP6), S477N (S), and L84S (NS8) illustrate mutations that emerged rapidly and then faded-out. We also demonstrate that most genes in SARS-CoV-2 have mutations with frequencies lower than 10% (**Fig.1**). Also, these mutations are summarized into **Table 1**, which illustrates nucleotide substitution producing the amino acid change, the frequency at the end of 2020 and their respective effects. (**Table 1**). These results indicate that only D614G (S) and P323L (NSP12) were fixed in the viral consensus sequence, many of the mutations either faded out or emerged and then stabilized at a frequency lower than 50%.

Geographic localization and timeline of the viral genes with mutations higher than 50% frequency.

To further study the mutations that have frequencies higher than 50%, we took the graphs of NSP12, S, and N genes from **Figure 1** and added worldwide geographic locations of the mutations provided from GISAID. These maps are useful to track down if a mutation is a localized and regional event or found worldwide. In the S protein, D614G is found worldwide with higher reported cases in the US, UK, and Australia, probably due to more large-scale testing, while A222V is mostly reported in the UK, but has not been reported in South America, Central and East African regions (**Fig. 2A**). Like D614G, P323L in the RdRp is also found worldwide, with higher reported cases in the US, UK, and Australia (**Fig. 2B**). The N gene doesn't have successful mutations that have attained reference strain status, but R203K, G204R and A220V all had a frequency of 50% or higher during the past twelve months. Even if R203K and G204R frequencies have decreasing since July of 2020 and stabilized in November of 2020, both

mutations are currently found worldwide. A220V emerged in August of 2020 and reached a frequency higher than 50% in October of 2020. Mutations G204R, R203K and A220V are reported at high frequency in the UK, but have not been detected in South America, Central, East, and South African regions (**Fig. 2C**). The analyses of these data illustrate the localization of the most prevalent mutations to date, which appear to be mostly present in Western and developed countries. This, however, is undoubtable attributable to overall more testing.

Localization and molecular interactions of recurrent S protein mutations. Here we illustrated the molecular interactions and spatial localization of the mutations in the S protein. PyMOL was used to model the structures of S protein and analyze the possible effects of specific mutations at given positions in the protein. **Figure 3A** presents an overview of the localization of the S protein mutations and their interactions with their environment. The A222V mutation has no reported effects on protein stability, neutralizing antibody escape, and affinity for hACE2 (**Table 1**). The substitution from A to V results in a low steric clash between neighboring residues (**Fig. 3B**). The S477N substitution in the RBD enables increased stability during hACE2-RBD interactions (**Table 1, Fig. 3C**). In the N-terminal domain (NTD), mutagenesis of L18F leads to a steric clash between neighboring residues (**Fig. 3D**). However, this does not appear to impact the stability of the protein given that no such effects have been reported for L18F so far (**Table 1**). In the closed conformation, D614 makes an ionic bond with K854 in the S2 subunit of another S protein monomer (**Fig. 3F**) (28). In the open conformation, D614 (or G614) doesn't make interactions or display steric clashes with neighboring residues (**Fig. 3E**).

The emergence of the B.1.1.7 variant in the UK. A new variant was discovered in late 2020 in the UK that displayed increased affinity to hACE2 and Nabs escape capabilities (24,29,30). Here we attempted to further investigate the B.1.1.7 variant by looking at S protein mutations of this variant in complex with Nabs and hACE2. We mapped the localization of the mutations with available Cryo-EM structures of the S protein and assessed the frequency of the variant by interrogating the GISAID database. There are nine mutations in the S protein out of the total 24 mutations in the B.1.1.7 SARS-CoV-2 genome (**Fig. 5A & 5E**). Deletions and mutations in the S protein of the B.1.1.7 variant, apart from D614G, emerged in October of 2020 and reached a frequency of 68% to 72% mid February (**Fig. 4, Fig. 5C, Table 2**). N501Y is found in the RBD and can interact with a lysine residue in hACE2 (**Fig. 5B & 5D, Fig. 8C**). The N501Y mutation is associated with an increased affinity to hACE2, along with an increase in infectivity and virulence (**Table 2**). **Figure 5E** illustrates the whole genome of SARS-CoV-2 with all nucleotide substitutions of the B.1.1.7 variant. The C913T, C5986T, C14676T, C15279T, C16176T in ORF1ab, and T26801C in M protein are synonymous mutations. Also, the C27972T mutation has a frequency of 71% and produces a premature stop codon in NS8 that inactivates the protein (Q27stop)

without obvious consequences (**Fig. 5E, Table 2**) (31). These results allow us to better understand the frequencies, localization, and interactions of mutations in the S protein of the B.1.1.7 variant. Importantly, for viruses, not only synonymous mutations are of important. Non-synonymous mutations can exercise very important roles at various stages of the viral infection cycle, such as replication and creating functional RNA loops that serve as docking points for ribonucleoproteins and primers.

The emergence of the B.1.351 variant in South Africa. During the emergence of the B.1.1.7 variant in the UK, another variant was emerging in South Africa, known as B.1.351 (21,22). Similar to **Figure 5**, we illustrate the mutations in S protein and their respective frequencies. The GISAID database was used to track down mutations and Cryo-EM structures of the S protein to model the effects of point mutations. Most of the mutations in the S protein of the B.1.351 variant are localized in the S1 subunit, with only A701V in the S2 subunit. Additionally, three mutations reside in the RBD, among which two of them are not found in the B.1.1.7 variant (K417N, E484K) (**Fig. 6A & 6B**). This variant contains the D614G and N501Y, which are also seen in the B.1.1.7 variant (**Fig. 5A**). Furthermore, many of the mutations found in the B.1.351 variant have global frequencies lower than 2%. The exception is D614G, L18F and N501Y (**Fig. 6C, Table 3**). In comparison to B.1.1.7 and P.1 variants, B.1.351 variant has not reached a frequency higher than 1% as of mid February 2021 (**Fig. 4**). By using the mutagenesis tool of PyMOL, we modeled the S protein in complex with hACE2, and with C103 and C121 Nabs, which are human recombinant class I & II neutralizing antibodies, respectively(32). Our *in silico* mutagenesis predicts that mutations in the RBD induce a loss of interactions with C102 Nab and C121 Nab. At the position 417 in the S protein, a loss of interaction is predicted between the RBD and C102 Nab when the K417 is mutated to Asn producing Nabs escape capability. (**Fig. 6D & 8A**). Another loss of interaction is predicted with the E484K mutation and the C121 Nab, which could lead to Nabs escape capability (**Fig. 6E & 8B**). As previously mentioned, N501Y is also found in the B.1.351 strain and our modeling predicts that it will have similar effects as those observed with the B.1.1.7 variant (**Fig. 6F**). **Figure 6G** illustrates the nucleotide substitutions of the B.1.351 variant.

The emergence of the P.1 variant in Brazil. Similar to the B.1.351 variant, the P.1 variant harbours the N501Y and E484K mutations, but position 417 of the S protein displays a threonine instead of a lysine residue (19,23). Similar to the UK and South African variants, we demonstrate mutations in the S protein producing the P.1 variant and their frequencies from December 2019 to 17th of February 2021 (**Fig. 7**). The P.1 variant harbours substitutions L18F, T20N, P26S, D138Y, and R190S in the NTD of the S protein. H655Y and T1027I are in the subdomain 2 (SD2) and S2 subunit, respectively (**Fig. 7A & 7B, Table 4**). Overall, P.1-specific mutations have worldwide frequencies less than 2% (**Fig. 7C, Table 4**). D614G, L18F and N501Y are not specific to P1. Similar to B.1.351, P.1 variant has low global frequency

with less than 1% of global variant frequency as of mid February 2021 (**Fig. 4**). We then modeled mutations to investigate interaction alterations with known recombinant neutralizing antibodies (32). The K417T mutation reduces interactions with neighboring residues in the C102 Nab and therefore we predict, as with K417N in B.1.1.7, an increased ability to escape neutralization (**Fig. 7D**). Also, the P.1 variant has E484K, and N501Y mutations in the RBD. We predict they will have the same effect reported for the B.1.1.7, and B.1.351 variants (**Fig. 7E & 7F**). In **Figure 7G**, we illustrate the synonymous, non-synonymous, and deletions in SARS-CoV-2 P.1 genome (**Table 4**).

DISCUSSION

Research on the effect of mutations in SARS-CoV-2 has been carried out since the appearance of the virus, and the emergence of new genetic variants that are more transmissible and resistant to antibody neutralization have highlighted the importance of studying these mutations. The number of sequenced viral genomes uploaded to the GISAID database grew fast from 131,417 at the end of September to 451,913 by January 30th, 2021 (27). GISAID is a formidable tool for tracking the emergence of mutations, identifying the region where it emerged, and track its spread around the globe. Given the risk mutations and new variants pose for neutralizing antibody therapy and vaccines for SARS-CoV-2, it is crucial to continuously monitor the susceptibility of these variants to neutralization by humoral and cellular immune responses either induced through natural exposure to the reference strain or induced by vaccination (24). Recent reports on the vaccine efficacy of the Moderna, Pfizer-BioNTech, and Oxford-AstraZeneca vaccines against the B.1.1.7 and B.1.351 variant is variable. All of these vaccines remain efficacious against the B.1.1.7 variant (30,33,34). However, the Oxford-AstraZeneca vaccine has displayed compromised efficacy against the B.1.351 variant with 21.4% (35). Preliminary data with the Pfizer-BioNTech and Moderna mRNA vaccines also show reduction of efficacy against B.1.351 (34,36). Furthermore, antibodies induced by the Pfizer-BioNTech vaccine appear to display a 15.1-fold decrease in neutralization efficacy against the P.1 variant (37) (**Table 5**). Nevertheless, humoral responses are only one component of the adaptive immune response. T and B cell responses have not been probed in detail against these variants at this time and may still provide robust protection.

Furthermore, there is likely epistatic mutations in the S protein. Epistasis is the combinatory effect of two or more mutations in a genome (41). Epistasis has previously been studied in the surface protein hemagglutinin (HA) of the influenza viruses, and have illustrated positive epistasis in 11 regions of the HA receptor-binding domain (42). In relation to the S protein of SARS-CoV-2, it could, for instance, allow the S protein to adopt a specific conformation when all the mutations are present, thereby producing

a unique folding of the protein. A recent study demonstrated the impact of antigenicity and infectivity of the D614G SARS-CoV-2 variants with a combination of different mutations occurring in the S protein (19). The study shows that D614G alone increases infectivity, but in combination with different other mutations in the S protein, these can either increase or decrease viral infectivity. Similar findings have been reported regarding sensitivity to Nabs. D614G alone has undetectable effects on Nabs escape. However, the combination of D614G with other mutations in S can enable Nabs escape. This data suggests that the continuous emergence of epistatic mutations in SARS-CoV-2 will likely be involved in further altering properties of the virus, including transmissibility, pathogenicity, stability and Nab resistance.

Our analyses have highlighted that several of the successful mutations analyzed had frequency trajectories that eventually plunged or stabilized at low frequencies. Only the D614G in the S protein and the P323L in the RdRp maintained their presence in the consensus sequence (**Fig. 1 & 2**). Analyses of the GISAID database also reveal which countries upload the most sequences to the database and are therefore carrying out the most testing and sequencing. The global frequency of mutations and variants in the database is therefore biased to represent the genetic landscape of the countries doing the most testing. Emergence of new variants may therefore go undetected until they leave their point of origin and enter countries with high testing and sequencing rates. This delayed notification constitutes a major obstacle in preventing the spread of nefarious variants that are potentially resistant to current vaccines and neutralizing antibody therapy.

In conclusion, our metadata analysis of emerging mutations has highlighted the natural upward and downward fluctuation in mutation prevalence. We also illustrate how mutations sometimes need to co-emerge in order to create a favorable outcome for virus propagation. Tracking mutations and the evolution of the SARS-CoV-2 genome is critical for the development and deployment of effective treatments and vaccines. Thus, it is the responsibility of all countries and governing jurisdictions to increase testing and sequencing and upload SARS-CoV-2 genomes to databases in real-time. On then will we have the most accurate information to inform policy and decisions makers about interventions required to blunt the global transmission of the virus and ensure that our tools remain effective against the all circulating variants.

307 **ACKNOWLEDGEMENTS**

308 The authors wish to thank Sean Li for helpful comments on our manuscript. M.-A.L. holds a Canada
 309 Research Chair in Molecular Virology and Intrinsic Immunity. This study was supported by a COVID-19
 310 Rapid Response grant to M-A Langlois by the Canadian Institute of Health Research (CIHR) and by a
 311 grant supplement by the Canadian Immunity Task Force (CITF).

312

313 **CONFLICTS OF INTERESTS**

314 The authors declare no competing interests.

REFERENCES

1. Zhu N, Zhang D, Wang W, Li X, Yang B, Song J, Zhao X, Huang B, Shi W, Lu R, Niu P, Zhan F, Ma X, Wang D, Xu W, Wu G, Gao GF, Tan W. 2020. A Novel Coronavirus from Patients with Pneumonia in China, 2019. *N Engl J Med* **382**:727–733.
2. Dong E, Du H, Gardner L. 2020. An interactive web-based dashboard to track COVID-19 in real time. *The Lancet Infectious Diseases* **20**:533–534.
3. Wu F, Zhao S, Yu B, Chen Y-M, Wang W, Song Z-G, Hu Y, Tao Z-W, Tian J-H, Pei Y-Y, Yuan M-L, Zhang Y-L, Dai F-H, Liu Y, Wang Q-M, Zheng J-J, Xu L, Holmes EC, Zhang Y-Z. 2020. A new coronavirus associated with human respiratory disease in China. *Nature* **579**:265–269.
4. Kim D, Lee J-Y, Yang J-S, Kim JW, Kim VN, Chang H. 2020. The Architecture of SARS-CoV-2 Transcriptome. *Cell* **181**:914-921.e10.
5. Duffy S. 2018. Why are RNA virus mutation rates so damn high? *PLOS Biology* **16**:e3000003.
6. Mandary MB, Masomian M, Poh CL. 2019. Impact of RNA Virus Evolution on Quasispecies Formation and Virulence. *Int J Mol Sci* **20**:4657.
7. Duffy S, Shackelton LA, Holmes EC. 2008. Rates of evolutionary change in viruses: patterns and determinants. *Nature Reviews Genetics* **9**:267–276.
8. Eckerle LD, Becker MM, Halpin RA, Li K, Venter E, Lu X, Scherbakova S, Graham RL, Baric RS, Stockwell TB, Spiro DJ, Denison MR. 2010. Infidelity of SARS-CoV Nsp14-Exonuclease Mutant Virus Replication Is Revealed by Complete Genome Sequencing. *PLOS Pathogens* **6**:e1000896.
9. Minskaia E, Hertzog T, Gorbalenya AE, Campanacci V, Cambillau C, Canard B, Ziebuhr J. 2006. Discovery of an RNA virus 3'→5' exoribonuclease that is critically involved in coronavirus RNA synthesis. *Proc Natl Acad Sci U S A* **103**:5108.
10. Ma Y, Wu L, Shaw N, Gao Y, Wang J, Sun Y, Lou Z, Yan L, Zhang R, Rao Z. 2015.

Structural basis and functional analysis of the SARS coronavirus nsp14–nsp10 complex.
Proc Natl Acad Sci USA 112:9436.

11. Rausch JW, Capoferri AA, Katusiime MG, Patro SC, Kearney MF. 2020. Low genetic diversity may be an Achilles heel of SARS-CoV-2. *Proc Natl Acad Sci USA* 117:24614.

12. Andreano E, Piccini G, Licastro D, Casalino L, Johnson NV, Paciello I, Monego SD, Pantano E, Manganaro N, Manenti A, Manna R, Casa E, Hyseni I, Benincasa L, Montomoli E, Amaro RE, McLellan JS, Rappuoli R. 2020. SARS-CoV-2 escape *in vitro* from a highly neutralizing COVID-19 convalescent plasma. *bioRxiv* 2020.12.28.424451.

13. Shi J, Wen Z, Zhong G, Yang H, Wang C, Huang B, Liu R, He X, Shuai L, Sun Z, Zhao Y, Liu P, Liang L, Cui P, Wang J, Zhang X, Guan Y, Tan W, Wu G, Chen H, Bu Z. 2020. Susceptibility of ferrets, cats, dogs, and other domesticated animals to SARS–coronavirus 2. *Science* 368:1016.

14. Li X, Giorgi EE, Marichannegowda MH, Foley B, Xiao C, Kong X-P, Chen Y, Gnanakaran S, Korber B, Gao F. 2020. Emergence of SARS-CoV-2 through recombination and strong purifying selection. *Sci Adv* 6:eabb9153.

15. Walls AC, Park Y-J, Tortorici MA, Wall A, McGuire AT, Velesler D. 2020. Structure, Function, and Antigenicity of the SARS-CoV-2 Spike Glycoprotein. *Cell* 181:281-292.e6.

16. Plante JA, Liu Y, Liu J, Xia H, Johnson BA, Lokugamage KG, Zhang X, Muruato AE, Zou J, Fontes-Garfias CR, Mirchandani D, Scharton D, Bilello JP, Ku Z, An Z, Kalveram B, Freiberg AN, Menachery VD, Xie X, Plante KS, Weaver SC, Shi P-Y. 2020. Spike mutation D614G alters SARS-CoV-2 fitness. *Nature* <https://doi.org/10.1038/s41586-020-2895-3>.

17. Zhang L, Jackson CB, Mou H, Ojha A, Peng H, Quinlan BD, Rangarajan ES, Pan A, Vanderheiden A, Suthar MS, Li W, Izard T, Rader C, Farzan M, Choe H. 2020. SARS-CoV-2 spike-protein D614G mutation increases virion spike density and infectivity. *Nature Communications* 11:6013.

18. Korber B, Fischer WM, Gnanakaran S, Yoon H, Theiler J, Abfalterer W, Hengartner N, Giorgi EE, Bhattacharya T, Foley B, Hastie KM, Parker MD, Partridge DG, Evans CM,

Freeman TM, de Silva TI, Angyal A, Brown RL, Carrilero L, Green LR, Groves DC, Johnson KJ, Keeley AJ, Lindsey BB, Parsons PJ, Raza M, Rowland-Jones S, Smith N, Tucker RM, Wang D, Wyles MD, McDanal C, Perez LG, Tang H, Moon-Walker A, Whelan SP, LaBranche CC, Saphire EO, Montefiori DC. 2020. Tracking Changes in SARS-CoV-2 Spike: Evidence that D614G Increases Infectivity of the COVID-19 Virus. *Cell* 182:812-827.e19.

19. Li Q, Wu J, Nie J, Zhang L, Hao H, Liu S, Zhao C, Zhang Q, Liu H, Nie L, Qin H, Wang M, Lu Q, Li X, Sun Q, Liu J, Zhang L, Li X, Huang W, Wang Y. 2020. The Impact of Mutations in SARS-CoV-2 Spike on Viral Infectivity and Antigenicity. *Cell*, 2020/07/17 ed. 182:1284-1294.e9.

20. Rambaut A, Loman N, Pybus O, Barclay W, Barrett J, Carabelli A, Connor T, Peacock T, Robertson D, Volz E, on behalf of CoG-UK. 2020. Preliminary genomic characterisation of an emergent SARS-CoV-2 lineage in the UK defined by a novel set of spike mutations. <https://virological.org/t/preliminary-genomic-characterisation-of-an-emergent-sars-cov-2-lineage-in-the-uk-defined-by-a-novel-set-of-spike-mutations/563>

21. O'Toole, A, Hill V, et al. (2021) Tracking the international spread of SARS-CoV-2 lineage B.1.1.7 and B.1.351/501Y-V2. <https://virological.org/t/tracking-the-international-spread-of-sars-cov-2-lineages-b-1-1-7-and-b-1-351-501y-v2/592>

22. Tegally H, Wilkinson E, Giovanetti M, Iranzadeh A, Fonseca V, Giandhari J, Doolabh D, Pillay S, San EJ, Msomi N, Mlisana K, von Gottberg A, Walaza S, Allam M, Ismail A, Mohale T, Glass AJ, Engelbrecht S, Van Zyl G, Preiser W, Petruccione F, Sigal A, Hardie D, Marais G, Hsiao M, Korsman S, Davies M-A, Tyers L, Mudau I, York D, Maslo C, Goedhals D, Abrahams S, Laguda-Akingba O, Alisoltani-Dehkordi A, Godzik A, Wibmer CK, Sewell BT, Lourenço J, Alcantara LCJ, Pond SLK, Weaver S, Martin D, Lessells RJ, Bhiman JN, Williamson C, de Oliveira T. 2020. Emergence and rapid spread of a new severe acute respiratory syndrome-related coronavirus 2 (SARS-CoV-2) lineage with multiple spike mutations in South Africa. *medRxiv* 2020.12.21.20248640.

- 396
- 397 23. Faria, Nuno R.; et al. (12 January 2021). Genomic characterisation of an emergent SARS-
398 CoV-2 lineage in Manaus: preliminary findings. *Virological*. Retrieved 23 January 2021.
399 [https://virological.org/t/genomic-characterisation-of-an-emergent-sars-cov-2-lineage-in-](https://virological.org/t/genomic-characterisation-of-an-emergent-sars-cov-2-lineage-in-manaus-preliminary-findings/586)
400 [mana-us-preliminary-findings/586](https://virological.org/t/genomic-characterisation-of-an-emergent-sars-cov-2-lineage-in-manaus-preliminary-findings/586)
- 401 24. Wang P, Liu L, Iketani S, Luo Y, Guo Y, Wang M, Yu J, Zhang B, Kwong PD, Graham BS,
402 Mascola JR, Chang JY, Yin MT, Sobieszczyk M, Kyratsous CA, Shapiro L, Sheng Z, Nair
403 MS, Huang Y, Ho DD. 2021. Increased Resistance of SARS-CoV-2 Variants B.1.351 and
404 B.1.1.7 to Antibody Neutralization. *bioRxiv* 2021.01.25.428137.
- 405 25. Yi C, Sun X, Ye J, Ding L, Liu M, Yang Z, Lu X, Zhang Y, Ma L, Gu W, Qu A, Xu J, Shi Z,
406 Ling Z, Sun B. 2020. Key residues of the receptor binding motif in the spike protein of
407 SARS-CoV-2 that interact with ACE2 and neutralizing antibodies. *Cellular & Molecular*
408 *Immunology* 17:621–630.
- 409 26. Gu H, Chen Q, Yang G, He L, Fan H, Deng Y-Q, Wang Y, Teng Y, Zhao Z, Cui Y, Li Y, Li
410 X-F, Li J, Zhang N-N, Yang X, Chen S, Guo Y, Zhao G, Wang X, Luo D-Y, Wang H, Yang
411 X, Li Y, Han G, He Y, Zhou X, Geng S, Sheng X, Jiang S, Sun S, Qin C-F, Zhou Y. 2020.
412 Adaptation of SARS-CoV-2 in BALB/c mice for testing vaccine efficacy. *Science* 369:1603.
- 413 27. Shu Y, McCauley J. 2017. GISAID: Global initiative on sharing all influenza data - from
414 vision to reality. *Euro Surveill* 22:30494.
- 415 28. Benton DJ, Wrobel AG, Xu P, Roustan C, Martin SR, Rosenthal PB, Skehel JJ, Gamblin SJ.
416 2020. Receptor binding and priming of the spike protein of SARS-CoV-2 for membrane
417 fusion. *Nature* 588:327–330.
- 418 29. Starr TN, Greaney AJ, Hilton SK, Ellis D, Crawford KHD, Dingens AS, Navarro MJ,
419 Bowen JE, Tortorici MA, Walls AC, King NP, Veasler D, Bloom JD. 2020. Deep Mutational
420 Scanning of SARS-CoV-2 Receptor Binding Domain Reveals Constraints on Folding and
421 ACE2 Binding. *Cell* 182:1295-1310.e20.
- 422 30. Muik A, Wallisch A-K, Sanger B, Swanson KA, Muhl J, Chen W, Cai H, Maurus D, Sarkar

- 423 R, Türeci Ö, Dormitzer PR, Şahin U. 2021. Neutralization of SARS-CoV-2 lineage B.1.1.7
424 pseudovirus by BNT162b2 vaccine–elicited human sera. *Science* eabg6105.
- 425 31. Pereira F. 2020. Evolutionary dynamics of the SARS-CoV-2 ORF8 accessory gene. *Infect*
426 *Genet Evol*, 2020/09/02 ed. 85:104525–104525.
- 427 32. Barnes CO, Jette CA, Abernathy ME, Dam K-MA, Esswein SR, Gristick HB, Malyutin AG,
428 Sharaf NG, Huey-Tubman KE, Lee YE, Robbiani DF, Nussenzweig MC, West AP,
429 Bjorkman PJ. 2020. SARS-CoV-2 neutralizing antibody structures inform therapeutic
430 strategies. *Nature* 588:682–687.
- 431 33. Emary KR, Golubchik T, Aley PK, Ariani CV, Angus BJ, Bibi S, Blane B, Bonsall D,
432 Cicconi P, Charlton S. 2021. Efficacy of ChAdOx1 nCoV-19 (AZD1222) vaccine against
433 SARS-CoV-2 VOC 202012/01 (B. 1.1. 7).
- 434 34. Wu K, Werner AP, Koch M, Choi A, Narayanan E, Stewart-Jones GBE, Colpitts T, Bennett
435 H, Boyoglu-Barnum S, Shi W, Moliva JJ, Sullivan NJ, Graham BS, Carfi A, Corbett KS,
436 Seder RA, Edwards DK. 2021. Serum Neutralizing Activity Elicited by mRNA-1273
437 Vaccine — *Preliminary Report*. *N Engl J Med* <https://doi.org/10.1056/NEJMc2102179>
- 438 35. Madhi SA, Baillie V, Cutland CL, Voysey M, Koen AL, Fairlie L, Padayachee SD, Dheda
439 K, Barnabas SL, Bhorat QE, Briner C, Kwatra G, Ahmed K, Aley P, Bhikha S, Bhiman JN,
440 Bhorat AE, Plessis J du, Esmail A, Groenewald M, Horne E, Hwa S-H, Jose A, Lambe T,
441 Laubscher M, Malahleha M, Masenya M, Masilela M, McKenzie S, Molapo K, Moultrie A,
442 Oelofse S, Patel F, Pillay S, Rhead S, Rodel H, Rossouw L, Taoushanis C, Tegally H,
443 Thombrayil A, Eck S van, Wibmer CK, Durham NM, Kelly EJ, Villafana TL, Gilbert S,
444 Pollard AJ, de Oliveira T, Moore PL, Sigal A, Izu A. 2021. Safety and efficacy of the
445 ChAdOx1 nCoV-19 (AZD1222) Covid-19 vaccine against the B.1.351 variant in South
446 Africa. *medRxiv* 2021.02.10.21251247.
- 447 36. Liu Y, Liu J, Xia H, Zhang X, Fontes-Garfias CR, Swanson KA, Cai H, Sarkar R, Chen W,
448 Cutler M, Cooper D, Weaver SC, Muik A, Sahin U, Jansen KU, Xie X, Dormitzer PR, Shi P-
449 Y. 2021. Neutralizing Activity of BNT162b2-Elicited Serum — *Preliminary Report*. *N Engl*
450 *J Med* <https://doi.org/10.1056/NEJMc2102017>.

37. Garcia-Beltran WF, Lam EC, Denis KSt, Nitido AD, Garcia ZH, Hauser BM, Feldman J, Pavlovic MN, Gregory DJ, Poznansky MC, Sigal A, Schmidt AG, Iafrate AJ, Naranbhai V, Balazs AB. 2021. Circulating SARS-CoV-2 variants escape neutralization by vaccine-induced humoral immunity. *medRxiv* 2021.02.14.21251704.
38. Polack FP, Thomas SJ, Kitchin N, Absalon J, Gurtman A, Lockhart S, Perez JL, Pérez Marc G, Moreira ED, Zerbini C, Bailey R, Swanson KA, Roychoudhury S, Koury K, Li P, Kalina WV, Cooper D, Frenck RW, Hammitt LL, Türeci Ö, Nell H, Schaefer A, Ünal S, Tresnan DB, Mather S, Dormitzer PR, Şahin U, Jansen KU, Gruber WC. 2020. Safety and Efficacy of the BNT162b2 mRNA Covid-19 Vaccine. *N Engl J Med* 383:2603–2615.
39. Baden LR, El Sahly HM, Essink B, Kotloff K, Frey S, Novak R, Diemert D, Spector SA, Rouphael N, Creech CB, McGettigan J, Khetan S, Segall N, Solis J, Brosz A, Fierro C, Schwartz H, Neuzil K, Corey L, Gilbert P, Janes H, Follmann D, Marovich M, Mascola J, Polakowski L, Ledgerwood J, Graham BS, Bennett H, Pajon R, Knightly C, Leav B, Deng W, Zhou H, Han S, Ivarsson M, Miller J, Zaks T. 2020. Efficacy and Safety of the mRNA-1273 SARS-CoV-2 Vaccine. *N Engl J Med* 384:403–416.
40. Voysey M, Clemens SAC, Madhi SA, et al. 2021. Safety and efficacy of the ChAdOx1 nCoV-19 vaccine (AZD1222) against SARS-CoV-2: an interim analysis of four randomised controlled trials in Brazil, South Africa, and the UK. *The Lancet* 397:99–111.
41. Phillips PC. 2008. Epistasis — the essential role of gene interactions in the structure and evolution of genetic systems. *Nature Reviews Genetics* 9:855–867.
42. Wu NC, Xie J, Zheng T, Nycholat CM, Grande G, Paulson JC, Lerner RA, Wilson IA. 2017. Diversity of Functionally Permissive Sequences in the Receptor-Binding Site of Influenza Hemagglutinin. *Cell Host & Microbe* 21:742-753.e8.
43. COVID-19 GENOMIC UK CONSORTIUM. 2021. COG-UK report on SARS-CoV-2 Spike mutations of interest in the UK. https://www.cogconsortium.uk/wp-content/uploads/2021/01/Report-2_COG-UK_SARS-CoV-2-Mutations.pdf
44. Zahradník J, Marciano S, Shemesh M, Zoler E, Chiaravalli J, Meyer B, Rudich Y, Dym O,

478 Elad N, Schreiber G. 2021. SARS-CoV-2 RBD *in vitro* evolution follows contagious
479 mutation spread, yet generates an able infection inhibitor. *bioRxiv* 2021.01.06.425392.

480 45. Kannan SR, Spratt AN, Quinn TP, Heng X, Lorson CL, Sönnnerborg A, Byraredy SN, Singh
481 K. 2020. Infectivity of SARS-CoV-2: there Is Something More than D614G? *J*
482 *Neuroimmune Pharmacol*, 2020/09/15 ed. 15:574–577.

483 46. Ugurel OM, Mutlu O, Sariyer E, Kocer S, Ugurel E, Inci TG, Ata O, Turgut-Balik D. 2020.
484 Evaluation of the potency of FDA-approved drugs on wild type and mutant SARS-CoV-2
485 helicase (Nsp13). *Int J Biol Macromol*, 2020/09/24 ed. 163:1687–1696.

486 47. Wu S, Tian C, Liu P, Guo D, Zheng W, Huang X, Zhang Y, Liu L. 2020. Effects of SARS-
487 CoV-2 mutations on protein structures and intraviral protein–protein interactions. *Journal of*
488 *Medical Virology* n/a.

489

490

TABLES

Table 1: Non-synonymous mutations in SARS-CoV-2 genes with a worldwide frequency >0.01%.

Gene	Genome Nucleotide Mutation	Codon	AA Mutation	Frequency (%) [*]	Effect
S	A23403G	GAT to GGT	D614G	99.70	Moderate increase in Transmissibility ⁴³
	C22227T	GCT to GTT	A222V	42.79	No mutational effect ⁴³
	C21615T	CTT to TTT	L18F	19.86	N/A
	G22992A	AGC to AAC	S477N	5.36	Spike-hACE2 complex stability and Nab interference ⁴²
NSP1	C22879A	AAC to AAA	N439K	4.09	Antibody Escape ability ¹⁹
	C21575T	CTT to TTT	L5F	1.44	N/A
	C21855T	TCT to TTT	S98F	2.74	N/A
	G22346T	GCT to TCT	A262S	1.64	N/A
NSP2	C335T	CGC to TGC	R24C	0.70	N/A
	A1163T	ATT to TTT	I120F	0.74	N/A
	G1210T	ATG to ATT	M135I	0.124	N/A
	C1059T	ACC to ATC	T85I	5.55	N/A
NSP3	T1947C	GTT to GCT	V381A	0.55	N/A
	C2453T	CTC to TTC	L550F	2.73	N/A
	C7926T	GCA to GTA	A1736V	1.23	N/A
	G2891A	GCA to ACA	A58T	0.03	N/A
NSP4	T7767C	ATC to ACC	I1683T	4.05	N/A
	C5622T	CCT to CTT	P968L	0.63	N/A
	G8371T	CAG to CAT	Q1884H	0.05	N/A
	C4002T	ACT to ATT	T428I	0.38	N/A
NSP5	C5388A	GCT to GAT	A890D	21.37	N/A
	G8083A	ATG to ATA	M1788I	1.23	N/A
	C3602T	CAC to TAC	H295Y	2.51	N/A
	C9246T	GCT to GTT	A231V	0.12	N/A
NSP6	G9526T	ATG to ATT	M324I	5.18	N/A
	A10323G	AAG to AGG	K90R	1.60	N/A
	G10097A	GGT to AGT	G15S	0.36	N/A
	G10265A	GGT to AGT	G71S	0.69	N/A
NSP7	C10319T	CTT to TTT	L89F	3.21	N/A
	C11109T	GCT to GTT	A46V	0.08	N/A
	C11396T	CTT to TTT	L142F	2.64	N/A
	G11083T	TTG to TTT	L37F	2.73	N/A
NSP8	C11195T	CTT to TTT	L75F	0.05	N/A
	G11230T	ATG to ATT	M86I	0.52	N/A
	G11801A	GGT to AGT	G277S	0.01	N/A
	G11132T	GCT to TCT	A54S	1.83	N/A
NSP9	11288-11296 del	N/A	ΔS106	22.50	N/A
	G12067T	ATG to ATT	M75I	0.171	N/A
	C11916T	TCA to TTA	S25L	0.06	N/A
	G12988T	ATG to ATT	M101I	3.20	N/A
NSP12	C14408T	CCT to CTT	P323L	99.53	Improve processivity, by interaction with NSP8 ⁴⁵
	G15766T	GTG to TTG	V776L	5.27	N/A
	G13993T	GCT to TCT	A185S	5.22	N/A
	G15598A	GTC to ATC	V720I	3.21	N/A
NSP13	G14202T	GAG to GAT	E254D	0.68	N/A
	C13730T	GCT to GTT	A97V	0.04	N/A
	C16289T	GCT to GTT	A18V	0.05	N/A
	G17019T	GAG to GAT	E261D	5.20	N/A
NSP14	C17104T	CAT to TAT	H290Y	4.20	N/A
	C17747T	CCT to CTT	P504L	0.10	Increase hydrophobicity of 2A domain ⁴⁶

Castonguay et al., 2020

Genome Evolution of SARS-CoV-2

	C17639T	TCA to TTA	S468L	0.02	N/A
	A17615G	AAG to AGG	K460R	10.20	N/A
	A16889G	AAA to AGA	K218R	5.18	N/A
	G18028T	GCA to TCA	A598S	3.21	N/A
NSP14	C18998T	GCA to GTA	A320V	0.02	N/A
	C18568T	CTC to TTC	L177F	0.16	N/A
	G19542T	ATG to ATT	M501I	0.71	N/A
	A18424G	AAT to GAT	N129D	3.00	N/A
NSP15	C19718T	ACA to ATA	T33I	1.78	N/A
NSP16	A21137G	AAG to AGG	K160R	0.39	N/A
	A21390G	TTA to TTG	R216C	2.77	N/A
N	C28932T	GCT to GTT	A220V	46.41	N/A
	G28580T	GAT to TAT	D103Y	0.13	N/A
	G28883C	GGA to CGA	G204R	29.55	Destabilizing N protein ⁴⁷
	C28311T	CCC to CTC	P13L	0.27	N/A
	C28869T	CCA to CTA	P199L	5.55	N/A
	GC28882AA	AGC to AAA	R203K	29.74	Destabilizing N protein ⁴⁷
	C28854T	TCA to TTA	S194L	4.65	Destabilizing N protein ⁴⁷
	C28977T	TCT to TTT	S235F	22.01	N/A
	GAT28282CTA	GAT to CTA	D3L	22.01	N/A
	G28975C	ATG to ATC	M234I	5.58	N/A
	C28472T	CCT to TCT	P67S	3.09	N/A
	G29399A	GCT to ACT	A376T	5.14	N/A
	C29366T	CCA to TCA	P365S	5.64	N/A
	G29402T	GAT to TAT	D377Y	1.29	N/A
	C28887T	ACT to ATT	T205I	1.47	N/A
	C29466T	GCA to GTA	A398V	0.78	N/A
M	A26530G	GAT to GGT	D3G	0.42	N/A
	C27046T	ACG to ATG	T175M	0.02	N/A
NS3	G25907T	GGT to GTT	G172V	2.98	N/A
	G25617T	AAG to AAT	K75N	0.39	N/A
	G25563T	CAG to CAT	Q57H	13.00	N/A
	C26060T	ACT to ATT	T223I	1.87	N/A
	G25429T	GTA to TTA	V13L	0.10	N/A
	G25996T	GTA to TTA	V202L	2.64	N/A
	A25505G	CAA to CGA	Q38R	2.46	N/A
	G25906C	GGT to CGT	G172R	2.32	N/A
NS7b	AT27866TA	CAT to CTA	H37L	0.058	N/A
	C27769T	TCA to TTA	S5L	1.68	N/A
NS8	C28087T	GCT to GTT	A65V	2.68	N/A
	T28144C	TTA to TCA	L84S	0.12	N/A
	C27964T	TCA to TTA	S24L	3.58	N/A
	G28077T	GTG to TTG	V62L	0.27	N/A
	C27972T	CAA to TAA	Q27stop	21.17	Inactivation of NS8 ²⁰
	G28048T	AGA to ATA	R52I	21.14	N/A

493

494 *Frequency of the mutation as of December 31, 2020.

495

Table 2: Non-synonymous, synonymous and deletion mutations in the B.1.1.7 variant

Variant	Gene	Genome Nucleotide Mutation ¹⁷	S or NS	AA mutation ¹⁷	Domain	Frequency* (%)	Effect
B.1.1.7	ORF1ab	C3267T	NS	T1001I		70.7	N/A
		C5388A	NS	A1708D		71.5	N/A
		T6954C	NS	I2230T		70	N/A
		11288-11296 del	NS	ΔS3675/ΔG3676/ΔF3677		69.2	N/A
		C913T	S			N/A	N/A
		C5986T	S			N/A	N/A
		C14676T	S			N/A	N/A
		C15279T	S			N/A	N/A
		C16176T	S			N/A	N/A
	Spike	21765- 21770	NS	ΔH69/ΔV70	NTD	70.8	Antibody escape ⁴³
		21991-21993	NS	ΔY144	NTD	0.01	Decrease infectivity, Antibody escape ¹⁹
		A23063T	NS	N501Y	RBD	71.9	Increase infectivity, virulence and affinity for hACE2 ⁴³
	ORF8	C23271A	NS	A570D	SD1	71.6	
		A23403G	NS	D614G	SD2	99.4	Moderate increase transmissibility ⁴³
		C23709A	NS	P681H	SD2	72.7	N/A
		C23709T	NS	T716I		72.1	N/A
		T24506G	NS	S982A	HR1	71.3	N/A
		G24914C	NS	D1118H		71.2	N/A
		C27972T	NS	Q27stop		71.2	Inactivation of NS8 ²⁰
		G28048T	NS	R52I		71	N/A
		A28111G	NS	Y73C		71.3	N/A
		T26801C	S			N/A	N/A
	M N	GAT28280CT A	NS	D3L		70.5	N/A
		C28977T	NS	S235F		71.4	N/A

*Frequency of the mutation as of February 17th 2021.

Table 3: Non-synonymous mutations and deletions in the B.1.351 variant

Variant	Gene	Genome Nucleotide Mutation ¹	A.A mutation ¹⁹	Domain	Frequency * (%)	Effect
B.1.351	<i>ORF1ab</i>	C1059T	T265I		7.3	N/A
		G5230T	K1655N		0.57	N/A
		A10323G	K3353R		1.7	N/A
	<i>Spike</i>	C21614T	L18F	NTD	4.5	N/A
		A21801C	D80A	NTD	0.47	N/A
		A22206G	D215G	NTD	0.51	N/A
		22286-22294	ΔL242/ΔA243/ΔL244	NTD	0.311	N/A
		G22299T	R246I	NTD	0	N/A
		G22813T	K417N	RBD	0.50	Antibody escape ²⁴
		G23012A	E484K	RBD	1.74	Antibody escape ⁴³
		A23063T	N501Y	RBD	71.9	Increase affinity for hACE2 ⁴³
		A23403G	D614G	SD2	99.4	Moderate increase in transmissibility ⁴³
		G23664T	A701V	S1/S2 – S2'	1.40	N/A
	<i>ORF3a</i>	G25563T	Q57H		10.2	N/A
		C25904T	S171L		1.29	N/A
	<i>E</i>	C26456T	P71L		0.55	N/A
	<i>N</i>	C28887T	T205I		2.5	N/A

*Frequency of the mutation as of February 17th 2021.

Table 4: Non-synonymous, synonymous and deletions in the P.1 variant.

Variant	Gene	Genome Nucleotide Mutation	S or NS	A.A mutation	Domain	Frequency* (%)	Effect
P.1	ORF1ab	T733C	S			N/A	N/A
		C2749T	S			N/A	N/A
		C3828T	NS	S1188L		0.22	N/A
		A5648C	NS	K1795Q		0.19	N/A
		11288-11296 del	NS	ΔS3675/ ΔG3676/ΔF3677		71.1	N/A
		C12778T	S			N/A	N/A
		C13860T	S			N/A	N/A
		G17259T	NS	E5665D		0.24	N/A
	Spike	C21614T	NS	L18F	NTD	4.5	N/A
		C21621A	NS	T20N	NTD	0.30	N/A
		C21638T	NS	P26S	NTD	0.44	N/A
		G21974T	NS	D138Y	NTD	0.28	N/A
		G22132T	NS	R190S	NTD	0.73	N/A
		A22812C	NS	K417T	RBD	0.11	N/A
		G23012A	NS	E484K	RBD	1.7	Antibody escape ⁴³
		A23063T	NS	N501Y	RBD	71.9	Increase affinity for hACE2 ⁴³
		A23403G	NS	D614G	SD2	99.4	Moderate increase in transmissibility ⁴³
		C23525T	NS	H655Y	SD2	0.25	N/A
		C24642T	NS	T1027I	S2	0.20	N/A
		ORF8	G28167A	NS	E92K		0.30
Ins28269-28273	S				N/A	N/A	
N		C28512G	NS	P80R		0.18	N/A

*Frequency of the mutation as of February 17th 2021.

517 **Table 5:** Efficacy of vaccines against SARS-CoV-2 variants.

Vaccine	hCoV-19/Wuhan/WIV-4/2019	B.1.1.7	B.1.351	P.1
Pfizer-BioNTech	95.0% ³⁸	~ 95.0% ³⁰	2/3 reduction in neutralization ³⁶	15.1-fold decrease ³⁷
Moderna	94.1% ³⁹	~ 94.1% ³⁴	6.4 reduction in ³⁴ titers	N/A
Oxford-AstraZeneca	70.4% ⁴⁰	74.6% ³³	21.9% ³⁵	N/A

518

519

FIGURE LEGENDS

Figure 1: Variation of mutation frequency in SARS-CoV-2 genes. The occurrence and frequency of mutations in various SARS-CoV-2 genes are presented for the time period between December 2019 to December 2020. SARS-CoV-2 genes are represented with non-structural proteins (NSPs) and the function on the genes in parentheses. Graphs were generated using RStudio.

Figure 2: Geographic location and timeline of dominant mutations in NSP12, S, and N genes. A) Frequency of S protein mutations with corresponding geographic maps. B) Frequency of RdRp mutations with corresponding geographic maps. C) Frequency of Nucleoprotein mutations with corresponding geographic maps. D) Mutations reaching a frequency higher than 50% between December 2019 and December 2020. A low frequency of reported cases of the mutations is represented in white, while higher frequencies are represented in red. All maps were taken from GISAID. Graphs were generated using RStudio and Biorender.

Figure 3: Structural rendering of most frequent mutation in the S protein. A) Surface representation of hACE2 (yellow) in complex with S protein trimers illustrated in grey, blue, and magenta. Recurrent mutations are represented in green. Cartoon representation of B) A222V, C) S477N, D) L18F, E) D614G in open conformation and F) D614G in closed conformation. Reference sequence residues are illustrated in green, and the mutant amino acid is represented in purple. The red circles illustrate the steric clash when the mutations are inserted into the structure. Graphs were generated using PyMOL.

Figure 4: Frequency of B.1.1.7, B.1.351, P.1, D614G, and reference variants. Database variants frequency were analyzed from December 2019, to February 17th, 2021. The hCoV-19/Wuhan is the reference strain, and hCoV-19/D614G is representing the D614G mutation in the S protein. B.1.1.7, B.1.351, and P.1 represent the UK, South African, and Brazilians variants. *(overlapping curves).

Figure 5: Frequency of B.1.1.7 spike protein mutations with structural confirmation and genome map. A & B) Colour representation of S protein subdomains with mutations of the B.1.1.7 variant in red. NTD (green), RBD (blue), SD1 (purple), SD2 (light blue), and S2 (magenta) are illustrated. The other S protein monomers are displayed in grey and white. C) Frequency of the mutations in the S protein,

B.1.1.7 variant from December 2019 to February 17th 2021. D) Interaction of the N501Y (red) mutation in the RBD (blue) of S protein with hACE2 (yellow). The dash lines indicate interactions with adjacent residues. E) Genome of the SARS-CoV-2 B.1.1.7 variant with identified nucleotide substitutions and deletions. Graphs were generated using Biorender, PyMOL, and RStudio. *(overlapping curves).

Figure 6: Frequency of B.1.351 spike protein mutations with structural confirmation and genome map. A & B) Colour representation of S protein subdomains with mutations of the B.1.351 variant in orange. NTD (green), RBD (blue), SD1 (purple), SD2 (light blue), and S2 (magenta) are illustrated. The other S protein monomers are illustrated in grey and white. C) Frequency of the mutations in the S protein B.1.351 variant from December 2019 to February 17th 2021. Interaction of D) 417N with C102 Nab (green), E) 484K with C121 Nab (light pink), and F) 501Y with hACE2 (yellow). The mutant residues are illustrated in orange, and the dashed lines represent interactions with adjacent residues. G) Genome of the SARS-CoV-2 B.1.351 variant with identified nucleotide substitutions or deletions. Graphs were generated using Biorender, PyMOL, and RStudio. *(overlapping curves).

Figure 7: Frequency of P.1 spike protein mutations with structural confirmation and genome map. A& B) Colour representation of S protein subdomains with mutations of the P.1 variant in black. NTD (green), RBD (blue), SD1 (purple), SD2 (light blue), and S2 (magenta) are illustrated. The other S protein monomers are illustrated in grey and white. C) Frequency of P.1 variant S protein mutations from December 2019 to February 17th 2021. Interaction of D) 417T with C102 Nab (green), E) 484K with C121 Nab (light pink), and F) 501Y with hACE2 (yellow). The mutations are coloured in black and interaction with adjacent residues are demonstrated by dashed lines. G) Genome of the SARS-CoV-2 P.1 variant with identified nucleotides substitution, deletions, and insertions. Figures were generated using Biorender, PyMOL and RStudio. *(overlapping curves).

Figure 8: Interactions of K417, E484, and N501 of the S protein with neutralizing antibodies and hACE2. A) Interaction of K417 (blue) with C102 Nab (green) residues. B) Interaction of E484 (blue) with C121 Nab (light pink). C) Interaction of N501 (blue) with hACE2 (yellow) Dashes lines indicate interactions between residues. The graphs were generated using PyMOL.

Figure 1

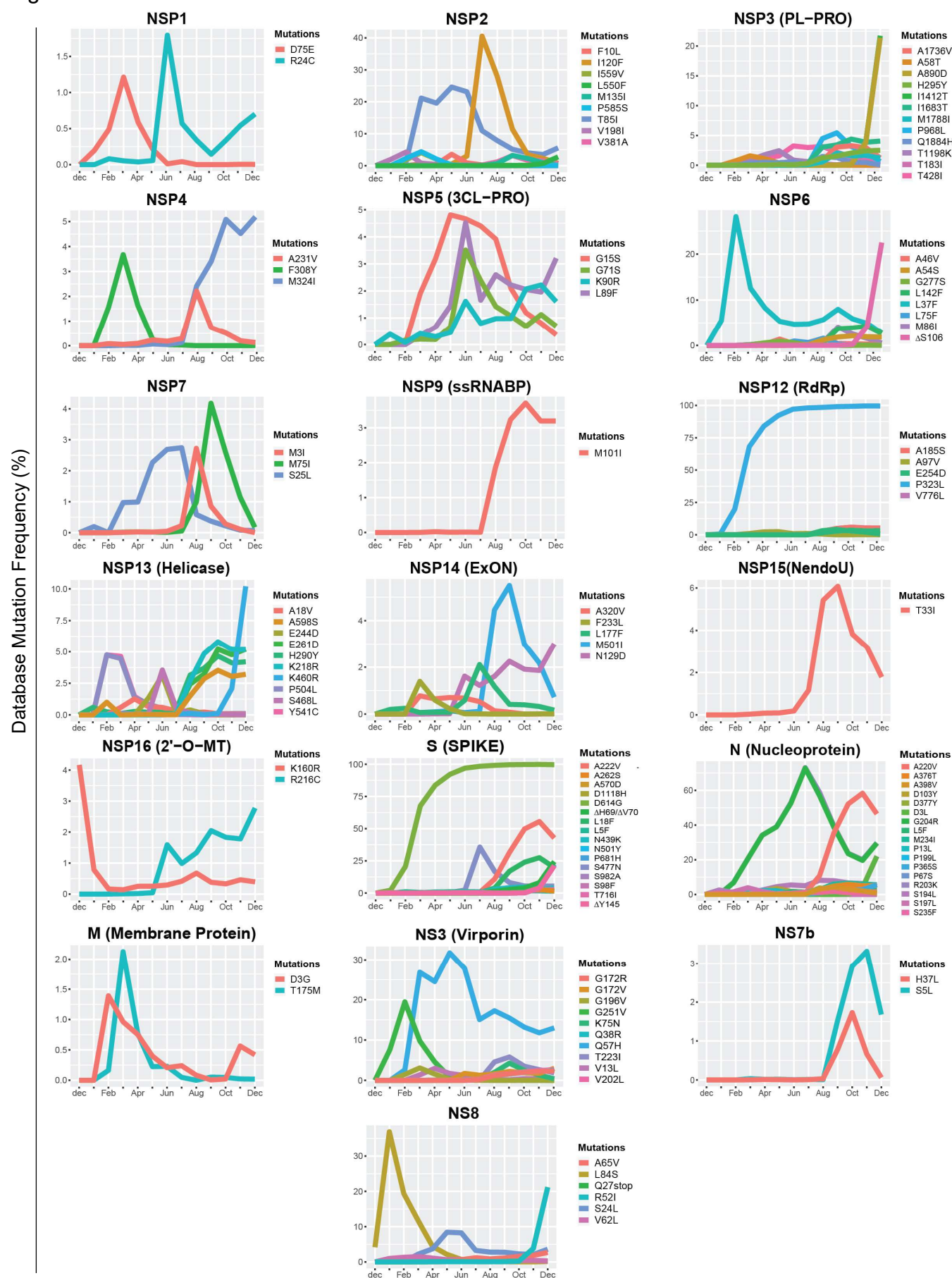


Figure 2

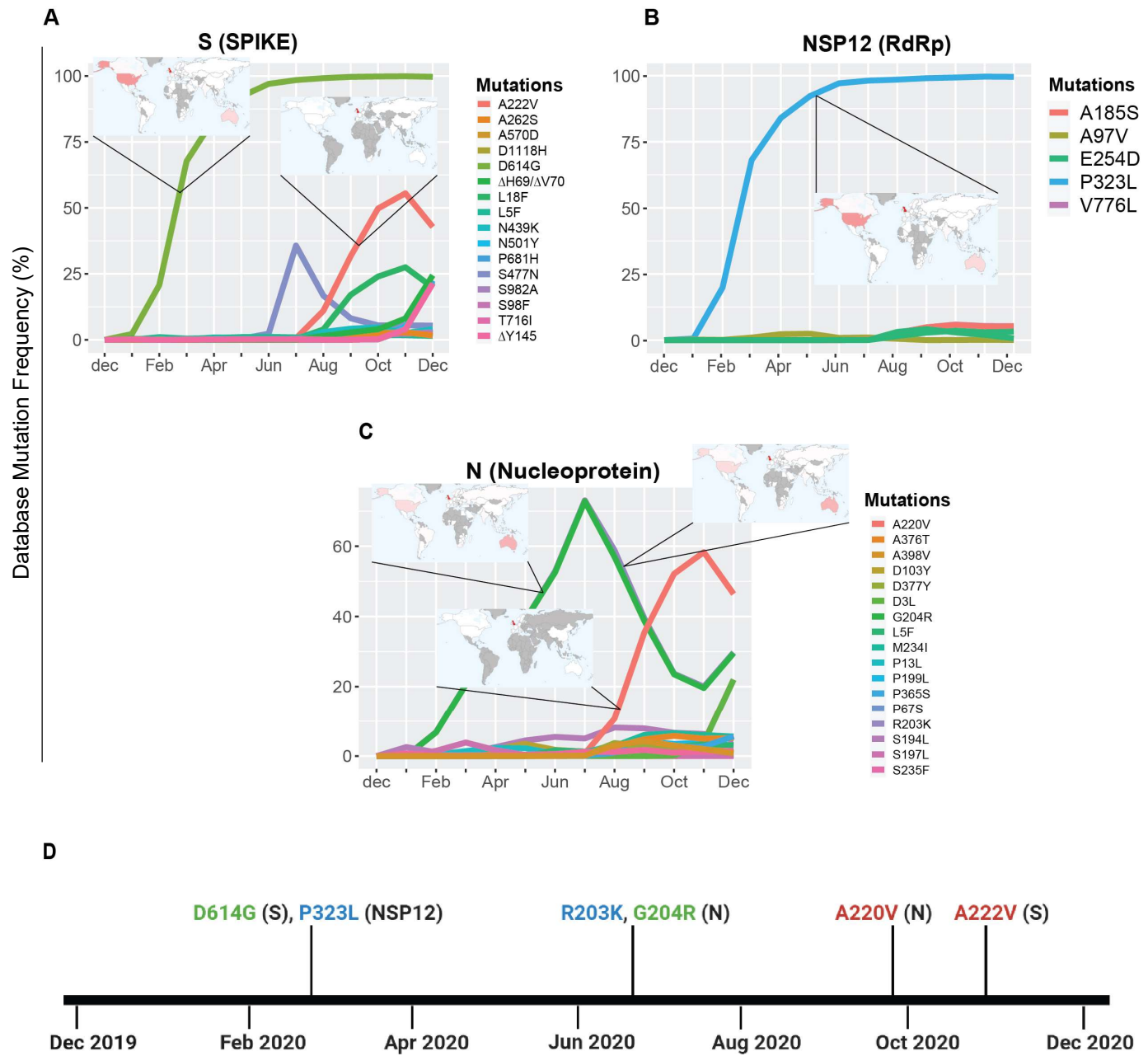


Figure 3

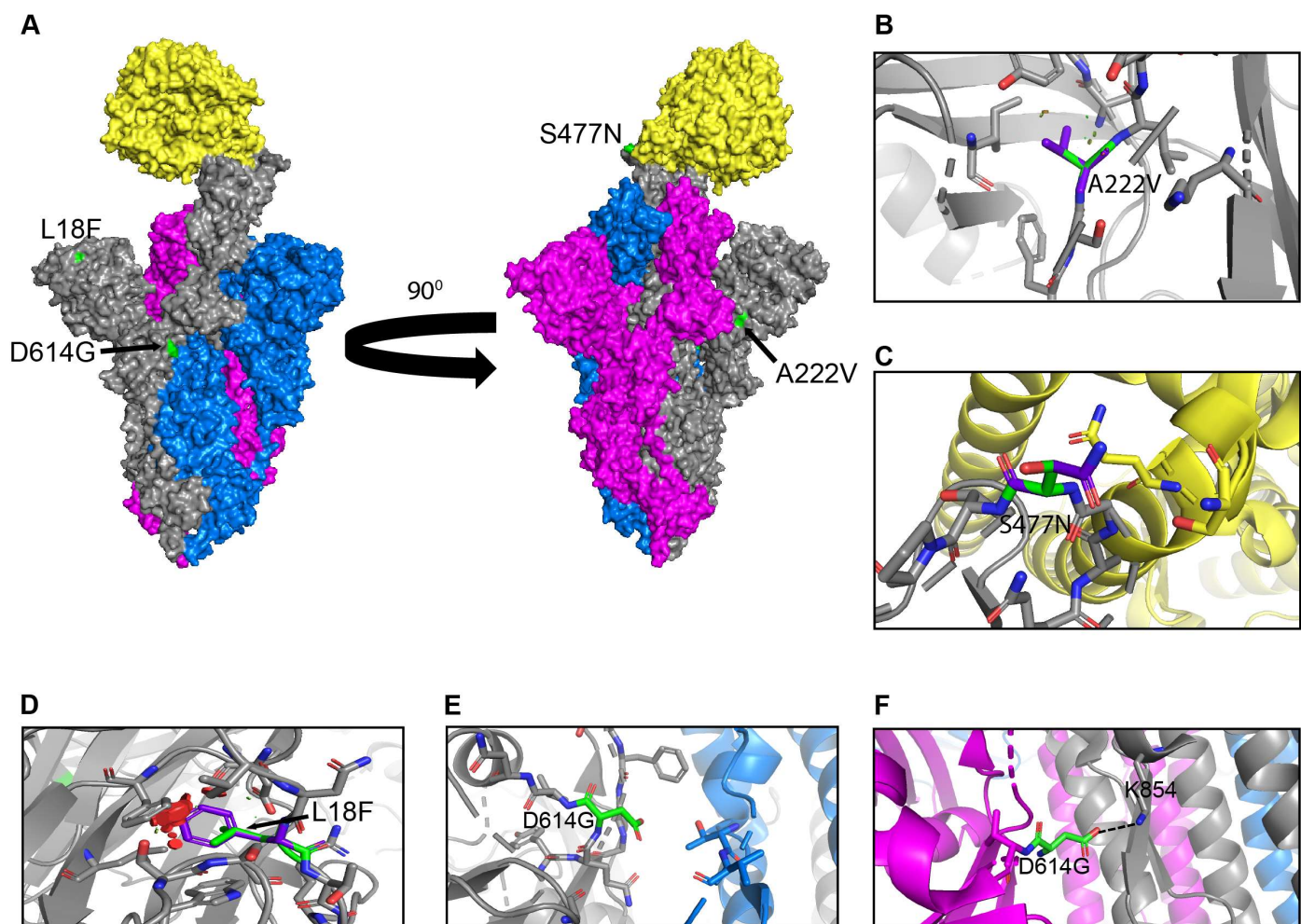


Figure 4

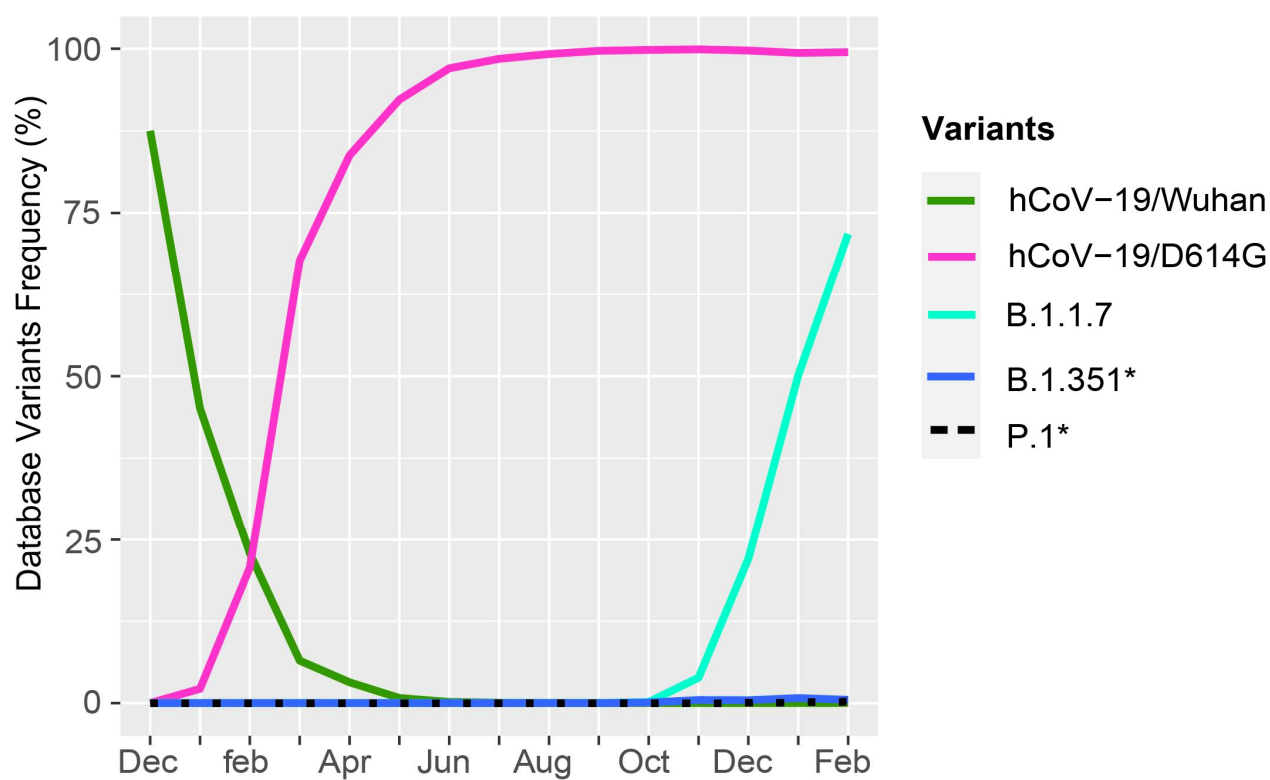


Figure 6

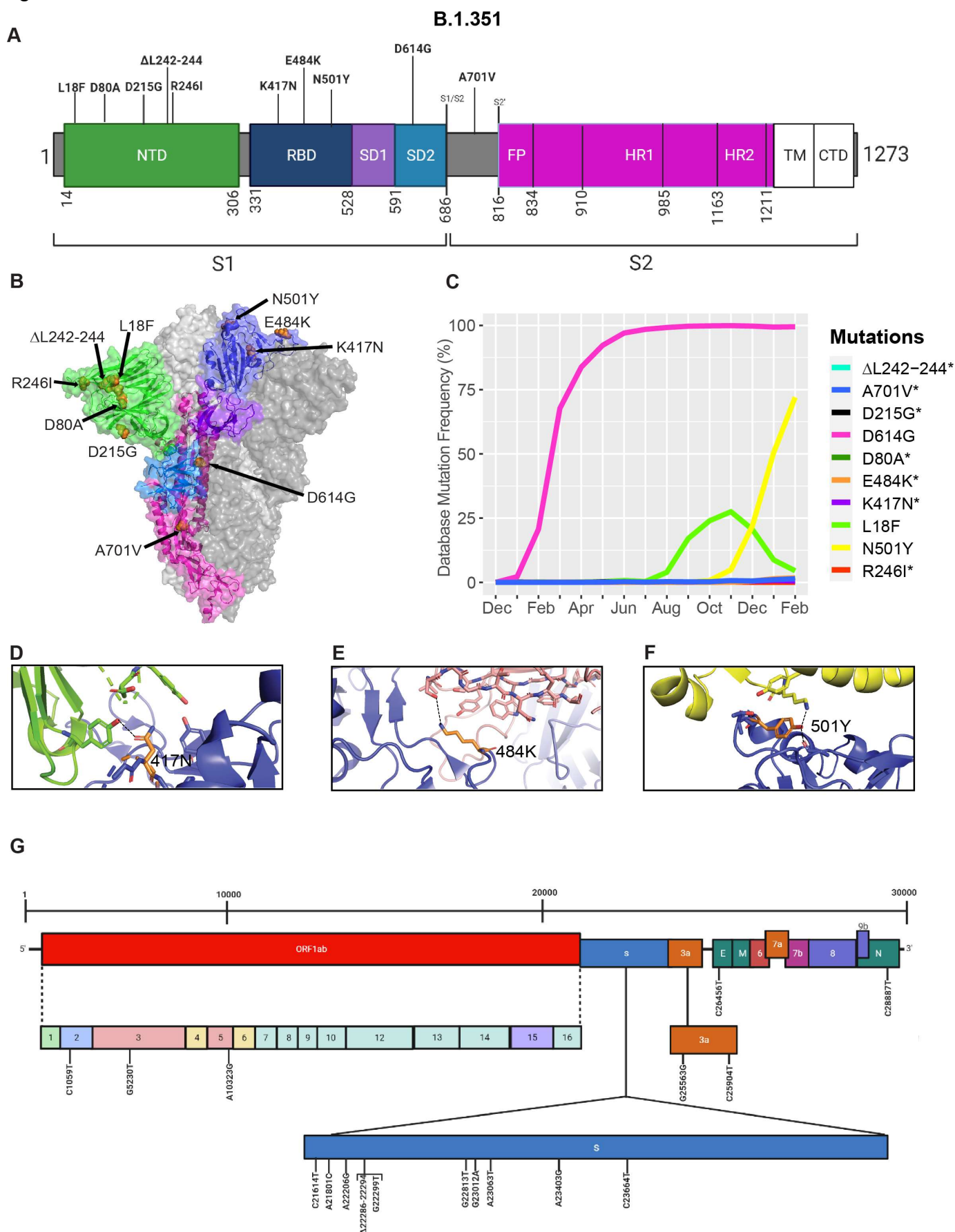


Figure 7

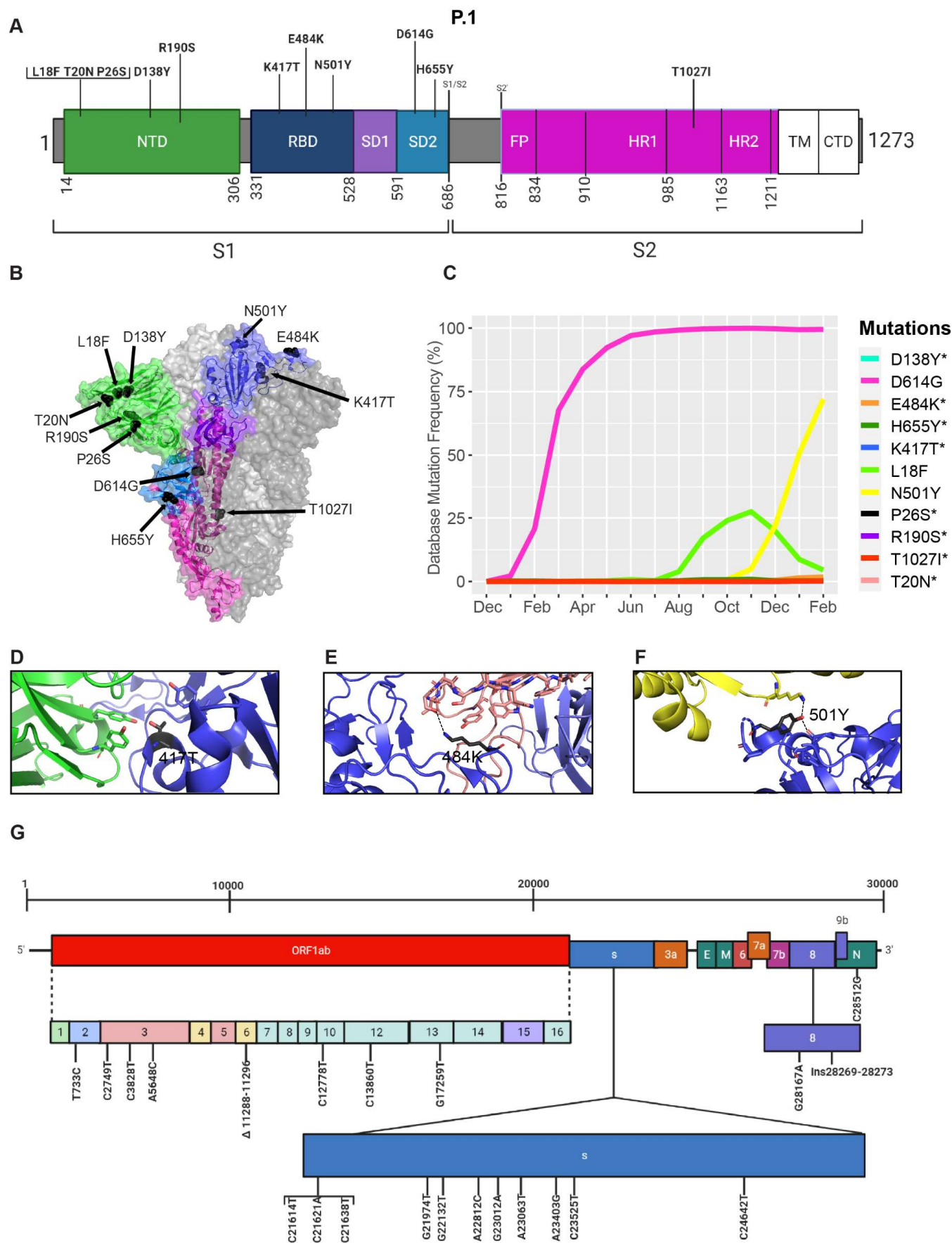


Figure 8

