A comprehensive evolutionary and epidemiological characterization of insertion and deletion mutations in SARS-CoV-2 genomes

Xue Liu,† Liping Guo, Tiefeng Xu, Xiaoyu Lu, Mingpeng Ma, Wenyu Sheng, Yinxia Wu, Hong Peng, Liu Cao, Fuxiang Zheng, Siyao Huang, Zixiao Yang, Jie Du, Mang Shi,*‡ and Deyin Guo*§

Centre for Infection and Immunity Study (CIIS), School of Medicine (Shenzhen), Shenzhen Campus of Sun Yat-sen University, Sun Yat-sen University, Shenzhen, Guangdong 518107, China

†https://orcid.org/0000-0001-9195-5408
‡https://orcid.org/0000-0002-6154-4437
§https://orcid.org/0000-0002-8297-0814

*Corresponding authors: E-mail: shim23@mail.sysu.edu.cn; guodeyin@mail.sysu.edu.cn

Abstract

SARS-CoV-2, which causes the current pandemic of respiratory illness, is evolving continuously and generating new variants. Nevertheless, most of the sequence analyses thus far focused on nucleotide substitutions despite the fact that insertions and deletions (indels) are equally important in the evolution of SARS-CoV-2. In this study, we analyzed 1,099,664 high-quality sequences of SARS-CoV-2 genomes to re-construct the evolutionary and epidemiological histories of indels. Our analysis revealed 289 circulating indel types (237 deletion and 52 insertion types, each represented by more than ten genomic sequences), among which eighteen were recurrent indel types, each represented by more than 500 genome sequences. Although indels were identified across the entire genome, most of them were identified in nsp6, S, ORF8, and N genes, among which ORF8 indel types had the highest frequencies of frameshift. Geographical and temporal analyses of these variants revealed a few alterations of dominant indel types, each accompanied by geographic expansion to different countries and continents, which resulted in the fixation of several types of indels in the field, including the current variants of concern. Evolutionary and structural analyses revealed that indels involving S N-terminal domain regions were linked to the 3/4 variants of concern, resulting in significantly altered S protein that might contribute to the selective advantage of the corresponding variant. In sum, our study highlights the important role of insertions and deletions in the evolution and spread of SARS-CoV-2.

Key words: SARS-CoV-2; deletions; insertions; evolution; molecular epidemiology

1. Introduction

A new type of betacoronavirus causing severe respiratory disease was identified in December 2019 (Zhou et al. 2020), which was later officially named as SARS-CoV-2 by the International Committee on Taxonomy of Viruses (NC_045512), and the disease it causes was named as coronavirus disease 2019 (COVID-19) by the World Health Organization (WHO) (Gorbalenya et al. 2020). The virus has since spread rapidly across the globe, causing recurrent epidemics in many countries and regions around the world. As of 24 September 2021, SARS-CoV-2 was circulating in 223 countries or regions, with more than 233,278,752 cases and 4,774,507 deaths reported thus far (Dong, Du, and Gardner 2020).

Like other ssRNA(+) viruses, SARS-CoV-2 is prone to genomic variation, including the substitution, insertions, and deletions. Substitutions have been intensively studied in relation to changes in the structure and/or function of the viral proteins, which in turn result in altered virulence, antigenic properties, or transmissibility of the virus (Hou et al. 2020; Plante et al. 2020). Based on substitutions, viruses were divided into more than 1,593 Pango lineages with shared sequence identity, phylogenetic relationships, and temporal and geographic structure (Rambaut et al. 2020). Several lineages defining substitutions N501Y and E484K cause amino acid changes that strengthened the binding of the receptor binding domain of S to the ACE2 receptor, making the variants 70 per cent more contagious than the predecessor lineage (Davies et al. 2021; Khan et al. 2021). Furthermore, among these lineages, WHO identified, based on transmissibility, pathogenicity and the impact on vaccines, several SARS-CoV-2 variants of concern (VOCs), including Alpha (B.1.1.7), Beta (B.1.351), Gamma (P.1), and Delta (B.1.617.2), and variants of interest (VOIs), including Epsilon (B.1.429 + B.1.427), Zeta (P.2), Eta (B.1.525), Theta (P.3), Iota (B.1.526), Kappa (B.1.617.1), Lambda (C.37), and Mu (B.1.621).
Nevertheless, despite intensive research on substitution, the role of insertions and deletions (indels) has not been systematically investigated. A few indels were identified within the VOCs. For example, B.1.1.7 strain contains two S protein deletions (del69-70HV and del145Y) in the N-terminal domain (NTD) (Shen et al. 2021); B.1.351 contains del242–244 in the S protein NTD domain; and B.1.1.7 and B.1.351 contained del145Y and del242–244, respectively, that render resistance to most NTD-directed monoclonal antibodies by previous strains (Wang et al. 2021b). Other reported indels included nsp1 del214-243 (Benedetti et al. 2020), nsp2 del268 (Bal et al. 2020), ORF6 34-nt deletion (Quèrromès et al. 2021), and ORF7a 81-nt deletion (Holland et al. 2020), and, amongst others, most of these indels resulted in virus progenies that have been spread to other patients. Interestingly, the largest indel reported so far is the 382-nucleotide deletion (∆382) in the ORF8 region, which appeared in Singapore in January and February of 2020 (Su et al. 2020), which terminated the translation of ORF8 at positions 28,229. It may result in a milder infection, which makes it suitable for design of attenuated vaccine (Young et al. 2020; Zinzula 2021). There are also reports that the del675-679 in S protein may restrict virus replication in Vero cells at the late phase (Liu et al. 2020).

Importantly, rapid genome sequencing and online sharing of SARS-CoV-2 genomes by public health and research teams worldwide had provided us invaluable insights into the ongoing evolution and epidemiology of the virus, as well as the global variations during the pandemic, and thus played an important role in virus surveillance and its eventual mitigation and control. The Global Initiative on Sharing All Influenza Data (GISAID) (Shu and McCauley 2017) database contains a large number of COVID-19 genome sequences, which make it possible to analyze and trace the sequence variation and evolution of SARS-CoV-2 on a global scale such that any variants with altered pathogenicity or antigenic properties can be promptly identified.

In order to systematically characterize the indels of SARS-CoV-2, we performed a genome-wide indels analyses on 1,031,249 complete-genome sequences of the SARS-CoV-2 collected from more than 166 countries or regions. Our analyses revealed the frequencies, genome distributions, and molecular characteristics of all indels that are circulating in the field. For highly prevalent indels, we further characterized the temporal and spatial dynamics and evolutionary histories of the corresponding variants. And structure and functional impacts of relevant proteins were subsequently evaluated.

**Figure 1.** Overview of indels within global SARS-CoV-2 genomes. The number of deletion (A) and insertion types (B) and the proportion of indels causing frameshift were described. (C) Distribution of deletions and insertions on different SARS-CoV-2 proteins. The N represents the number of indels sequences.
2. Results

2.1 A general characterization of circulating SARS-CoV-2 genomic indels

Compared to the prototype strain, a total of 3,854 types of deletions and 891 types of insertions were detected among the 1,031,249 SARS-CoV-2 genome sequences (Tables S1 and S2). Among these, only 237 deletion types and 52 insertion types were regarded as ‘circulating’ genomic variant based on our criterion for ‘circulating’ indels that exist in more than ten genomic sequences. And the numbers were reduced to seventy deletion types and twenty insertion types if we set the threshold of detection frequencies higher at 50 (Fig. 1). Among the 237 types of deletions, 34.18 per cent (81/237) caused frameshift and 65.82 per cent (156/237) did not (Fig. 1A). On the other hand, a total of twenty-five frameshifts (48.08 per cent) and twenty-seven non-frameshift (51.92 per cent) were observed in the fifty-two insertions types (Fig. 1B). Generally, insertion occurs less frequently than deletion, with the ratio of deletion:insertion as 31.33:1, and the ratio of non-frameshift indels:frameshift indels as 3.89:1. The frameshift frequency of deletion mutation in the genes coding for accessory proteins (37.97 per cent) is significantly higher than that of non-structural proteins (0.3 per cent) and structural proteins (0.28 per cent). The latter genes are essential for viral propagation, and the indels detected in these regions may represent sequencing errors and dead-end genomic products. Strikingly, the sequence counts for frameshift indels in ORF8 were one or two orders of magnitude higher than other protein-coding genes (Fig. 1C).

2.2 Genome-wide diversity of indels

We further characterized the distribution of indels on SARS-CoV-2 genomes with the exception of 5′ and 3′ UTR as these regions are prone to have sequencing errors (Fig. 2). For deletions, circulating forms were detected in all protein-coding genes with the exception of non-structural proteins nsp7, nsp10, and nsp11, which contained no deletion. On the other hand, higher frequencies of deletions were detected in structural genes encoding the spike protein (n = 32), N protein (n = 18), as well as non-structural protein genes, such as nsp3 (n = 29), and accessory genes ORF7a

Figure 2. The distribution of indels on SARS-CoV-2 genome. The sequence number (outer plot) and length (inner plot) of deletion (A) and insertion (B) types were described. The histograms above the outer plots show the frequencies of indel types along the entire genome. The deletion types associated with each gene is marked with different colors.
Table 1. Recurrent deletion or insertion types (RDT or RIT) for SARS-CoV-2.

| Name         | Region | Start position and indels nucleotides | Indels of nucleotides | Frequency |
|--------------|--------|---------------------------------------|-----------------------|-----------|
| RDT-nspl     | nsp1   | pos_686-9                             | AAGTCATT    | 1,771     |
| RDT-nspl-1   | nsp2   | pos_1598-6                            | GGTCTT      | 528       |
| RDT-nspl-2   | nsp2   | pos_1605-3                            | ATG         | 854       |
| RDT-nspl6    | nspl6  | pos_11288-9                           | TCTGGTTTT   | 228,125   |
| RDT-S-1      | S      | pos_21765-6                           | TACATG      | 220,758   |
| RDT-S-2      | S      | pos_21991-3                           | TTA         | 126,048   |
| RDT-S-3      | S      | pos_22029-6                           | AGTTCA      | 3,931     |
| RDT-S-4      | S      | pos_22189-3                           | TAT         | 556       |
| RDT-S-5      | S      | pos_22281-9                           | CTTIACTTG   | 887       |
| RDT-ORF8/F   | ORF8   | pos_26155-3                           | GTT         | 631       |
| RDT-ORF8     | ORF8   | pos_27205-3                           | TTT         | 1,014     |
| RDT-ORF8/N   | IGR8/n | pos_28271-1                           | A           | 865       |
| RDT-ORF8/N   | IGR8/n | pos_28278-3                           | A           | 127,020   |
| RDT-ORF8/N   | IGR8/n | pos_28280-6                           | CTG         | 1,201     |
| RDT-ORF8/N   | IGR8/n | pos_28286-7                           | TTTCCG      | 930       |
| RDT-ORF8/N   | IGR8/n | pos_28285-2                           | TG          | 13,744    |
| RIT-ORF8     | ORF8   | pos_28255-2                           | TC          | 861       |
| RIT-ORF8/N   | IGR8/n | pos_28263-4                           | AACA        | 5,582     |

Note: IGR8/n, the intergenic region between ORF8 and N gene; *the number following the ‘+’ and ‘+’ signs indicates the number of deleted and inserted nucleotides, respectively.

(n = 27), ORF8 (n = 26), and ORF3a (n = 22) (Fig. 2A). Deletions with the most successful epidemiological outcome were detected in nspl pos_11288-9 (22.12 per cent of 1,031,249 sequences), S pos_21765-6 (21.41 per cent), S pos_21991-3 (12.22 per cent), and intergenic region IGR8/n pos_28271-1 (12.32 per cent). The most common lengths for deletions are 3 nt (36.71 per cent), 1 nt (18.14 per cent), 6 nt (11.81 per cent), 9 nt (10.55 per cent), and 2 nt (5.06 per cent). The 1-nt and 2-nt indels were mostly detected in the non-coding regions and accessory proteins, which do not disrupt the translation of viral proteins essential for viral replication. Interestingly, our data also revealed a number of large-fragment deletions (>50 nt in length), which is mainly identified in the genes encoding accessory proteins ORF7a and ORF8 (Fig. 2A).

As for insertions, they were discovered in thirteen genes, with most types discovered in ORF8 gene (n = 8), followed by spike gene (n = 7), and nsp3 gene (n = 6) (Fig. 2B). The three types of insertions with most genome sequences were all associated with ORF8, including pos_28252+2 (1.33 per cent), ORF8 pos_28255+2 (0.08 per cent), and IGR8/n pos_28263+4 (0.54 per cent). And the most common insertion length included 3 nt (32.69 per cent), 1 nt (19.23 per cent), and 2 nt (15.38 per cent).

2.3 Temporal and spatial dynamics of highly prevalent genomic variants of SARS-CoV-2

We characterized the molecular epidemiological features of fifteen deletion and three insertion types with more than 500 sequences (Tables S1 and S2), which we named as recurrent deletion or insertion types (RDT or RIT) (Table 1). Interestingly, indel types with the highest frequencies included pos_11288-9 (n = 228,125), pos_21765-6 (n = 220,758), pos_21991-3 (n = 126,048), and pos_28271-1 (n = 127,020), located in nspl, S, and IGR8/n regions, respectively.

To reveal epidemiological dynamics, we mapped the distributions of RDT or RIT through time and across different geographical locations (Fig. 3). For RDTs, the earliest occurrence (i.e. RDT-nspl) appeared in the USA and Canada in January 2020, but its abundance level remained low (<0.5 per cent) since then (Fig. 3A). Between January and October 2020, other eleven earlier RDTs began to emerge, most of which had spread to multiple countries and continents. And among these, the RDT-nspl appeared in thirty-three countries, reaching 1.7 per cent of total sequences in March 2020. Nevertheless, these RDTs all disappeared or diminished significantly in numbers by the end of 2020. Indeed, they were gradually replaced by the RDT-nspl, RDT-S-1, RDT-S-2 and RDT-ORF8/N, which emerged in Feb 2020, appearing in Netherlands and Portugal initially and later spreading to more than eighty countries to become the dominant (>10 per cent) types in the field (Fig. 3A, Fig. S1). As of 29 May 2021, all six continents contained these four variants, with the most abundant type, namely RDT-nspl, reaching 22 per cent of total sequences.

As for RITs, the earliest type, namely RIT-ORF8-1, appeared in April 2020 in Spain and USA and later spread to forty-six countries and six continents, with the highest prevalence recorded in Mar 2021 (Fig. 3B). Other two types, RIT-ORF8/N and RIT-ORF8-2, appeared in October 2020 and November 2020, respectively. In the field, RIT-ORF8-1 remained the most dominant insertion type until May 2021, when the proportion of RIT-ORF8/N (2.4 per cent) exceeded that of RIT-ORF8-1 (1.5 per cent). As of 29 May 2021, RIT-ORF8/N had spread to thirty-three countries and five continents, and its population was still expanding (Fig. S2).

2.4 Phylogenetic analysis of SARS-CoV-2 genomes based on indel mutations

We further analyzed the evolutionary history of RDTs and RITs by mapping them onto a phylogenetic tree that described the major circulating lineages (Fig. 4). Generally, there were strong associations between the circulating lineages defined by nucleotide substitutions and recurrent indel variants (Fig. 4). For example, eight RDTs and two RITs were associated with B.1.1.7 (Alpha variant) (Fig. 4), which was also labeled as a VOC by WHO. The other VOCs contained one or two RDTs or RITs, among which RDT-S-1, RDT-S-2 were identified in B.1.1.7 (Alpha variant), RDT-S-3 were identified in B.1.617.2 (Delta variant), and RDT-S-5 were identified in B.1.351 (Beta variant). Interestingly, majority of these RDTs or RITs identified in VOCs were associated with S proteins. On the other hand, among the five VOIs registered by WHO, only one...
Figure 3. Temporal and spatial dynamics of the RDTs and RITs. The temporal (upper panel) and geographic (lower panel) distribution of RDTs (A) and RITs (B). For temporal distribution, the sequence counts of RDT and RIT are normalized against total sequences counts in each month. For geographic distributions, the size of the circle/pie chart is proportional to the log10(N + 1) transformation of the total sequence count and, therefore, reflects the size of sampling. For clarity, the geographic distributions are shown for January (2020), April (2020), October (2020), January (2021), and May (2021) months, whereas a more complete temporal and geographic change can be found in Figures S1 and S2 for RDT and RIT, respectively.
Virus Evolution

Figure 4. Distribution of RDT and RIT in Pango lineage. The phylogenetic tree is constructed using the nextstrain augur pipeline and based on a set of reference sequences downloaded from GISAID. For the definition of variants of concern (red) and variants of interest (CornflowerBlue), please refer to the official website of WHO (https://www.who.int/en/activities/tracking-SARS-CoV-2-variants/). The number of RDTs and RITs shown in the heatmap are transformed using (log(N+1)).

(i.e. B.1.525 (Eta)) contained recurrent indel variants, which are RDT-ORF6 and RDT-N, located in ORF6 and N genes, respectively (Fig. 4). For the rest of lineages, we identified indel types in S, namely RDT-S-4, pos_22205+9, and pos_22206+9, within B.1.36, B.1.214.2, and A.2.5, respectively. Among these, pos_22205+9 and pos_22206+9 were not defined as RIT because their associated sequences, 305 and 348, respectively, were below the 500 threshold. Interestingly, more than half of the RDT and RIT defined in this study appeared with more than one occurrence, in multiple and paraphyletic lineages (Fig. 4, Table S3), suggesting multiple and independent occurrence of these indel types.

2.5 Structural modeling of spike glycoprotein with recurrent indels

We next evaluated the impact of major types of indels on Spike protein structure and function. Specifically, sequence comparisons revealed that RDT-S-1, RDT-S-2, RDT-S-4, and RDT-S-5 caused the deletion of 69–70HV, 145Y, 210I, and 241–243LLA from S proteins, RDT-S-3 caused the replacement of EFR with G at 156–158aa, and pos_22289-6 caused deletion at 242–243ALA and pos_22205+9 and pos_22206+9 caused insertions at 215 (TDR) and 215 (AAGY) (Fig. 5A). Specifically, the del210I caused the loss of 2.5 Å of hydrogen bonds between I210 and F186 (Fig. 5B4); the del241-243LLA caused the loss of 2.7 Å, 2.7 Å, and 2.8 Å between L241, A243, and G103, T240, and I100 (Fig. 5B5); the insertion of ins215TDR caused 215-TDR217 to form 3.1 Å, 2.8 Å, 3.3 Å, 3.5 Å, and 3.2 Å hydrogen bonds with Y269, T29, and Y91 (Fig. 5B7), and ins215AAGY causes Y218 to form 3.1 Å, 2.8 Å, and 3.0 Å of hydrogen bonds with Y91, T95, and A267 (Fig. 5B8). Importantly, these deletions and insertions of spike glycoprotein may impede the function of the loop region and the N-terminal and C-terminal ends of the β-pleated sheet.

3. Discussion

Our study examined 1,031,249 complete-genome sequences of SARS-CoV-2 collected from across the world and revealed a remarkable number of indels across the entire genome of the virus. Our result demonstrated that insertions and deletions, like nucleotide substitutions, are important driving forces that contribute to the diversity of SARS-CoV2 viruses, some of which have selective advantages such that they were later fixed and became dominant types in the field (Aleem, Akbar Samad, and Slenker 2021). For example, it has been demonstrated that the deletion RDT-S-1 in Alpha variant B.1.1.7 resulted in increased spike infectivity (Meng et al. 2021). The deletion RDT-S-2 in Alpha variant B.1.1.7 resulted in increased spike escape neutralization mediated by mAbs targeting the antigenic supersite (Zost et al. 2020; McCallum et al. 2021). On the other hand, the most dominant variant currently circulating in the field (>35.36 per cent of sequences as of 29 May 2021) with a significantly higher R0 (Liu and Rocklov 2021), namely Delta variant (B.1.617.2), contained a recurrent deletion type RDT-S-3 located at S protein and resulted in immune
Figure 5. Structural analysis of spike glycoprotein with recurrent indels. (A) The multiple sequences alignments display of RDT-S-1, RDT-S-2, RDT-S-3, RDT-S-4, RDT-S-5, pos_22289-6, pos_22205+9, pos_22206+9. (B) Tertiary structure of the recurrent indels of spike glycoprotein. B1–B8 are different S protein NTD indels tertiary structure align with template. B1–B8 align with template PDB: 7CWU (green). Deletion or insertion area (red), amino acid change region (pink), corresponding normal area by amino acid change region (blue).
that it increases S1/S2 cleavage and results in higher spike infectivity. One study that focused on the H69/V70 deletion of the Alpha variant revealed within S protein might cause increase in infectivity. One study that renders selective advantage of the virus is that the indels that were resulted from sequencing artifacts, it is possible that some of the circulating type of indels are due to sequencing errors (i.e. the frameshift indels of ORF1ab), although the number of such occurrence is most likely very low.

4. Material and methods
4.1 Data collection and processing
As of 29 May 2021, a total of 1,099,664 high-quality SARS-CoV-2 genome sequences were downloaded from the GISAID website (Shu and McCauley 2017) before filtering low-quality sequences by the following options: (1) complete; (2) high coverage; (3) and low coverage excl. To do unbiased genomic variation analysis, we did further filtering and deleted those sequences with more than fifty consecutive N bases (50 Ns). Following the QC steps, 1,031,249 sequences were included in the study, which were sampled from 166 countries or regions, including the USA (n = 271,494), the UK (n = 244,460), Germany (n = 83,160), Denmark (n = 69,766), and Sweden (n = 39,418), amongst others (Table S4).

4.2 Genomic indels analysis
Genomic indels were defined based on the genome of prototype SARS-CoV-2 strain identified from Wuhan, namely Wuhan-Hu-1 (NC_045512.2) (Wu et al. 2020). Multiple sequences alignments were performed using the progressive method (FFT-NS-2) implemented in MAFFT (version 7.4) (Katoh et al. 2002). The whole-genome indels analysis was carried out using the pipeline implemented in the CoVa software (version 0.2) (Young et al. 2020). Indels that appeared in 5' and 3' UTRs were excluded from the analyses. Seqtk program (https://github.com/lh3/seqtk) was used to extract the genome sequences with indels and subjected to a second CoVa to remove false positives. These steps were repeated two or three times before the final manual inspection of the alignment involving major types of indels.

For each reliable indel identified, the naming follows the pattern ‘gene pos_genomic_position ± length’ to indicate the gene and genomic position of occurrence, whether they are insertions or deletions, as well as how many bases are involved, which was exemplified by S pos_21765-6, ORF8 pos_28252+2.

4.3 Phylogenetic analyses
We used nextstrain augur tool (Hadfield et al. 2018) for phylogenetic analyses, which contained SARS-CoV-2 pango lineage reference strains in the GISAID database that described the major historical and current genomic variants defined by fixed nucleotide substitutions. We then map the indel information to these major lineages using pango nomenclature program (Rambaut et al. 2020). All of the modifications were implemented by the iTOL software (Letunic and Bork 2019).

4.4 Structural prediction and analysis
SARS-CoV-2 S proteins were aligned using mafft software and visualized with texshade software (Beitz 2000). The structural models for spike proteins with indels were constructed using the computer-guided homology modeling method implemented in SWISS-MODEL online server (Waterhouse et al. 2018) using Cryo-EM structure of SARS-CoV-2 spike proteins trimer (PDB ID: 7CWU) (Wang et al. 2021a), the prototype S protein, as the template. The similarity between all sequences and the template were greater than 99.45 per cent, GMQE (Global Model Quality Estimate) were greater than 0.67, and QMEANDisCo Global were 0.72 ± 0.05. The visualization of modeled structure were carried
out by PyMOL (Schrodinger, LLC. (2015), The PyMOL Molecular Graphics System, Version 1.8.) in here or UCSF chimera software (Petterson et al. 2004). Prediction of potential impact on biological function was carried out by PROVEAN (Protein Variation Effect Analyzer) (Choi and Chan 2015). To understand the implications of the amino acid indels in the mutants, we constructed the hydrogen bond changes by PyMOL software in the three-dimensional structure of the indels region.

**Supplementary data**

Supplementary data is available at Virus Evolution online.

**Acknowledgement**

We are grateful to the submitters from many laboratories for the sequences in the GISAID database.

**Funding**

This study was supported by the National Natural Science Foundation of China (#32041002), Shenzhen Science and Technology Program (KQTD2018041114323605, JSGG20200225150431472 and KQTD2020082014582023), Guangdong Zhuhai Talents Program (#2016J065Y540), Guangdong Province ‘Pearl River Talent Plan’ Innovation and Entrepreneurship Team Project (2019ZT08Y464), and National Key Research and Development Program of China.

**Conflict of interest:** The authors have declared no competing interests.

**References**

Aleem, A., Akbar Samad, A. B., and Slender, A. K. (2021) Emerging Variants of SARS-CoV-2 and Novel Therapeutics against Coronavirus (COVID-19). Treasure Island, FL: StatPearls Publishing.

Bal, A. et al. (2020) ‘Molecular Characterization of SARS-CoV-2 in the First COVID-19 Cluster in France Reveals an Amino Acid Deletion in Nsp2 (Asp268del)’, Clinical Microbiology and Infection, 26: 960–2.

Beitz, E. (2000) ‘TeXshade: Shading and Labeling of Multiple Sequence Alignments Using LaTeX2e’, Bioinformatics, 16: 135–9.

Benedetti, F. et al. (2020) ‘Emerging of a SARS-CoV-2 Viral Strain with a Deletion in nsp1’, Journal of Translational Medicine, 18: 329.

Chi, X. et al. (2020) ‘A Neutralizing Human Antibody Binds to the N-terminal Domain of the Spike Protein of SARS-CoV-2’, Science, 369: 650–5.

Choi, Y. and Chan, A. P. (2015) ‘PROVEAN Web Server: A Tool to Predict the Functional Effect of Amino Acid Substitutions and Indels’, Bioinformatics, 31: 2745–7.

Davies, N. G. et al. (2021) ‘Estimated Transmissibility and Impact of SARS-CoV-2 Lineage B.1.1.7 In England’, Science, 372: eabg3055.

Dong, E., Du, H., and Gardner, L. (2020) ‘An Interactive Web-Based Dashboard to Track COVID-19 in Real Time’, The Lancet Infectious Diseases, 20: 533–4.

Gong, Y.-N. et al. (2020) ‘SARS-CoV-2 Genomic Surveillance in Taiwan Revealed Novel ORF8-Deletion Mutant and Clade Possibly Associated with Infections in Middle East’, Emerging Microbes and Infections, 9: 1457–66.

Gorbatenya, A. E. et al. (2020) ‘The Species Severe Acute Respiratory Syndrome-Related Coronavirus: Classifying 2019-nCoV and Naming It SARS-CoV-2’, Nature Microbiology, 5: 536–44.

Hadsfield, J. et al. (2018) ‘Nextstrain: Real-Time Tracking of Pathogen Evolution’, Bioinformatics, 34: 4121–3.

Holland, L. A. et al. (2020) ‘An 81-Nucleotide Deletion in SARS-CoV-2 ORF7a Identified from Sentinel Surveillance in Arizona (January to March 2020)’, Journal of Virology, 94: e00711–20.

Hou, Y. J. et al. (2020) ‘SARS-CoV-2 D614G Variant Exhibits Efficient Replication Ex Vivo and Transmission in Vivo’, Science, 370: 1464–8.

Katoh, K. et al. (2002) ‘MAFFT: A Novel Method for Rapid Multiple Sequence Alignment Based on Fast Fourier Transform’, Nucleic Acids Research, 30: 3059–66.

Khan, A. et al. (2021) ‘Higher Infectivity of the SARS-CoV-2 New Variants Is Associated with K417N/T, E484K, and N501Y Mutants: An Insight from Structural Data’, Journal of Cellular Physiology, 236: 7045–57.

Letunic, I., and Bork, P. (2019) ‘Interactive Tree Of Life (iTOl) v4: Recent Updates and New Developments’, Nucleic Acids Research, 47: W256–9.

Liu, Y., and Rocklov, J. (2021) ‘The Reproductive Number of the Delta Variant of SARS-CoV-2 Is Far Higher Compared to the Ancestral SARS-CoV-2 Virus’, Journal of Travel Medicine, 28: taab124.

Liu, Z. et al. (2020) ‘Identification of Common Deletions in the Spike Protein of Severe Acute Respiratory Syndrome Coronavirus 2’, Journal of Virology, 94: e00790–20.

McCallum, M. et al. (2021) ‘N-terminal Domain Antigenic Mapping Reveals a Site of Vulnerability for SARS-CoV-2’, Cell, 184: 2332–47 e16.

Meng, B. et al. (2021) ‘Recent Emergence of SARS-CoV-2 Spike Deletion H69/V70 and Its Role in the Alpha Variant B.1.1.7’, Cell reports, 35: 109292.

Pereira, F. (2020) ‘Evolutionary Dynamics of the SARS-CoV-2 ORF8 Accessory Gene’, Infection Genetics and Evolution, 85: 104525.

Petterson, E. F. et al. (2004) ‘UCSF Chimera: A Visualization System for Exploratory Research and Analysis’, Journal of Computational Chemistry, 25: 1605–12.

Plante, J. A. et al. (2021) ‘Spike Mutation D614G Alters SARS-CoV-2 Fitness’, Nature, 592: 116–21.

Quéromès, G. et al. (2021) ‘Characterization of SARS-CoV-2 ORF6 Deletion Variants Detected in a Nosocomial Cluster during Routine Genomic Surveillance, Lyon, France’, Emerging Microbes and Infections, 10: 167–77.

Rambaut, A. et al. (2020) ‘A Dynamic Nomenclature Proposal for SARS-CoV-2 Lineages to Assist Genomic Epidemiology’, Nature Microbiology, 5: 1403–7.

Shen, L. et al. (2021) ‘Rapidly Emerging SARS-CoV-2 B.1.1.7 Sub-lineage in the United States of America with Spike Protein D178H and Membrane Protein V70L Mutations’, Emerging Microbes and Infections, 10: 1293–9.

Shu, Y., and McCauley, J. (2017) ‘GISAID: Global Initiative on Sharing All Influenza Data – From Vision to Reality’, Eurosurveillance, 22: 30494.

Su, Y. C. F. et al. (2020) ‘Discovery and Genomic Characterization of a 382-Nucleotide Deletion in ORF7b and ORF8 during the Early Evolution of SARS-CoV-2’, mBio, 11: e01610–20.

Tang, X. L. et al. (2020) ‘On the Origin and Continuing Evolution of SARS-CoV-2’, National Science Review, 7: 1012–23. National Science Review, 2020, 1012–23. doi: 10.1093/nsr/nwaa036.

Torjesen, I. (2021) ‘Covid-19: Delta Variant Is Now UK’s Most Dominant Strain and Spreading through Schools’, BMJ, 373: n1445. https://www.bmj.com/content/373/bmj.n1445.long.

Wang, N. et al. (2021a) ‘Structure-based Development of Human Antibody Cocktails against SARS-CoV-2’, Cell Research, 31: 101–3.

Wang, P. et al. (2021b) ‘Antibody Resistance of SARS-CoV-2 Variants B.1.351 and B.1.1.7’, Nature, 593: 130–5.
Waterhouse, A. et al. (2018) ‘SWISS-MODEL: Homology Modelling of Protein Structures and Complexes’, Nucleic Acids Research, 46: W296–303.

Wu, F. et al. (2020) ‘A New Coronavirus Associated with Human Respiratory Disease in China’, Nature, 579: 265–9.

Young, B. E. et al. (2020) ‘Effects of a Major Deletion in the SARS-CoV-2 Genome on the Severity of Infection and the Inflammatory Response: An Observational Cohort Study’, The Lancet, 396: 603–11.

Zhou, P. et al. (2020) ‘A Pneumonia Outbreak Associated with a New Coronavirus of Probable Bat Origin’, Nature, 579: 270–3.

Zinzula, L. (2021) ‘Lost in Deletion: The Enigmatic ORF8 Protein of SARS-CoV-2’, Biochemical and Biophysical Research Communications, 538: 116–24.

Zost, S. J. et al. (2020) ‘Potently Neutralizing and Protective Human Antibodies against SARS-CoV-2’, Nature, 584: 443–9.