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

Shengyuan Dang[1,2], Lili Ren[1,2], Jianwei Wang[1,2]

[1]National Health Commission of the People's Republic of China Key Laboratory of Systems Biology of Pathogens and Christophe Mérieux Laboratory, Institute of Pathogen Biology, Chinese Academy of Medical Sciences & Peking Union Medical College, Beijing 100730, China;
[2]Key Laboratory of Respiratory Disease Pathogenomics, Chinese Academy of Medical Sciences and Peking Union Medical College, Beijing 100730, China.

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

Access this article online

Quick Response Code:

Website: www.cmj.org

DOI: 10.1097/CM9.0000000000002158

Correspondence to: Lili Ren, Chinese Academy of Medical Sciences & Peking Union Medical College, No. 9 Dong Dan San Tiao, Dongcheng District, Beijing 100730, China
E-Mail: renliliipb@163.com

Chinese Medical Journal 2022;135(10)
Received: 23-12-2021; **Online:** 05-07-2022 **Edited by:** Peifang Wei

host. NSP1 can degrade host cell messenger RNA (mRNA) and inhibit interferon (IFN) signal transduction;[5] 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.[6] NSP4 forms a double membrane vesicle (DMV);[7] NSP5, a 3C protease, cleaves transcription activator 2 and inhibits IFN signal transduction.[8] NSP6 inhibits the expansion of autophagy bodies and promotes DMV formation.[9,10] NSPs 7, 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-κB antagonist that inhibits the production of interleukin 6 and 8.[21] ORF3 can coordinate with ORF7a to antagonize IFN-I 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.[30,31] M protein has three transmembrane regions 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 reticular 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. Referencing 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 Hotpots

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].[43-45] 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.[3] 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

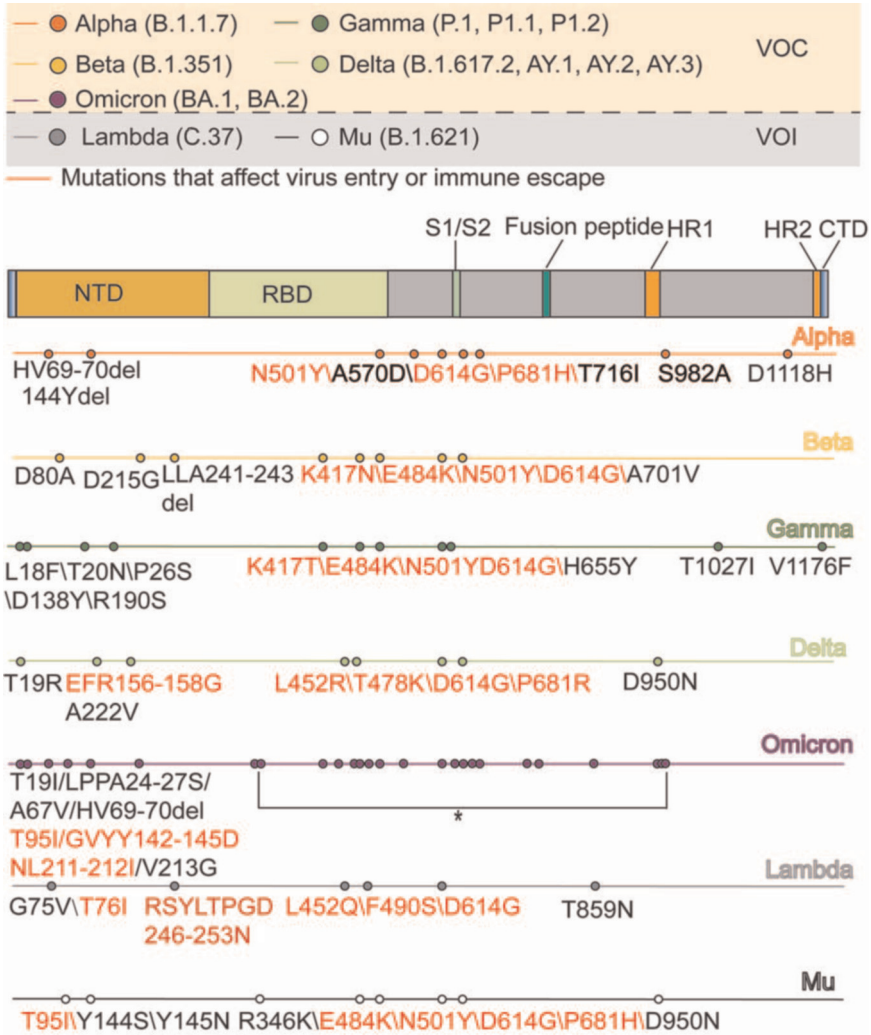

Figure 1: Mutation hotspots of the reported VOIs and VOCs on the S protein. S protein mutations include G339D, S371L/F, S373P, S375F, T376A, D405N, R408S, K417N, N440K, G446S, S477N, T478K, E484A, Q493R, G496S, Q498R, N501Y, Y505H, T547K, D614G, H655Y, N679K, P681H, N764K, D796Y, N856K, Q954H, N969K, and L981F. The amino acid positions were determined according to reference sequence, Wuhan-Hu-1 (NC_045512.2). CTD: C-terminal domain; NTD: N-terminal domain; HR: Heptad repeat; RBD: Receptor binding domain; VOC: Variant of concern; VOI: Variant of interest.

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.[50] A 382-nucleotide deletion (Q27*) on *orf8* gene of Alpha strain has been reported relating to milder infection.[51] 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.[52-54] The B.1.1.7 viral strain quickly became the dominant epidemic strain in Europe in the second half of 2020.[55,56]

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.[57,58] VOC Beta exhibits significant immune-escaping ability through its major mutation site at E484K.[59] 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.[60-63] K417 is an important site on the S protein which contributes to the electrostatic binding energies that are resulted from the salt

bridges that form with the ACE2-D30 site, and mutations at this site will directly change the S protein binding ability.[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.[66] In March 2020, N439K first emerged in Scotland, then the B.1.258 strain with this mutation appeared independently in the United States in October 2020. N439K enhanced the binding affinity with the hACE2 receptor, and this mutation also enables escaping from host NAbs. Of the other functional mutation, Q57H in ORF3a has been reported to cause the truncation of ORF3a, which also facilitates viral evading the induction of human cytokine.[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 remain the critical antigenic escape sites for class 1 and 2 antibodies, respectively.[56]

VOC Gamma

VOC Gamma, also called the P.1 lineage or N501Y.V3, was identified in Brazil in January 2021.[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.[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.[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.[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.[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. [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.[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 BBV152 vaccines,[74] partially due to evading of NTD antigenic sites-induced neutralization antibodies (eg, NTD-18, NTD-20, NTD-69, and NTD-71).[69,75-78] 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.[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 *in vitro*.[85,86] Replication of the Delta strain in animal models showed higher levels of the viral subgenome and virulence compared with the Wuhan-Hu-1 strain.[87] Delta strain infections showed higher syncytia rates in cell models compared with those of Alpha strain.[88] Such characteristics may result in a high risk of hospitalization in patients infected with Delta strain.[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).[90] 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.[91] Compared with the Delta variant, S protein of Omicron strain is less efficiently cleaved and much depending on endocytosis to enter cell.[92] Q498R and N501Y mutations have been shown to significantly increase the binding affinity with ACE2 *in vitro*.[93] Omicron variant has strong immune evasion capabilities, owing to three amino acid deletions on the NSP6 105 to 107 locus.[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 relating to the decrease of mortality and increase in viral transmission activity.[95] S A67V and S T95I 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.[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_{50}) to NAbs is 11.9% of the strain that has only the S D614G mutation.[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.[99-101]

VOI 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.[40] 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.[102] 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.[103-105] 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 NAbs levels.[106]

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.[40] 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 (eg, 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.[107] 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.[1,108-114] 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.[115] BANAL-52 has no furin cleavage site in S protein.[115] SARS-CoV-2 may transmit from humans to animals, such as minks and wild white-tailed deer.[116,117] During the transmissions, recombination may happen and it is common in betacoronaviruses.[118] The potential interclade recombination in SARS-CoVs has been predicted at the early stage of COVID-19 outbreak based on Bolotie.[119] The interlineage recombination has been viewed between B.1.1.7 and many other lineages (eg, 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.[120] Compared with other lineages epidemic simultaneously in UK, genomic fragments of B.1.1.7 lineage have higher transmission rates.[120] SARS-CoV-2 genome recombination is also observed in a COVID-19 patient, who was coinfected with Beta and Delta variants.[121] The potential recombination regions are between *orf1ab* and *s* genes.[121] 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.[122] Although the recombination events happened at low level, however, it is really a critical risk factor generating novel viral strain with antigenic shift.[123] 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.[124,125]

Perspectives

SARS-CoV-2 replication in humans was confirmed to involve quasispecies, which contributes to the emergence of dominant epidemic strains.[126] 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.[127] 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.[128] 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.[129-132]

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. However,

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[135]	*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[135]	*In vitro*	Delta, Omicron	Global
E484A/K	Resistant to mAbs and NAbs[60-62,79]	*In vivo* (mouse)	Beta, Gamma, Omicron, 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[57,58]	*In vitro* and *in vivo* (mouse)	Alpha, Beta, Gamma, Omicron, Mu	Asia, Africa
F490S	Resistant to NAbs[138]	*In vitro*	Lambda	South America
D614G	Enhance binding affinity with ACE2 and S protein expression[46-49]	*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 fusogenicity[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.

the ability gaps on deep-sequencing among different geographical regions or countries may cause the lacking of monitoring on mutants and inaccurate data analyses. Thus, SARS-CoV-2 mutation surveillance is important for the next phase of COVID-19. Nextstrain is a powerful tool for real-time tracking of viral evolution by providing the worldwide distributions and evolution of SARS-CoV-2.[133] The National Genomics Data Center in China has constructed a COVID-19 information database to track epidemics of SARS-CoV-2 and gene mutations. A 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 immunogenecity 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.

Funding

This review was supported by the Medical and Health Science and Technology Innovation Project of Chinese Academy of Medical Sciences (No. 2021-I2M-1-040).

Conflicts of interest

None.

References

1. Ren LL, Wang YM, Wu ZQ, Xiang ZC, Guo L, Xu T, *et al*. Identification of a novel coronavirus causing severe pneumonia in human: a descriptive study. Chin Med J 2020;133:1015–1024. doi: 10.1097/cm9.0000000000000722.
2. Wu Z, Jin Q, Wu G, Lu J, Li M, Guo D, *et al*. SARS-CoV-2's origin should be investigated worldwide for pandemic prevention. Lancet 2021;398:1299–1303. doi: 10.1016/s0140-6736(21)02020-1.
3. Global Initiative of Sharing All Influenza Data. Available from: https://www.gisaid.org/. [Accessed on December 22, 2021].
4. Masters PS. The molecular biology of coronaviruses. Adv Virus Res 2006;66:193–292. doi: 10.1016/s0065-3527(06)66005-3.
5. Tanaka T, Kamitani W, DeDiego ML, Enjuanes L, Matsuura Y. Severe acute respiratory syndrome coronavirus nsp1 facilitates efficient propagation in cells through a specific translational shutoff of host mRNA. J Virol 2012;86:11128–11137. doi: 10.1128/jvi.01700-12.
6. Lei J, Kusov Y, Hilgenfeld R. Nsp3 of coronaviruses: structures and functions of a large multi-domain protein. Antiviral Res 2017;149:58–74. doi: 10.1016/j.antiviral.2017.11.001.
7. Beachboard DC, Anderson-Daniels JM, Denison MR. Mutations across murine hepatitis virus nsp4 alter virus fitness and membrane modifications. J Virol 2014;89:2080–2089. doi: 10.1128/jvi.02776-14.
8. Zhu X, Wang D, Zhou J, Pan T, Chen J, Yang Y, *et al*. Porcine deltacoronavirus nsp5 antagonizes type I interferon signaling by cleaving STAT2. J Virol 2017;91:e00003–e00017. doi: 10.1128/jvi.00003-17.
9. Angelini MM, Akhlaghpour M, Neuman BW, Buchmeier MJ. Severe acute respiratory syndrome coronavirus nonstructural proteins 3, 4, and 6 induce double-membrane vesicles. mBio 2013;4:e00524–e00613. doi: 10.1128/mBio.00524-13.
10. Cottam EM, Whelband MC, Wileman T. Coronavirus NSP6 restricts autophagosome expansion. Autophagy 2014;10:1426–1441. doi: 10.4161/auto.29309.

11. Kirchdoerfer RN, Ward AB. Structure of the SARS-CoV nsp12 polymerase bound to nsp7 and nsp8 co-factors. Nat Commun 2019;10:2342. doi: 10.1038/s41467-019-10280-3.
12. Ahn DG, Choi JK, Taylor DR, Oh JW. Biochemical characterization of a recombinant SARS coronavirus nsp12 RNA-dependent RNA polymerase capable of copying viral RNA templates. Arch Virol 2012;157:2095–2104. doi: 10.1007/s00705-012-1404-x.
13. te Velthuis AJ, van den Worm SH, Snijder EJ. The SARS-coronavirus nsp7+nsp8 complex is a unique multimeric RNA polymerase capable of both de novo initiation and primer extension. Nucleic Acids Res 2011;40:1737–1747. doi: 10.1093/nar/gkr893.
14. Zeng Z, Deng F, Shi K, Ye G, Wang G, Fang L, *et al*. Dimerization of coronavirus nsp9 with diverse modes enhances its nucleic acid binding affinity. J Virol 2018;92:e00692–e00718. doi: 10.1128/jvi.00692-18.
15. Bouvet M, Lugari A, Posthuma CC, Zevenhoven JC, Bernard S, Betzi S, *et al*. Coronavirus nsp10, a critical co-factor for activation of multiple replicative enzymes. J Biol Chem 2014;289:25783–25796. doi: 10.1074/jbc.M114.577353.
16. Jia Z, Yan L, Ren Z, Wu L, Wang J, Guo J, *et al*. Delicate structural coordination of the severe acute respiratory syndrome coronavirus nsp13 upon ATP hydrolysis. Nucleic Acids Res 2019;47:6538–6550. doi: 10.1093/nar/gkz409.
17. Chen Y, Cai H, Pan J, Xiang N, Tien P, Ahola T, *et al*. Functional screen reveals SARS coronavirus nonstructural protein nsp14 as a novel cap N7 methyltransferase. Proc Natl Acad Sci U S A 2009;106:3484–3489. doi: 10.1073/pnas.0808790106.
18. Deng X, Hackbart M, Mettelman RC, O'Brien A, Mielech AM, Yi G, *et al*. Coronavirus nonstructural protein 15 mediates evasion of dsRNA sensors and limits apoptosis in macrophages. Proc Natl Acad Sci U S A 2017;114:E4251–E4260. doi: 10.1073/pnas.1618310114.
19. Zhang L, Li L, Yan L, Ming Z, Jia Z, Lou Z, *et al*. Structural and biochemical characterization of endoribonuclease nsp15 encoded by middle east respiratory syndrome coronavirus. J Virol 2018;92:e00893–e00918. doi: 10.1128/jvi.00893-18.
20. Shi P, Su Y, Li R, Liang Z, Dong S, Huang J. PEDV nsp16 negatively regulates innate immunity to promote viral proliferation. Virus Res 2019;265:57–66. doi: 10.1016/j.virusres.2019.03.005.
21. Wu Z, Cheng L, Xu J, Li P, Li X, Zou D, *et al*. The accessory protein ORF3 of porcine epidemic diarrhea virus inhibits cellular interleukin-6 and interleukin-8 productions by blocking the nuclear factor-κB p65 activation. Vet Microbiol 2020;251:108892. doi: 10.1016/j.vetmic.2020.108892.
22. Dedeurwaerder A, Olyslaegers DAJ, Desmarets LMB, Roukaerts IDM, Theuns S, Nauwynck HJ. ORF7-encoded accessory protein 7a of feline infectious peritonitis virus as a counteragent against IFN-α-induced antiviral response. J Gen Virol 2014;95 (Pt 2):393–402. doi: 10.1099/vir.0.058743-0.
23. Zou D, Xu J, Duan X, Xu X, Li P, Cheng L, *et al*. Porcine epidemic diarrhea virus ORF3 protein causes endoplasmic reticulum stress to facilitate autophagy. Vet Microbiol 2019;235:209–219. doi: 10.1016/j.vetmic.2019.07.005.
24. Lin C, Gu J, Wang H, Zhou J, Li J, Wang S, *et al*. Caspase-dependent apoptosis induction via viral protein ORF4 of porcine circovirus 2 binding to mitochondrial adenine nucleotide translocase 3. J Virol 2018;92:e00238–e00318. doi: 10.1128/jvi.00238-18.
25. Miorin L, Kehrer T, Sanchez-Aparicio MT, Zhang K, Cohen P, Patel RS, *et al*. SARS-CoV-2 Orf6 hijacks Nup98 to block STAT nuclear import and antagonize interferon signaling. Proc Natl Acad Sci U S A 2020;117:28344–28354. doi: 10.1073/pnas.2016650117.
26. Lei X, Dong X, Ma R, Wang W, Xiao X, Tian Z, *et al*. Activation and evasion of type I interferon responses by SARS-CoV-2. Nat Commun 2020;11:3810. doi: 10.1038/s41467-020-17665-9.
27. Schaecher SR, Mackenzie JM, Pekosz A. The ORF7b protein of severe acute respiratory syndrome coronavirus (SARS-CoV) is expressed in virus-infected cells and incorporated into SARS-CoV particles. J Virol 2006;81:718–731. doi: 10.1128/jvi.01691-06.
28. Flower TG, Buffalo CZ, Hooy RM, Allaire M, Ren X, Hurley JH. Structure of SARS-CoV-2 ORF8, a rapidly evolving immune evasion protein. Proc Natl Acad Sci U S A 2021;118:e2021785118. doi: 10.1073/pnas.2021785118.
29. Pancer K, Milewska A, Owczarek K, Dabrowska A, Kowalski M, Łabaj PP, *et al*. The SARS-CoV-2 ORF10 is not essential in vitro or in vivo in humans. PLoS Pathog 2020;16:e1008959. doi: 10.1371/journal.ppat.1008959.
30. Benton DJ, Wrobel AG, Xu P, Roustan C, Martin SR, Rosenthal PB, *et al*. Receptor binding and priming of the spike protein of SARS-CoV-2 for membrane fusion. Nature 2020;588:327–330. doi: 10.1038/s41586-020-2772-0.
31. Li F. Structure, function, and evolution of coronavirus spike proteins. Annu Rev Virol 2016;3:237–261. doi: 10.1146/annurev-virology-110615-042301.
32. Neuman BW, Kiss G, Kunding AH, Bhella D, Baksh MF, Connelly S, *et al*. A structural analysis of M protein in coronavirus assembly and morphology. J Struct Biol 2010;174:11–22. doi: 10.1016/j.jsb.2010.11.021.
33. Nieto-Torres JL, DeDiego ML, Verdiá-Báguena C, Jimenez-Guardeño JM, Regla-Nava JA, Fernandez-Delgado R, *et al*. Severe acute respiratory syndrome coronavirus envelope protein ion channel activity promotes virus fitness and pathogenesis. PLoS Pathog 2014;10:e1004077. doi: 10.1371/journal.ppat.1004077.
34. Schoeman D, Fielding BC. Is there a link between the pathogenic human coronavirus envelope protein and immunopathology? A review of the literature. Front Microbiol 2020;11:2086. doi: 10.3389/fmicb.2020.02086.
35. Schoeman D, Fielding BC. Coronavirus envelope protein: current knowledge. Virol J 2019;16:69. doi: 10.1186/s12985-019-1182-0.
36. Szelazek B, Kabala W, Kus K, Zdzalik M, Twarda-Clapa A, Golik P, *et al*. Structural characterization of human coronavirus NL63 N protein. J Virol 2017;91:e02503–e2516. doi: 10.1128/jvi.02503-16.
37. Candido DS, Claro IM, de Jesus JG, Souza WM, Moreira FRR, Dellicour S, *et al*. Evolution and epidemic spread of SARS-CoV-2 in Brazil. Science 2020;369:1255–1260. doi: 10.1126/science.abd2161.
38. Resource for Coronavirus; 2019. Available from: https://ngdc.cncb.ac.cn/ncov/. [Accessed on December 22, 2021].
39. Domingo E, Perales C. Viral quasispecies. PLoS Genet 2019;15:e1008271. doi: 10.1371/journal.pgen.1008271.
40. World Health Organization. Available from: https://www.who.int/. [Accessed on December 22, 2021].
41. Lau SY, Wang P, Mok BW, Zhang AJ, Chu H, Lee AC, *et al*. Attenuated SARS-CoV-2 variants with deletions at the S1/S2 junction. Emerg Microbes Infect 2020;9:837–842. doi: 10.1080/22221751.2020.1756700.
42. Cheng MH, Zhang S, Porritt RA, Noval Rivas M, Paschold L, Willscher E, *et al*. Superantigenic character of an insert unique to SARS-CoV-2 spike supported by skewed TCR repertoire in patients with hyperinflammation. Proc Natl Acad Sci U S A 2020;117:25254–25262. doi: 10.1073/pnas.2010722117.
43. Tai W, He L, Zhang X, Pu J, Voronin D, Jiang S, *et al*. Characterization of the receptor-binding domain (RBD) of 2019 novel coronavirus: implication for development of RBD protein as a viral attachment inhibitor and vaccine. Cell Mol Immunol 2020;17:613–620. doi: 10.1038/s41423-020-0400-4.
44. Shang J, Ye G, Shi K, Wan Y, Luo C, Aihara H, *et al*. Structural basis of receptor recognition by SARS-CoV-2. Nature 2020;581:221–224. doi: 10.1038/s41586-020-2179-y.
45. Rockx B, Donaldson E, Frieman M, Sheahan T, Corti D, Lanzavecchia A, *et al*. Escape from human monoclonal antibody neutralization affects in vitro and in vivo fitness of severe acute respiratory syndrome coronavirus. J Infect Dis 2010;201:946–955. doi: 10.1086/651022.
46. Korber B, Fischer WM, Gnanakaran S, Yoon H, Theiler J, Abfalterer W, *et al*. Tracking changes in SARS-CoV-2 spike: evidence that D614G increases infectivity of the COVID-19 virus. Cell 2020;182:812–827.e19. doi: 10.1016/j.cell.2020.06.043.
47. Weissman D, Alameh MG, de Silva T, Collini P, Hornsby H, Brown R, *et al*. D614G spike mutation increases SARS CoV-2 susceptibility to neutralization. Cell Host Microbe 2020;29:23–31.e4. doi: 10.1016/j.chom.2020.11.012.
48. Ou J, Zhou Z, Dai R, Zhang J, Zhao S, Wu X, *et al*. V367F mutation in SARS-CoV-2 spike RBD emerging during the early

transmission phase enhances viral infectivity through increased human ACE2 receptor binding affinity. J Virol 2021;95: e0061721. doi: 10.1128/jvi.00617-21.
49. Yurkovetskiy L, Wang X, Pascal KE, Tomkins-Tinch C, Nyalile TP, Wang Y, *et al*. Structural and functional analysis of the D614G SARS-CoV-2 spike protein variant. Cell 2020;183:739–751.e8. doi: 10.1016/j.cell.2020.09.032.
50. Hemachudha P, Petcharat S, Ampoot W, Ponpinit T, Paitoonpong L, Hemachudha T. Genetic variations from successive whole genome sequencing during COVID-19 treatment in five individuals. New Microbes New Infect 2022;45:100950. doi: 10.1016/j.nmni.2022.100950.
51. Young BE, Fong SW, Chan YH, Mak TM, Ang LW, Anderson DE, *et al*. Effects of a major deletion in the SARS-CoV-2 genome on the severity of infection and the inflammatory response: an observational cohort study. Lancet 2020;396:603–611. doi: 10.1016/s0140-6736(20)31757-8.
52. Leary S, Gaudieri S, Parker MD, Chopra A, James I, Pakala S, *et al*. Generation of a novel SARS-CoV-2 sub-genomic RNA due to the R203K/G204R variant in nucleocapsid: homologous recombination has potential to change SARS-CoV-2 at both protein and RNA level. Pathog Immun 2021;6:27–49. doi:10.20411/pai.v6i2.460.
53. Mourier T, Shuaib M, Hala S, Mfarrej S, Alofi F, Naeem R, *et al*. Saudi Arabian SARS-CoV-2 genomes implicate a mutant Nucleocapsid protein in modulating host interactions and increased viral load in COVID-19 patients. J medRxiv 2021. doi: 10.1101/2021.05.06.21256706.
54. Wu H, Xing N, Meng K, Fu B, Xue W, Dong P, *et al*. Nucleocapsid mutations R203K/G204R increase the infectivity, fitness, and virulence of SARS-CoV-2. Cell Host Microbe 2021;29:1788–1801.e6. doi: 10.1016/j.chom.2021.11.005.
55. Leung K, Shum MH, Leung GM, Lam TT, Wu JT. Early transmissibility assessment of the N501Y mutant strains of SARS-CoV-2 in the United Kingdom, October to November 2020. Euro Surveill 2021;26:2002106. doi: 10.2807/1560-7917.Es.2020.26.1.2002106.
56. Zhao S, Lou J, Cao L, Zheng H, Chong MKC, Chen Z, *et al*. Quantifying the transmission advantage associated with N501Y substitution of SARS-CoV-2 in the U.K.: an early data-driven analysis. J Travel Med 2021;28:taab011. doi: 10.1093/jtm/taab011.
57. Ramanathan M, Ferguson ID, Miao W, Khavari PA. SARS-CoV-2 B.1.1.7 and B. 1. 351 spike variants bind human ACE2 with increased affinity. Lancet Infect Dis 2021;21:1070. doi: 10.1016/s1473-3099(21)00262-0.
58. Niu Z, Zhang Z, Gao X, Du P, Lu J, Yan B, *et al*. N501Y mutation imparts cross-species transmission of SARS-CoV-2 to mice by enhancing receptor binding. Signal Transduct Target Ther 2021;6:284. doi: 10.1038/s41392-021-00704-2.
59. Li Q, Nie J, Wu J, Zhang L, Ding R, Wang H, *et al*. SARS-CoV-2 501Y.V2 variants lack higher infectivity but do have immune escape. Cell 2021;184:2362–2371.e9. doi: 10.1016/j.cell.2021.02.042.
60. Huang B, Dai L, Wang H, Hu Z, Yang X, Tan W, *et al*. Neutralization of SARS-CoV-2 VOC 501Y V2 by human antisera elicited by both inactivated BBIBP-CorV and recombinant dimeric RBD ZF2001 vaccines. J bioRxiv 2021. doi: 10.1101/2021.02.01.429069.
61. Greaney AJ, Loes AN, Crawford KHD, Starr TN, Malone KD, Chu HY, *et al*. Comprehensive mapping of mutations in the SARS-CoV-2 receptor-binding domain that affect recognition by polyclonal human plasma antibodies. Cell Host Microbe 2021;29:463–476.e6. doi: 10.1016/j.chom.2021.02.003.
62. Wang P, Nair MS, Liu L, Iketani S, Luo Y, Guo Y, *et al*. Antibody resistance of SARS-CoV-2 variants B.1.351 and B.1.1.7. Nature 2021;593:130–135. doi: 10.1038/s41586-021-03398-2.
63. Vogel M, Augusto G, Chang X, Liu X, Speiser D, Mohsen MO, *et al*. Molecular definition of severe acute respiratory syndrome coronavirus 2 receptor-binding domain mutations: receptor affinity versus neutralization of receptor interaction. Allergy 2021;77:143–149. doi: 10.1111/all.15002.
64. Amin M, Sorour MK, Kasry A. Comparing the binding interactions in the receptor binding domains of SARS-CoV-2 and SARS-CoV. J Phys Chem Lett 2020;11:4897–4900. doi: 10.1021/acs.jpclett.0c01064.
65. Khan A, Zia T, Suleman M, Khan T, Ali SS, Abbasi AA, *et al*. Higher infectivity of the SARS-CoV-2 new variants is associated with K417N/T, E484K, and N501Y mutants: an insight from structural data. J Cell Physiol 2021;236:7045–7057. doi: 10.1002/jcp.30367.
66. Cao Y, Yisimayi A, Bai Y, Huang W, Li X, Zhang Z, *et al*. Humoral immune response to circulating SARS-CoV-2 variants elicited by inactivated and RBD-subunit vaccines. Cell Res 2021;31:732–741. doi: 10.1038/s41422-021-00514-9.
67. Chu DKW, Hui KPY, Gu H, Ko RLW, Krishnan P, Ng DYM, *et al*. Introduction of ORF3a-Q57H SARS-CoV-2 variant causing fourth epidemic wave of COVID-19, Hong Kong, China. Emerg Infect Dis 2021;27:1492–1495. doi: 10.3201/eid2705.210015.
68. Faria NR, Mellan TA, Whittaker C, Claro IM, Candido DDS, Mishra S, *et al*. Genomics and epidemiology of the P.1 SARS-CoV-2 lineage in Manaus, Brazil. Science 2021;372:815–821. doi: 10.1126/science.abh2644.
69. Wang P, Casner RG, Nair MS, Wang M, Yu J, Cerutti G, *et al*. Increased resistance of SARS-CoV-2 variant P.1 to antibody neutralization. Cell Host Microbe 2021;29:747–751.e4. doi: 10.1016/j.chom.2021.04.007.
70. Dejnirattisai W, Zhou D, Supasa P, Liu C, Mentzer AJ, Ginn HM, *et al*. Antibody evasion by the P.1 strain of SARS-CoV-2. Cell 2021;184:2939–2954.e9. doi: 10.1016/j.cell.2021.03.055.
71. Boehm E, Kronig I, Neher RA, Eckerle I, Vetter P, Kaiser L. Novel SARS-CoV-2 variants: the pandemics within the pandemic. Clin Microbiol Infect 2021;27:1109–1117. doi: 10.1016/j.cmi.2021.05.022.
72. Nextstrain. Available from: https://nextstrain.org/. [Accessed on December 22, 2021].
73. Shen L, Bard JD, Triche TJ, Judkins AR, Biegel JA, Gai X. Emerging variants of concern in SARS-CoV-2 membrane protein: a highly conserved target with potential pathological and therapeutic implications. Emerg Microbes Infect 2021;10:885–893. doi: 10.1080/22221751.2021.1922097.
74. Farinholt T, Doddapaneni H, Qin X, Menon V, Meng Q, Metcalf G, *et al*. Transmission event of SARS-CoV-2 delta variant reveals multiple vaccine breakthrough infections. BMC Med 2021;19:255. doi: 10.1186/s12916-021-02103-4.
75. Dejnirattisai W, Zhou D, Ginn HM, Duyvesteyn HME, Supasa P, Case JB, *et al*. The antigenic anatomy of SARS-CoV-2 receptor binding domain. Cell 2021;184:2183–2200.e22. doi: 10.1016/j.cell.2021.02.032.
76. Planas D, Veyer D, Baidaliuk A, Staropoli I, Guivel-Benhassine F, Rajah MM, *et al*. Reduced sensitivity of SARS-CoV-2 variant Delta to antibody neutralization. Nature 2021;596:276–280. doi: 10.1038/s41586-021-03777-9.
77. Li M, Lou F, Fan H. SARS-CoV-2 variants of concern delta: a great challenge to prevention and control of COVID-19. Signal Transduct Target Ther 2021;6:349. doi: 10.1038/s41392-021-00767-1.
78. Flemming A. SARS-CoV-2 Delta variant excels at membrane fusion, but not immune evasion. Nat Rev Immunol 2021;21:761. doi: 10.1038/s41577-021-00654-4.
79. Liu Z, VanBlargan LA, Bloyet LM, Rothlauf PW, Chen RE, Stumpf S, *et al*. Identification of SARS-CoV-2 spike mutations that attenuate monoclonal and serum antibody neutralization. Cell Host Microbe 2021;29:477–488.e4. doi: 10.1016/j.chom.2021.01.014.
80. Chen J, Wang R, Wang M, Wei GW. Mutations strengthened SARS-CoV-2 infectivity. J Mol Biol 2020;432:5212–5226. doi: 10.1016/j.jmb.2020.07.009.
81. Starr TN, Greaney AJ, Hilton SK, Ellis D, Crawford KHD, Dingens AS, *et al*. Deep mutational scanning of SARS-CoV-2 receptor binding domain reveals constraints on folding and ACE2 binding. Cell 2020;182:1295–1310.e20. doi: 10.1016/j.cell.2020.08.012.
82. Greaney AJ, Starr TN, Gilchuk P, Zost SJ, Binshtein E, Loes AN, *et al*. Complete mapping of mutations to the SARS-CoV-2 spike receptor-binding domain that escape antibody recognition. Cell Host Microbe 2021;29:44–57.e9. doi: 10.1016/j.chom.2020.11.007.
83. Li Q, Wu J, Nie J, Zhang L, Hao H, Liu S, *et al*. The impact of mutations in SARS-CoV-2 spike on viral infectivity and antigenicity. Cell 2020;182:1284–1294.e9. doi: 10.1016/j.cell.2020.07.012.

84. Starr TN, Greaney AJ, Dingens AS, Bloom JD. Complete map of SARS-CoV-2 RBD mutations that escape the monoclonal antibody LY-CoV555 and its cocktail with LY-CoV016. Cell Rep Med 2021;2:100255. doi: 10.1016/j.xcrm.2021.100255.
85. Mlcochova P, Kemp SA, Dhar MS, Papa G, Meng B, Ferreira I, *et al*. SARS-CoV-2 B.1.617. 2 Delta variant replication and immune evasion. Nature 2021;599:114–119. doi: 10.1038/s41586-021-03944-y.
86. Guo L, Wang G, Wang Y, Zhang Q, Ren L, Gu X, *et al*. SARS-CoV-2 specific antibody and T cell responses 1 year after infection in people recovered from COVID-19: a longitudinal cohort study. Lancet Microbe 2022;3:e348–e356. doi: 10.1016/S2666-5247(22)00036-2.
87. Mohandas S, Yadav PD, Shete A, Nyayanit D, Sapkal G, Lole K, *et al*. SARS-CoV-2 Delta variant pathogenesis and host response in Syrian hamsters. Viruses 2021;13:1773. doi: 10.3390/v13091773.
88. Rajah MM, Hubert M, Bishop E, Saunders N, Robinot R, Grzelak L, *et al*. SARS-CoV-2 Alpha, Beta, and Delta variants display enhanced Spike-mediated syncytia formation. EMBO J 2021;40:e108944. doi: 10.15252/embj.2021108944.
89. Bager P, Wohlfahrt J, Rasmussen M, Albertsen M, Krause TG. Hospitalisation associated with SARS-CoV-2 delta variant in Denmark. Lancet Infect Dis 2021;21:1351. doi: 10.1016/s1473-3099(21)00580-6.
90. Martin DP, Lytras S, Lucaci AG, Maier W, Grüning B, Shank SD, et al. Selection Analysis Identifies Significant Mutational Changes in Omicron That are Likely to Influence Both Antibody Neutralization and Spike Function (Part 1 of 2). Available from: https://virological.org/t/selection-analysis-identifies-significant-mutational-changes-in-omicron-that-are-likely-to-influence-both-antibody-neutralization-and-spike-function-part-1-of-2/771. [Accessed on December 22, 2021].
91. Gong SY, Chatterjee D, Richard J, Prévost J, Tauzin A, Gasser R, *et al*. Contribution of single mutations to selected SARS-CoV-2 emerging variants spike antigenicity. Virology 2021;563:134–145. doi: 10.1016/j.virol.2021.09.001.
92. Meng B, Abdullahi A, Ferreira I, Goonawardane N, Saito A, Kimura I, *et al*. Altered TMPRSS2 usage by SARS-CoV-2 Omicron impacts tropism and fusogenicity. Nature 2022;603:706–714. doi: 10.1038/s41586-022-04474-x.
93. Zahradník J, Marciano S, Shemesh M, Zoler E, Harari D, Chiaravalli J, *et al*. SARS-CoV-2 variant prediction and antiviral drug design are enabled by RBD in vitro evolution. Nat Microbiol 2021;6:1188–1198. doi: 10.1038/s41564-021-00954-4.
94. Benvenuto D, Angeletti S, Giovanetti M, Bianchi M, Pascarella S, Cauda R, *et al*. Evolutionary analysis of SARS-CoV-2: how mutation of Non-Structural Protein 6 (NSP6) could affect viral autophagy. J Infect 2020;81:e24–e27. doi: 10.1016/j.jinf.2020.03.058.
95. Oulas A, Zanti M, Tomazou M, Zachariou M, Minadakis G, Bourdakou MM, *et al*. Generalized linear models provide a measure of virulence for specific mutations in SARS-CoV-2 strains. PLoS One 2021;16:e0238665. doi: 10.1371/journal.pone.0238665.
96. Omotuyi OI, Olujide O, Nash O, Afolabi EO, Oyinloye B, Fatumo S, *et al*. SARS-CoV-2 Omicron spike glycoprotein receptor binding domain exhibits super-binder ability with ACE2 but not convalescent monoclonal antibody. Comput Biol Med 2022;142:105226. doi: 10.1016/j.compbiomed.2022.
97. Cui Z, Liu P, Wang N, Wang L, Fan K, Zhu Q, *et al*. Structural and functional characterizations of infectivity and immune evasion of SARS-CoV-2 Omicron. Cell 2022;185:860–871. doi: 10.1016/j.cell.2022.01.019.
98. Zhang L, Li Q, Liang Z, Li T, Liu S, Cui Q, *et al*. The significant immune escape of pseudotyped SARS-CoV-2 variant Omicron. Emerg Microbes Infect 2021;11:1–5. doi: 10.1080/22221751.2021.2017757.
99. Cao YR, Wang J, Jian F, Xiao T, Song W, Yisimayi A, *et al*. B.1.1.529 escapes the majority of SARS-CoV-2 neutralizing antibodies of diverse epitopes. Research Square 2021. doi: 10.21203/rs.3.rs-1148985/v1.
100. Redd A, Nardin A, Kared H, Bloch EM, Abel B, Pekosz A, *et al*. Minimal cross-over between mutations associated with Omicron variant of SARS-CoV-2 and CD8+ T cell epitopes identified in COVID-19 convalescent individuals. mBio 2022;13:e0361721. doi: 10.1128/mbio.03617-21.
101. Guo L, Zhang Q, Zhang CY, Huang TX, Ren LL, Cao B, *et al*. Assessment of antibody and T Cell responses to the SARS-CoV-2 virus and Omicron variant in unvaccinated individuals recovered from COVID-19 infection in Wuhan, China. JAMA Netw Open 2022;5:e229199. doi: 10.1001/jamanetworkopen.2022.9199.
102. Schiaffino F, Ferradas C, Jara LM, Salvatierra G, Dávila-Barclay A, Sanchez-Carrion C, *et al*. First detection and genome sequencing of SARS-CoV-2 Lambda (C.37) variant in symptomatic domestic cats in Lima, Peru. Front Vet Sci 2021;8:737350. doi: 10.3389/fvets.2021.737350.
103. Lamptey J, Oyelami FO, Owusu M, Nkrumah B, Idowu PO, Adu-Gyamfi EA, *et al*. Genomic and epidemiological characteristics of SARS-CoV-2 in Africa. PLoS Negl Trop Dis 2021;15:e0009335. doi: 10.1371/journal.pntd.0009335.
104. Padilla-Rojas C, Jimenez-Vasquez V, Hurtado V, Mestanza O, Molina IS, Barcena L, *et al*. Genomic analysis reveals a rapid spread and predominance of lambda (C.37) SARS-COV-2 lineage in Peru despite circulation of variants of concern. J Med Virol 2021;93:6845–6849. doi: 10.1002/jmv.27261.
105. Aleem A, Akbar Samad AB, Slenker AK. Emerging variants of SARS-CoV-2 and novel therapeutics against coronavirus (COVID-19). In: StatPearls [Internet]. Treasure Island: StatPearls Publishing; 2022.
106. Kimura I, Kosugi Y, Wu J, Zahradnik J, Yamasoba D, Butlertanaka EP, *et al*. The SARS-CoV-2 Lambda variant exhibits enhanced infectivity and immune resistance. Cell Rep 2022;38:110218. doi: 10.1016/j.celrep.20-21.110218.
107. Xie X, Han JB, Ma G, Feng XL, Li X, Zou QC, *et al*. Emerging SARS-CoV-2 B.1.621/Mu variant is prominently resistant to inactivated vaccine-elicited antibodies. Zool Res 2021;42:789–791. doi: 10.24272/j.issn.2095-8137.2021.343.
108. Zhou P, Yang XL, Wang XG, Hu B, Zhang L, Zhang W, *et al*. A pneumonia outbreak associated with a new coronavirus of probable bat origin. Nature 2020;579:270–273. doi: 10.1038/s41586-020.
109. Zhou P, Yang XL, Wang XG, Hu B, Zhang L, Zhang W, *et al*. Addendum: a pneumonia outbreak associated with a new coronavirus of probable bat origin. Nature 2020;588:E6. doi: 10.1038/s41586-020-2951-z.
110. Latinne A, Hu B, Olival KJ, Zhu G, Zhang L, Li H, *et al*. Origin and cross-species transmission of bat coronaviruses in China. Nat Commun 2020;11:4235. doi: 10.1038/s41467-020-17687-3.
111. Andersen KG, Rambaut A, Lipkin WI, Holmes EC, Garry RF. The proximal origin of SARS-CoV-2. Nat Med 2020;26:450–452. doi: 10.1038/s41591-020-0820-9.
112. Wacharapluesadee S, Tan CW, Maneeorn P, Duengkae P, Zhu F, Joyjinda Y, *et al*. Evidence for SARS-CoV-2 related coronaviruses circulating in bats and pangolins in Southeast Asia. Nat Commun 2021;12:972. doi: 10.1038/s41467-021-21240-1.
113. Lytras S, Xia W, Hughes J, Jiang X, Robertson DL. The animal origin of SARS-CoV-2. Science 2021;373:968–970. doi: 10.1126/science.abh0117.
114. Holmes EC, Goldstein SA, Rasmussen AL, Robertson DL, Crits-Christoph A, Wertheim JO, *et al*. The origins of SARS-CoV-2: a critical review. Cell 2021;184:4848–4856. doi: 10.1016/j.cell.2021.08.017.
115. Temmam S, Vongphayloth K, Salazar EB, Munier S, Bonomi M, Regnault B, *et al*. Bat coronaviruses related to SARS-CoV-2 and infectious for human cells. Nature 2022;604:330–336. doi: 10.1038/s41586-022-04532-4.
116. Oude Munnink BB, Sikkema RS, Nieuwenhuijse DF, Molenaar RJ, Munger E, Molenkamp R, *et al*. Transmission of SARS-CoV-2 on mink farms between humans and mink and back to humans. Science 2021;371:172–177. doi: 10.1126/science.abe5901.
117. Kuchipudi SV, Surendran-Nair M, Ruden RM, Yon M, Nissly RH, Vandegrift KJ, *et al*. Multiple spillovers from humans and onward transmission of SARS-CoV-2 in white-tailed deer. Proc Natl Acad Sci U S A 2022;119:e2121644119. doi: 10.1073/pnas.2121644119.
118. Zhang Y, Li J, Xiao Y, Zhang J, Wang Y, Chen L, *et al*. Genotype shift in human coronavirus OC43 and emergence of a novel genotype by natural recombination. J Infect 2015;70:641–650. doi: 10.1016/j.jinf.2014.12.005.

119. Varabyou A, Pockrandt C, Salzberg SL, Pertea M. Rapid detection of inter-clade recombination in SARS-CoV-2 with Bolotie. Genetics 2021;218:iyab074. doi: 10.1093/genetics/iyab074.
120. Jackson B, Boni MF, Bull MJ, Colleran A, Colquhoun RM, Darby AC, *et al*. Generation and transmission of interlineage recombinants in the SARS-CoV-2 pandemic. Cell 2021;184:5179–5188. e8. doi: 10.1016/j.cell.2021.08.014.
121. He Y, Ma W, Dang S, Chen L, Zhang R, Mei S, *et al*. Possible recombination between two variants of concern in a COVID-19 patient. Emerg Microbes Infect 2022;11:552–555. doi: 10.1080/22221751.2022.2032375.
122. Kreier F. Deltacron: the story of the variant that wasn't. Nature 2022;602:19. doi: 10.1038/d41586-022-00149-9.
123. VanInsberghe D, Neish AS, Lowen AC, Koelle K. Recombinant SARS-CoV-2 genomes circulated at low levels over the first year of the pandemic. Virus Evol 2021;7:veab059. doi: 10.1093/ve/veab059.
124. Kosakovsky Pond SL, Posada D, Gravenor MB, Woelk CH, Frost SD. GARD: a genetic algorithm for recombination detection. Bioinformatics 2006;22:3096–3098. doi: 10.1093/bioinformatics/btl474.
125. Lole KS, Bollinger RC, Paranjape RS, Gadkari D, Kulkarni SS, Novak NG, *et al*. Full-length human immunodeficiency virus type 1 genomes from subtype C-infected seroconverters in India, with evidence of intersubtype recombination. J Virol 1999;73:152–160. doi: 10.1128/jvi.73.1.152-160.1999.
126. Shen Z, Xiao Y, Kang L, Ma W, Shi L, Zhang L, *et al*. Genomic diversity of severe acute respiratory syndrome-coronavirus 2 in patients with coronavirus disease 2019. Clin Infect Dis 2020;71:713–720. doi: 10.1093/cid/ciaa203.
127. Vlachogiannis NI, Verrou KM, Stellos K, Sfikakis PP, Paraskevis D. The role of A-to-I RNA editing in infections by RNA viruses: possible implications for SARS-CoV-2 infection. Clin Immunol 2021;226:108699. doi: 10.1016/j.clim.2021.108699.
128. Kosuge M, Furusawa-Nishii E, Ito K, Saito Y, Ogasawara K. Point mutation bias in SARS-CoV-2 variants results in increased ability to stimulate inflammatory responses. Sci Rep 2020;10:17766. doi: 10.1038/s41598-020-74843-x.
129. Menéndez-Arias L. Mutation rates and intrinsic fidelity of retroviral reverse transcriptases. Viruses 2009;1:1137–1165. doi: 10.3390/v1031137.
130. Smith EC, Blanc H, Surdel MC, Vignuzzi M, Denison MR. Coronaviruses lacking exoribonuclease activity are susceptible to lethal mutagenesis: evidence for proofreading and potential therapeutics. PLoS Pathog 2013;9:e1003565. doi: 10.1371/journal.ppat.1003565.
131. Sanjuán R, Nebot MR, Chirico N, Mansky LM, Belshaw R. Viral mutation rates. J Virol 2010;84:9733–9748. doi: 10.1128/jvi.00694-10.
132. Duffy S, Shackelton LA, Holmes EC. Rates of evolutionary change in viruses: patterns and determinants. Nat Rev Genet 2008;9:267–276. doi: 10.1038/nrg2323.
133. Hadfield J, Megill C, Bell SM, Huddleston J, Potter B, Callender C, *et al*. Nextstrain: real-time tracking of pathogen evolution. Bioinformatics 2018;34:4121–4123. doi: 10.1093/bioinformatics/bty407.
134. Aktas E. Bioinformatics analysis unveils certain mutations implicated in spike sructure damage and ligand-binding site of severe acute respiratory syndrome coronavirus 2. Bioinform Biol Insights 2021;15:11779322211018200. doi: 10.1177/11779322211018200.
135. Wilhelm A, Toptan T, Pallas C, Wolf T, Goetsch U, Gottschalk R, *et al*. Antibody-mediated neutralization of authentic SARS-CoV-2 B.1.617 variants harboring L452R and T478K/E484Q. Viruses 2021;13:1693. doi: 10.3390/v13091693.
136. Weisblum Y, Schmidt F, Zhang F, DaSilva J, Poston D, Lorenzi JC, *et al*. Escape from neutralizing antibodies by SARS-CoV-2 spike protein variants. Elife 2020;9:e61312. doi: 10.7554/eLife.61312.
137. Focosi D, Novazzi F, Genoni A, Dentali F, Gasperina DD, Baj A, *et al*. Emergence of SARS-COV-2 spike protein escape mutation Q493R after treatment for COVID-19. Emerg Infect Dis 2021;27:2728–2731. doi: 10.3201/eid2710.211538.
138. Wang M, Zhang L, Li Q, Wang B, Liang Z, Sun Y, *et al*. Reduced sensitivity of the SARS-CoV-2 Lambda variant to monoclonal antibodies and neutralizing antibodies induced by infection and vaccination. Emerg Microbes Infect 2022;11:18–29. doi: 10.1080/22221751.2021.2008775.
139. Braun KM, Moreno GK, Halfmann PJ, Hodcroft EB, Baker DA, Boehm EC, *et al*. Transmission of SARS-CoV-2 in domestic cats imposes a narrow bottleneck. PLoS Pathog 2021;17:e1009373. doi: 10.1371/journal.ppat.1009373.
140. Saito A, Irie T, Suzuki R, Maemura T, Nasser H, Uriu K, *et al*. Enhanced fusogenicity and pathogenicity of SARS-CoV-2 Delta P681R mutation. Nature 2022;602:300–306. doi: 10.1038/s41586-021-04266-9.

How to cite this article: Dang S, Ren L, Wang J. Functional mutations of SARS-CoV-2: implications to viral transmission, pathogenicity and immune escape. Chin Med J 2022;135:1213–1222. doi: 10.1097/CM9.0000000000002158