Functional mutations of SARS-CoV-2: implications to viral transmission, pathogenicity and immune escape

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Abstract

The pandemic of coronavirus disease 2019 caused by severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) has led to major public health challenges globally. The increasing viral lineages identified indicate that the SARS-CoV-2 genome is evolving at a rapid rate. Viral genomic mutations may cause antigenic drift or shift, which are important ways by which SARS-CoV-2 escapes the human immune system and changes its transmissibility and virulence. Herein, we summarize the functional mutations in SARS-CoV-2 genomes to characterize its adaptive evolution to inform the development of vaccination, treatment as well as control and intervention measures.

Keywords: Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2); Mutation; Variants of concern; Variants of interest; Adaptive evolution

Introduction

Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) was confirmed as the causative agent of coronavirus disease 2019 (COVID-19) in 2020.[1] The SARS-CoV-2 pandemic has caused major public health problems globally.[2] Thanks to the next-generation sequencing technology, an unprecedented large-scale virus sequencing campaign has been carried out and massive virus genome data have been accumulated in a short period of time. Based on the globally shared virus sequence resources, the variants with epidemic potential had been detected in time, which informs surveillance of the viral transmissibility and pathogenicity, the development and implementation of vaccination strategies, and the improvement of the control and prevention measures to block the viral spread. As of December 2021, more than six million SARS-CoV-2 mutation sequences have been uploaded to public datasets.[3]

The increasing viral lineages identified indicate that the SARS-CoV genome is evolving at a rapid rate. Viral gene mutations can cause antigenic drift or shift, which are important ways by which SARS-CoV-2 escapes the human immune system and changes its transmission and virulence mechanisms. Herein, we summarize the functional mutations of SARS-CoV-2 and viral genome recombination events to better understand its adaptive evolutionary characteristics.

Characteristics of the SARS-CoV-2 Genome and Encoding Proteins

SARS-CoV-2 contains non-segmented, single-stranded, positive-sense RNA, with a length of approximately 29,900 nucleotides (nt). Its genomic structure is similar to other betacoronaviruses, which have a standard eukaryotic 5'-terminal cap structure and a 3'-poly-A tail. The genome contains 14 open reading frames (ORFs), encoding 16 nonstructural proteins (NSPs) and four main structural proteins: the spike (S), membrane (M), envelope (E), and nucleocapsid (N) proteins.[4] Two-thirds of the whole genome from the 5'-terminus encodes polyproteins 1a and 1ab. The polyproteins are autoproteolytic and processed to form independently folded NSPs, which assemble to form the replicase-transcriptase complex for viral replication.

NSPs are responsible for transcription, replication, and maintaining complex interaction mechanisms with the
host. NSP1 can degrade host cell messenger RNA (mRNA) and inhibit interferon (IFN) signal transduction;[26] NSP3 can cleave polypeptides, facilitating escape from human natural immune responses and promoting expression of cytokines such as translation initiation factors eukaryotic initiation factor 4A and eukaryotic initiation factor 3. [26] NSP4 forms a double membrane vesicle (DMV);[27] NSP5, a 3C protease, cleaves transcription activator 2 and inhibits IFN signal transduction.[28] NSP6 inhibits the expansion of autophagy bodies and promotes DMV formation.[19,20] NSP7, 8, and 12 act synergistically with each other; NSP12 is an RNA-dependent RNA polymerase (RdRp), and NSPs 7 and 8 assist NSP12.[11-13] NSP9 binds to RNA as a dimer.[14] NSP10 is the scaffold protein of NSPs 14 and 16.[15] NSP13 is an RNA helicase involved in unwinding during viral replication and coordinates with NSP12 to improve viral replication efficiency.[16] NSP14 caps viral mRNA during viral replication as an exoribonuclease (N7 MTase).[17] NSP15 is an endonuclease and assists the virus in escaping recognition by the double-stranded (ds) RNA sensor.[18,19] NSP16 inhibits host innate immune responses by inhibiting IFN-induced proteins with tetrapeptide repeats family members (IFIT1, IFIT2, and IFIT3), thus escaping melanoma differentiation-associated gene 5.[20] ORF3 is a nuclear factor-xB antagonist that inhibits the production of interleukin 6 and 8.[21] ORF3 can coordinate with ORF7a to antagonize IFN-1 for immune escape.[22] ORF3 can induce endoplasmic reticulum-dependent autophagy by prolonging the cellular S phase, promoting vesicular formation, and in turn promoting viral proliferation.[23] ORF4 is a mitochondrial-targeting protein that induces apoptosis via interaction between the mitochondria and adenine translocator 3.[24] ORF6 inhibits nuclear translocation of signal transducer and activator of transcription 1 and 2 to block IFN signal transduction, thus helping the virus replicate smoothly and escape immune responses.[25,26] ORF7b is a viral structural protein and assists ORF7a with its functions.[27] ORF8 directly binds to major histocompatibility complex class I, blocking antigen presentation to achieve immune escape.[28] Few functional studies regarding ORF10 are available; some studies suggest that ORF10 should not be regarded as a protein-coding gene and that its genome annotation should be changed.[29]

S protein, a trimeric homologous protein on the surface of viral particles, binds cell surface receptors to mediate membrane fusion.[10,31] M protein has three transmembrane domains and is the most abundant protein in viral particles.[32] It binds with the S and N proteins to maintain the viral particle morphology. E protein is mainly involved in viral protein assembly and release.[33,34] E protein expression induces interleukin production and is related to viral virulence. For example, E protein inhibits activation of endoplasmic reticulum stress and unfolded protein responses during infection, thus playing an antiapoptotic role and enabling the virus to continue replicating and survive in cells. E protein also promotes activation of inflammatory bodies through Ca+ transportation, thus triggering human inflammatory responses.[35] N protein packages viral genomic RNA and is the main structural component of the viral capsid. N protein antagonizes IFN and inhibits host innate immunity by inhibiting retinoic acid-induced gene protein I recognition.[36] However, the functions of the proteins encoded by the CoV genome have not been fully elucidated and deserve intensive investigations, and mutations in some gene regions lead to changes in viral replication efficiency, virulence, transmission, and other functions.

The SARS-CoV-2 mutation rate is considered to be 1.13 × 10^{-3}/site/year, which is much lower than that of the influenza B virus (2.2 × 10^{-3}/site/year).[37] ORF6 has the highest amino acid mutation frequency (2.62 sites/year), and M protein has the lowest (2.15 sites/year) of all viral-encoding proteins.[38] The viral replication intensity, transmission, and infectivity are closely related to viral genomic mutations. Referring to the Wuhan-Hu-1 strain (NC045512.2), mutations have occurred on nearly all sites of the new lineages of the viral genome. Additionally, under human immune pressure, many unfixed mutations referred to as quasispecies, appear during infection.[39] After much accumulation and evolution, some mutations fixed in the variants, which may become new dominant strains.

**Variants of Concern (VOCs) and Their Mutation Hotspots**

In late 2020, the World Health Organization (WHO) announced variants of interest (VOIs) and VOCs to attract global attention and ongoing responses to SARS-CoV-2 variants.[40] For example, in the early stage of the SARS-CoV-2 epidemic (February 2020), a strain with a 15 to 30 nt deletion at the S1/S2 junction was found in Hong Kong of China with a decreasing ability to infect host cells. Such mutation lasted only a very short time, and the strain never dominated. However, its characteristic of reduced infectivity in cells makes it to be a potential attenuated vaccine model or laboratory experimental model.[41] A strain found in Europe with D839Y/N/E mutations in the S protein exhibits enhanced ability to interact with T cells.[42] The SARS-CoV-2 S protein is the main antigenic protein. The receptor-binding domain (RBD) region on the S protein, responsible for binding to the human angiotensin-converting enzyme 2 (hACE2) receptor, is the major antigenic region that induces neutralizing antibodies (NAbs). The amino acid mutations in S region, especially in the RBD region, may change antigenic sites, enable escaping recognition of host anti-viral antibodies and change the transmission ability, infectivity and virulence of SARS-CoV-2 [Figure 1].[43,44,45] The mutation characteristics for VOCs and VOIs are listed in Table 1.

**VOC Alpha**

Compared with the prototype Wuhan-Hu-1 strain, the first new site mutation to attract global attention was D614G in S protein. The D614G mutation was reported to significantly improve expression of the viral S protein in cells, and its intracellular replication enabled a higher viral titer by changing the RBD conformational enhancement and ACE2-binding ability.[46-49] D614G mutation has been found in epidemic viral strains worldwide since March 2020.[31] In addition to D614G, S protein region also contains S V367F and S D364Y mutations in lineages B.1 and B.1.1. These mutations may be related to the
increased viral transmission ability. Compared with Wuhan-Hu-1 strain, the S V367F mutation significantly enhanced the binding ability to hACE2. B.1.1.7 was derived from the B.1 and B.1.1 strains and was first found in the United Kingdom. When the B.1.1.7 viral strain occurred again in August 2020, it was named VOC Alpha and N501Y.V1. In addition to D614G, other mutations in S region include N501Y, H69 deletion, V70 deletion, Y144 deletion, A570D, P681H, T716I, S982A, and D1118H. Functional site mutations were also reported in viral genes other than S. The P314L in ORF1b, firstly identified in VOC Alpha, then shared in all VOCs and VOIs strain, was reported to increase the affinity of favipiravir and remdesivir. A 382-nucleotide deletion (Q27∗) on orf8 gene of Alpha strain has been reported relating to milder infection. The sites of R203K and G204R in N protein shared in VOCs (Gamma, Omicron) and VOI lambda have been considered increasing the ability of viral transmission. The B.1.1.7 viral strain quickly became the dominant epidemic strain in Europe in the second half of 2020.

**VOC Beta**

In August 2020, the B.1.351 lineage, firstly identified in South Africa, was named VOC Beta or N501Y.V2. VOC Beta is characterized with the mutations of D80A, D215G, LLA241-243del, K417N, E484K, N501Y, D614G and A701V in S protein, with K417N, E484K and N501Y as the main representative mutations. Eighty percent of VOC Beta strains in South Africa had these mutations. N501Y mutation combined with Y41 and K353 enhance the hACE2-binding ability by >5 folds, which may also contribute to interspecies transmission as per the findings in a mouse infection model. VOC Beta exhibits significant immune-escaping ability through its major mutation site at E484K. E484K mutation leads to amino acid transformation from a negative to positive charge. Although it does not affect ACE2 binding, it can reduce the neutralization ability of the humoral immunity induced by the Wuhan-Hu-1 strain by >10 folds.
bridges that form with the ACE2-D30 site, and mutations at this site will directly change the S protein binding affinity.\textsuperscript{[64,65]} The K417N mutation enhances the ability of the S protein to bind with ACE2, which compromises most of the neutralizing activity through reduced polar contact with complementarity determining regions.\textsuperscript{[65]} In March 2020, N439K has been reported to cause the truncation of ORF3a, which also facilitates viral evading the induction of human cytokine.\textsuperscript{[67]} Multiple reported monoclonal antibodies, regn10933 (REGN, USA), regn10987 (REGN, USA), ly-cov555 (Lilly, USA), and S309 (Vir, USA), as well as sotrovimab (GSK, UK), urgently authorized by the US Food and Drug Administration, can be escaped because of the N439K mutation of the VOC Beta strain. K417 and E484 remained the critical antigenic escape sites for class 1 and 2 antibodies, respectively.\textsuperscript{[66]}

**VOC Gamma**

VOC Gamma, also called the P.1 lineage or N501Y.V3, was identified in Brazil in January 2021.\textsuperscript{[68]} Its main mutation sites include L18F, T20N, P26S, D138Y, R190S, K417T, E484K, N501Y, D614G, H655Y, T1027I and V1176F in the S protein. L18F, T20N and D138Y mutations reduce the effects of NAbs induced by the N-terminal domain (NTD) of the S protein.\textsuperscript{[69]} Mutations (K417T, E484K, N501Y) in the RBD region enhance the affinity with hACE2. Moreover, these mutations also have been known as NAb-escaping mutant sites. For example, E484K is associated with a higher capacity to evade host neutralizing activity.\textsuperscript{[69,70]} VOC Gamma reached 40% in sequenced cases in February 2021 in South America, and with the emergence of Delta strain in summer of 2021, the proportion of Gamma strain in the world declined precipitously.\textsuperscript{[71]}

**VOC Delta**

The first Delta sequence (B.1.617.2) was uploaded in October 2020. The WHO defined it as VOI Delta on 4 April 2021, then redefined it as VOC Delta on 11 May 2021.\textsuperscript{[40]} VOC Delta remains the dominant epidemic strain, accounting for 81% of all epidemic strains worldwide as of December 2021 when we prepared this review.\textsuperscript{[72]} The Delta strain contains the major mutation sites including T19R, EFR156-158G, L452R, T478K, D614G, P681R and D950N, in S protein, and I82T in M protein. M I82T may increase viral pathogenicity.\textsuperscript{[73]} The NAbs levels in serum from Beta or Gamma strain infected patients are reduced when tested by Delta strain. Breakthrough infections of Delta mutants have been reported in individuals vaccinated with the Pfizer BNT162b2, Moderna mRNA-1273, and Covaxin BV152 vaccines,\textsuperscript{[74]} partially due to evading of NTD antigenic sites-induced neutralization antibodies (eg, NTD-18, NTD-20, NTD-69, and NTD-71).\textsuperscript{[69,73,78]} L452R, EFR156-158G, and T478K mutations permit Delta strain partially resistant to NAbs, increasing S protein expression on the cellular membrane and enhancing the binding affinity with the ACE2 receptor.\textsuperscript{[79-84]} Compared with Wuhan-Hu-1 strain (with D614G mutation), the Delta strain is 6-fold less sensitive to host NAbs retrieved from recovered COVID-19 patients infected by Wuhan-Hu-1 strain, and 8-fold less sensitive to inactivated vaccine-elicited antibodies \textit{in vitro}.\textsuperscript{[83,86]} Replication of the Delta strain in animal models showed higher levels of the viral subgenome and virulence compared with the Wuhan-Hu-1 strain.\textsuperscript{[87]} Delta strain infections showed higher synctia rates in cell models compared with those of Alpha strain.\textsuperscript{[88]} Such characteristics may result in a high risk of hospitalization in patients infected with Delta strain.\textsuperscript{[89]}

**VOC Omicron**

Omicron (BA.1, BA.2) was listed as a “variant under monitoring” on 24 November 2021, and the WHO quickly designated it as VOC within 2 days. Omicron has more mutation sites in the S protein (35 sites) than any other VOC strain, demonstrating adaptive evolution under the pressure of human immunity. Omicron has three unique cluster mutation regions at the RBD (amino acid sites G339D, S371L, S373P, and S375F), receptor-binding motif (amino acid sites Q493R, G496S, Q498R, and Y505H) and fusion domain (amino acid sites N764K, N856K, Q954H, N969K, and L981F).\textsuperscript{[89]} Among all mutation sites on the S protein, the HV69-70del mutation is also found in VOC Alpha. The cluster of mutations at the S1-S2 furin cleavage site (H655Y, N679K, and P681H) is reportedly related to viral transmissibility.\textsuperscript{[90]} Compared with the Delta variant, S protein of Omicron strain is less efficiently cleaved and much depending on endocytosis to enter cell.\textsuperscript{[92]} Q498R and N501Y mutations have been shown to significantly increase the binding affinity with ACE2 \textit{in vitro}.\textsuperscript{[95]} Omicron variant has strong immune evasion capabilities, owing to three amino acid deletions on the NSP6 105 to 107 locus.\textsuperscript{[94]} In addition to N R203K and N G204R mutations, a new mutation P13L in N protein has been found in the Omicron strain, which is considered related to the decrease of mortality and increase in viral transmissibility activity.\textsuperscript{[95]} S A67V and S T93I mutations in VOC Omicron have been found in other VOCs. VOC Omicron has strong ACE2-binding ability via pi-pi (Om-RBD-Y501/ACE2-Y41) and salt-bridge (Om-RBD-K493/ACE2-Y41) interactions.\textsuperscript{[96,97]} Compared with other VOCs (Alpha, Beta, Gamma, and Delta) and VOIs (Lambda and Mu), Omicron has significantly decreased sensitivity to NAbs, and its 50% effective dilution (ED\textsubscript{50}) to NAbs is 11.9% of the strain that has only the S D614G mutation.\textsuperscript{[98]} Although it has many mutations and causes significant humoral immune evasion, the antibodies of nearly all individuals with existing anti-SARS-CoV-2 CD8+ T-cell responses should recognize VOC Omicron, so vaccines and cellular immunity to Omicron variant remain effective.\textsuperscript{[99-101]}

**VOL Strains and Their Mutation Hotpots**

The list of VOI strains has been frequently updated. VOIs Lota (B.1.526), Kappa (B.1.617.1), Epsilon (B.1.427/ B.1.429) and Eta (B.1.525) have been removed since being
defined. As of December 2021, only VOI Mu (B.1.621) and VOI Lambda (C.37) are being monitored by the WHO. As for the frequently modified VOI strains list, several reasons may be considered. Viral genome is still in rapid evolution in human beings, and these VOI strains were announced when the strain contains some considered functional mutations. However, the VOI may be transient epidemic by the effective non-pharmaceutical interventions, vaccination, and weak environmental adaptivity or replaced rapidly by VOC strains.

**VOI Lambda**

VOI Lambda (C.37) was firstly identified in December 2020 in Peru and was defined by the WHO on June 14, 2021. This strain has high infectivity and immune-escape abilities and has been detected in North America, Europe, and the Middle East. When it was firstly identified in Peru, VOI Lambda showed high infectivity and pathogenicity, and the mortality caused by this strain ranked in the top level worldwide in its first epidemic in August 2021. Lambda strain has G75V, T76I, RSYLTPGD246-253N, L452Q, F490S, D614G, and T859N mutations in S protein. The T76I and L452Q mutations increase the infectivity, whereas RSYLTPGD246-253N deletion allows SARS-CoV-2 to escape the humoral immune responses induced by BNT162b2 vaccination (after the second vaccination) with an average of 1.5-fold reduction in NAb levels.

**VOI Mu**

VOI Mu (B.1.621) was first found in Colombia in January 2021 and was defined by the WHO on August 30, 2021. VOI Mu has since been found in North America and Europe. This strain carries many mutations that have been seen in other VOCs or VOIs (e.g., E484K, N501Y, P681H, D950N, R346K, and D614G). The new mutations included T95I, Y144S, and Y145N in the S protein. VOI Mu has lower infectivity than does the Delta strain; however, it has higher immune resistance to inactivated vaccine-elicited antibodies. It has two amino-acid deletions on ORF3a, which generate a stop codon.

**Genome Recombination**

Many studies have indicated that cross-species transmission is the most likely source of SARS-CoV-2, and more evidence supports a zoonotic origin. The bat coronavirus, BANAL-52, has been reported to have a higher similarity (96.8%) than that of RaTG13 (96.1%) compared with the SARS-CoV-2 strain obtained from France in 2020 (BetaCoV/France/IDF0372/2020, GISAID accession number EPI_ISL_406596), which binds more efficiently to the hACE2 protein Wuhan-Hu-1. BANAL-52 has no furin cleavage site in S protein. BANAL-52 may transmit from humans to animals, such as minks and wild white-tailed deer. During the transmissions, recombination may happen and it is common in betacoronaviruses. The potential inter-clade recombination in SARS-CoVs has been predicted at the early stage of COVID-19 outbreak based on Bolotin. The interlineage recombination has been viewed between B.1.1.7 and many other lineages (e.g., B.1.177, B.1.36.28, B.1.36.17, and B.1.177.9) based on the sequences obtained in UK in 2020 and early 2021. Compared with other lineages epidemic simultaneously in UK, genomic fragments of B.1.1.7 lineage have higher transmission rates. SARS-CoV-2 genome recombination is also observed in a COVID-19 patient, who was coinfected with Beta and Delta variants. The potential recombination regions are between orf1ab and s genes. Very recently, Kostrikis et al reported the emerging of “Deltacron,” generated from the recombination of Omicron and Delta strains. However, this finding is still in debate on whether there exists contamination in laboratory. Although the recombination events happened at low level, however, it is really a critical risk factor generating novel viral strain with antigenic shift. The tools, for example, SimPlot and GARD, are commonly used for recombination analysis based on viral genome, which emphasizes the importance of enhancing viral gene sequencing ability.

**Perspectives**

SARS-CoV-2 replication in humans was confirmed to involve quasispecies, which contributes to the emergence of dominant epidemic strains. Therefore, surveillance of quasispecies’ viral genomes would help find and characterize these strains earlier. The epidemic SARS-CoV-2 mutants may contain functional mutation sites influencing the effects of antibodies and vaccines. Therefore, the surveillance on the genomic mutations will help to predict viral evolutionary trends.

In addition to herd immunity stress, host RNA editing functions may also contribute to viral gene mutations. SARS-CoV-2 has codon preference. RNA editing mediated by endogenous deaminase can help the human body resist viral invasion. The RNA-specific adenosine deaminase acting on RNA and apolipoprotein B mRNA editing enzyme catalytic polypeptide families mediate RNA editing. Their editing modes are A-to-U and C-to-U, respectively. These mutation modes are related to cytokines, such as tumor necrosis factor-α, which is related to interleukin-6 production and enhancement. With RNA editing, the human body can interfere with or terminate viral replication. Therefore, host RNA editing may change the nucleotide of SARS-CoV-2 genome, accelerating SARS-CoV-2 gene mutations and promoting generation of new mutants. Moreover, the low error correction ability of SARS-CoV-2 RdRp, abnormal base pairing, genomic length and nucleic acid damage tendencies can lead to or affect mutations during viral epidemics.

Host immune pressure which results from the induced anti-viral antibodies may accelerate viral gene mutations. Therefore, researchers must be vigilant regarding functional mutations and viral recombination within species to prevent recurrence of a viral pandemic.

Since the COVID-19 outbreak, a key problem in SARS-CoV-2 surveillance is to confirm the characteristics of functional mutations. Developing deep sequencing data is critical for the identification of viral mutants.
H655Y Resistant to mAbs \[139\]
F490S Resistant to NAbs \[138\]

Angiotensin-converting enzyme 2; mAbs: Monoclonal antibodies; NAbs: Neutralizing antibodies.

D614G Enhance binding af
N501Y Enhance binding af

E484A/K Resistant to mAbs and NAbs \[60-62,79\]
T478K Resistant to mAbs \[135\]
S477N Resistant to mAbs \[79\]

N440K Escape from NAbs \[136\]
K417N/T Enhance binding af
L452R Resistant to mAbs and NAbs \[135\]
Y144del Resistant to mAbs \[62\]
P681R Enhance viral fusogenicity \[140\]
Q498R Enhance binding af
Q493R Resistant to mAbs \[137\]

2. The National Genomics Data Center in China has the worldwide distributions and evolution of SARS-CoV-2. Nextstrain is a powerful tool for real-time tracking of viral evolution by providing the next phase of COVID-19. Nextstrain is a powerful platform is also needed so that researchers can integrate deep-sequencing data and artificial intelligence to predict viral evolution and analyze quasispecies, which facilitate early precaution of VOCs and precision prevention or control of new outbreaks. Cell and animal models are also needed to test the immunogenicity and pathogenesis by integration of mutants and reverse genetic analysis. Researchers should also monitor animals that have close contact with humans and may be infected with SARS-CoV-2 to prevent it from infecting humans after mutation and recombination in animals.

In conclusion, we summarized functional gene mutations in SARS-CoV-2 VOCs and VOIs. Current knowledge of viral mutants suggests that the SARS-CoV-2 genome remains unstable. Future new lineages with functional mutations are predicted to emerge.

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**Table 1: Major S protein mutations in variants of interest (VOIs) and variants of concern (VOCs).**

| Variant | Clinical and biological characteristics | Experimental model | Strain with this variant | Main epidemic continents |
|---------|----------------------------------------|--------------------|-------------------------|-------------------------|
| G75V*   | Damage to S protein structure \[134\]  | Bioinformatics prediction | Lambda                  | South America           |
| Y144del | Resistant to mAbs \[62\]              | In vitro           | Alpha, Omicron          | Asia, Africa            |
| L452R   | Resistant to mAbs and NAbs \[131\]    | In vitro           | Delta                   | Global                  |
| K417N/T | Enhance binding affinity with ACE2  \[64,65\] | Bioinformatics prediction | Beta, Gamma             | Asia, Africa            |
| N440K   | Escape from NAbs \[136\]             | In vitro           | Omicron                 | Asia, Africa            |
| S477N   | Resistant to mAbs \[79\]             | In vitro           | Omicron                 | Asia, Africa            |
| T478K   | Resistant to mAbs \[133\]            | In vitro           | Delta, Omicron          | Global                  |
| E484A/K | Resistant to mAbs and NAbs \[60-62,79\] | In vitro (mouse)   | Beta, Gamma, Mu         | Africa                  |
| Q493R   | Resistant to mAbs \[137\]            | In vivo (human)    | Omicron                 | Asia, Africa            |
| Q498R   | Enhance binding affinity with ACE2 \[93\] | In vitro           | Omicron                 | Asia, Africa            |
| N501Y   | Enhance binding affinity with ACE2, interspecies transmission \[17,58\] | In vitro           | Alpha, Beta, Gamma, Mu  | Asia, Africa            |
| F490S   | Resistant to NAbs \[138\]            | In vitro           | Lambda                  | South America           |
| D614G   | Enhance binding affinity with ACE2 and S protein expression \[66-49\] | In vitro           | Alpha, Beta, Gamma, Delta, Omicron, Lambda, Mu | Global |
| H655Y   | Resistant to mAbs \[139\]            | In vivo (cat)      | Gamma, Omicron          | South America           |
| P681R   | Enhance viral fusogeneity \[140\]    | In vivo (hamster)  | Delta                   | Global                  |

* The amino acid positions were determined according to reference sequence, Wuhan-Hu-1 (NC_045512.2).
† S protein: Spike protein. ACE2: Angiotensin-converting enzyme 2; mAbs: Monoclonal antibodies; NAbs: Neutralizing antibodies.

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