Mutations in the genome of severe acute respiratory syndrome coronavirus 2: implications for COVID-19 severity and progression

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Abstract
Coronaviridae is a large family of enveloped, positive-strand RNA viruses that has plagued the world since it was discovered in humans in the 1960s. The recent severe acute respiratory syndrome coronavirus (SARS-CoV)-2 pandemic has already exceeded the number of combined cases and deaths witnessed during previous SARS-CoV and Middle East respiratory syndrome-CoV epidemics in the last two decades. This narrative review focuses on genomic mutations in SARS-CoV-2 and their impact on the severity and progression of COVID-19 in light of reported data in the literature. Notable SARS-CoV-2 mutations associated with open reading frames, the S glycoprotein, and nucleocapsid protein, currently circulating globally, are discussed along with emerging mutations such as those in the SARS-CoV-2 VUI 202012/01 variant in the UK and other European countries, the 484K.V2 and P.1 variants in Brazil, the B.1.617 variant in India, and South African variants 501Y.V2 and B.1.1.529 (omicron). These variants have the potential to influence the receptor binding domain, host–virus fusion, and SARS-CoV-2 replication. Correlating these mutations with disease dynamics could help us understand their pathogenicity and design appropriate therapeutics.

Keywords
Severe acute respiratory syndrome coronavirus-2, coronavirus disease 2019, genome, mutation, disease progression, pathogenicity

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Introduction

Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), the causative agent of the coronavirus disease 2019 (COVID-19) global pandemic, belongs to the Betacoronavirus genus that frequently undergoes genomic changes during transmission between hosts. SARS-CoV-2 genetically resembles the bat coronavirus isolate RaTG13 (approximately 96% sequence identity), Pangolin-CoV (approximately 91% sequence identity), and two previous coronaviruses: SARS-CoV and Middle East respiratory syndrome coronavirus (MERS-CoV) (approximately 79% and 52% sequence identity, respectively). The SARS-CoV-2 viral genome encodes several open reading frames (ORFs): ORF3a, ORF6, ORF7a, ORF7b, ORF8, ORF10, and ORF1a/ORF1ab located in the 5’ untranslated region (UTR). It also encodes four structural viral proteins: spike (S), envelope (E), membrane (M), and nucleocapsid (N) located in the 3’ UTR.

The high cross-species spillover and mutagenicity of human coronaviruses likely steered an outbreak of SARS-CoV-2 in late December 2019 at the wholesale animal and seafood market in Wuhan, China. Since then, SARS-CoV-2 has caused havoc worldwide and was declared a global public health emergency by the World Health Organization (WHO). At the time of writing (November 27, 2021), SARS-CoV-2 cases exceed 261 million and have claimed nearly 5 million lives worldwide. It has spread across several age groups within a short period, but the disease is more severe in older individuals, indicating the highly lethal nature of the virus in individuals with low immunity. It also impairs multiple organs including the heart, brain, liver, and kidney, facilitating the development of multi-organ failure.

The second worldwide wave of COVID-19 increased interest in SARS-CoV-2 research, particularly in delineating the components of the SARS-CoV-2 genome to discern whether alterations have the potential to influence virulence and infectivity. Early phylogenetic analyses of S, V, and G superclades mined from different geographic outbreaks in Europe, China, and the United States showed little indication of local adaptation, suggesting that evolution in SARS-CoV-2 is largely caused by founder effects and genetic drift. However, some studies forecast potential structural variations at the nucleotide and protein levels. Interestingly, a report by Shen et al. identified intra-host viral mutations post-COVID-19 infection and suggested that these could reflect evolution of the virus to escape host immunity.

RNA viruses are the most prevalent of human infectious viruses. The lack of proof-reading activity of RNA-dependent RNA polymerases results in the accumulation of mutations at a level more than a million times greater than their hosts. This high degree of variation leads to the continuous production of variants with a complex mixture of different but closely related genomes known as quasi-species. Mutants composing the quasi-species replicate and are subjected to competitive selection and cooperation. Some mutants may carry phenotypic characteristics that affect the severity and transmissibility of the disease.

SARS-CoV-2 has a moderate mutation rate, which is below the expected range of other RNA viruses and more similar to that of some small single-stranded DNA viruses. This is consistent with a role for nonstructural protein (NSP)14 in RNA proofreading or repair, which has 3’–5’ exonuclease activity and has been responsible for the fixation of several mutations in the genome, such as D414G. Observations of RNA viruses have previously shown them to have error-prone polymerases and lack
RNA proofreading functions, resulting in low replication fidelity that enables rapid evolution and adaptation to new environments. However, NSP14 is responsible for proof-reading in SARS-CoV-2, and thereby a high fidelity of genome replication that might contribute to sluggish evolution.\textsuperscript{23} Other mutations in the \textit{ORF7a} gene (T14I, V29L, S36F, V71I, S81L, V93F, E95K, P99S, and I110T)\textsuperscript{24,25} and \textit{NSP2} gene (F190L, P193S, R207C, E217G, T224I, and T265I)\textsuperscript{26,27} have enabled the classification of multiple SARS-CoV-2 lineages.\textsuperscript{28} Prominent SARS-CoV-2 mutations with high transmissibility include those present in ORFs, the S glycoprotein, and nucleocapsid proteins. These, together with emerging variants such as SARS-CoV-2 VUI 202012/01 in the UK and other European countries, SARS-CoV-2 501Y.V2 in South Africa, and 484K.V2 and P.1 in Brazil are currently circulating worldwide.\textsuperscript{29,30} Figure 1 represents the regional distribution of SARS-CoV-2 variants in new genomic sequences.\textsuperscript{31} Genomic mutations in SARS-CoV-2 could enhance viral pathogenicity, so may be involved in the development and progression of COVID-19.\textsuperscript{32}

Identifying and understanding the role of different viral mutations that influence disease progression and severity is best achieved through a comprehensive evaluation of the literature. This narrative review presents recent updates on SARS-CoV-2 mutations and their association with the changing dynamics of COVID-19 severity and outcomes. PubMed and ScienceDirect databases were searched to extract research articles written in the English language with the following keywords alone and in combination using Boolean operators (AND, OR, NOT): ‘Severe Acute Respiratory Syndrome Coronavirus 2’, ‘SARS-CoV-2’,

\begin{figure}[h]
\centering
\includegraphics[width=\textwidth]{figure1.png}
\caption{Regional distribution of SARS-CoV-2 variants in new genomic sequences (Updated February 04, 2022).\textsuperscript{107} The current regional predominance of different SARS-CoV-2 variants in new sequences. With the exception of Africa, where the delta variant dominates in new sequences, the omicron variant is dominant in all regions.}
\end{figure}
‘Coronavirus Disease’, ‘Coronavirus Disease 2019’, ‘COVID-19’, ‘Gene Mutation’, ‘Mutation’, ‘Variants’, ‘Severity’, ‘Severe Disease’, ‘Progression’, ‘Progressive Disease’, ‘Hospitalization’, and ‘Mortality’. The abstracts of identified articles were thoroughly reviewed to determine their relevance, and the reference sections were also reviewed where possible to identify additional relevant articles.

Mutations in the SARS-CoV-2 genome

The SARS-CoV-2 genome encodes several ORFs. Polyproteins pp1a and pp1ab are encoded by two-thirds of the genome, as part of the first ORF. The remaining ORFs encode both structural (S, M, E, and N) and accessory proteins. Of the four structural proteins, the S (spike) protein is central to viral attachment to host cells and cellular entry via the angiotensin-converting enzyme 2 (ACE2) receptor. It is the main target protein for antiviral antibodies and vaccine development. In contrast, accessory proteins such as 3a, 3b, 6, 7a, and 7b facilitate the evasion of SARS-CoV-2 from host immune responses. The ability of viruses to adapt to new niches primarily reflects their rapid evolution of beneficial mutations to better fit an unfavorable environment. The evolution rate or rate of fixation of mutations in CoV is around $10^{-6}$ to $10^{-7}$ substitutions/nucleotide/year, which is similar to the $8.4 \times 10^{-4}$ seen in other RNA viruses. As viral variants appear over time, diverse lineages are shaped and then clustered to form clades and subclades, as evidenced by the more than 245,000 SARS-CoV-2 genomic sequences available in the Global Initiative on Sharing Influenza Data (GISAID) database on December 7, 2020. SARS-CoV-2 was initially grouped into L and S types based on two single nucleotide polymorphisms in ORF1ab and ORF8, but is now categorized into V, G, GR, GH, T, and O clades. The SARS-CoV-2 genome is not immune to the high mutation rate common to other RNA viruses because of mutations in NSP14, which primarily assists in proof-reading and high genomic fidelity; as different mutations have been documented during transmission across the globe. This in turn enhanced the potential of SARS-CoV-2 transmission, infectivity, and virulence.

SARS-CoV-2 variant D614G

Mutations in the ORF encoding the SARS-CoV-2 S protein are well documented. The S protein mediates binding of the virus to the cellular ACE2 receptor through the receptor binding domain (RBD), and thus coordinates the host–virus interaction. The D614G mutation of the S glycoprotein was first reported in March 2020. Initially, it was found only sporadically, but was rapidly transmitted across global boundaries. In combination with other mutations, the D614G mutation was reported to increase the infectivity and antigenicity of SARS-CoV-2. Studies have demonstrated an association between high viral loads in the upper respiratory tract and the D614G mutation, and a large population-based phylogenetic analysis of more than 25,000 sequences from the UK recently showed that SARS-CoV-2 harboring the D614G mutation is transmitted more rapidly than the original wild-type virus. Animal model studies have demonstrated the higher infectivity and pathogenic potential of SARS-CoV-2 carrying the D614G mutation, while there is also evidence of enhanced virus survival and fitness, infectivity and transmissibility, and high viral load. Additionally, the D614G mutation may have epistatic interactions with other mutations. For example, a retrospective genomic sequencing
study from Italy identified a combination of D614G and S939F mutations in the S protein of SARS-CoV-2 in patients with COVID-19. In contrast to D614G, S939F only influences the immune response mildly; however, the substantial modulation of T-lymphocytes and enrichment of potential epitopes for some human leukocyte antigen (HLA) alleles have been reported.51

Other important mutations

A study by Islam et al. in 2020 reported 1516 synonymous and nonsynonymous nucleotide mutations spanning the entire SARS-CoV-2 genome, of which 1247 are located within the coding region and 269 in the noncoding region. The nonsynonymous mutations were shown to be causative of 744 amino acid substitutions, while 412 amino acid substitutions in ORF1ab and 228 in S, M, E, and N were also reported. Additionally, amino acid substitutions were found in accessory proteins ORF3a, ORF6, ORF7ab, ORF8, and ORF10.27 Similar ORF mutations were also reported in a study on Indian SARS-CoV-2 isolates by Singh et al.52 Sheikh et al. reported that the 5' region of SARS-CoV-2 RNA is more vulnerable to genetic alterations than the 3' region, and is an important orchestrator of viral evolution and diversity.42 The mutations are thought to assist SARS-CoV-2 in immune escape from neutralizing antibodies, and may increase transmission via higher ACE2 receptor affinity.53,54

Several other mutations in the S protein have also been identified; these include Q57H and V367 variants from French isolates, and A930V, G1124V, and R407I variants from Indian isolates.43,44,55 These genetic alterations result in a wide range of adaptive features, such as high infectivity, pathogenicity, and replication, a stronger host–virus interaction and host–receptor affinity, and changes to genomic evolution and phylogenetic cluster divergence.41,47,54 SARS-CoV-2 isolates from Brazil, Germany, and the USA were reported to harbor G251V, G392D, and L84S mutations, respectively.43 Of these, only G251V has been identified worldwide.27 Mutations in the RBD of the S glycoprotein, S477N, N439K, V483A, and V367F, are suggested to increase the binding affinity of SARS-CoV-2 to the ACE2 receptor, facilitating transmission and infectivity.42 Other mutations have been described in the N gene, which mediates SARS-CoV-2 capsid formation.56,57 For example, G204R, R203K, and S197L were first described in India, Chile, and the USA, respectively, and then spread worldwide.27 They are hypothesized to play an important part in modulating the function of protein–protein interactions and structural assembly of the N protein.56

P323L is a key mutation in the SARS-CoV-2 viral RNA dependent RNA polymerase gene (RdRp), which encodes an enzyme that facilitates SARS-CoV-2 genome replication and exhibits a proof-reading function. RdRp mutations may therefore enhance viral infectivity and disease severity, and P323L has been associated with a high mutation rate.58

Newly emerging mutations

VUI 202012/01 (alpha variant)

The new phylogenetic cluster lineage B.1.1.7 (also known as the alpha variant or SARS-CoV-2 VUI 202012/01 [Variant Under Investigation, Year 2020, Month 12, Variant 01]) circulated rapidly in the UK during autumn 2020.59 This exhibited multiple S glycoprotein alterations including deletion 69_70, deletion 145, and mutations A570D, N501Y, D614G, T716I, P681H, S982A, and D1118H.60 Additionally, the N501Y mutation is found in the RBD of the S protein, while P681H and deletion
69_70 mutations are located in the furin cleavage site, indicating their possible role in influencing ACE2 receptor affinity.46 The first case infected with this variant was identified on 20 September 2020. About 28% of SARS-CoV-2 cases in the UK were attributed to this variant between September and December 2020, and genomic models indicated that it was 56% more contagious than other variants.32

**501Y.V2 (beta variant)**

Another SARS-CoV-2 variant, 501Y.V2 (also known as the beta variant or 20C/501Y.V2 of B.1.351 lineage), was identified that derived from South Africa. This variant was found to have a high binding ability to human ACE2 receptors,61 and to possess three mutations in the binding site of the S protein: E484K, K417N, and N501Y. It was reported to have spread to Australia, France, South Korea, Sweden, Switzerland, and the UK.62 Concerns have been raised that the mutations might render SARS-CoV-2 vaccines to be less effective,63,64 but a study by Xie et al. showed no absence of antibody neutralization efficacy in the recently licensed Pfizer BioNTech vaccine.65

**Variant 484K.V2**

More recently, B.1.1.28 subclade variants 484K.V2 and P.1 were identified in Brazil. 484K.V2 mutations are located in the S protein and have a phylogenetic origins of July 2020.66 The variant has thus far reached the UK, USA, Singapore, Norway, Denmark, Argentina, Canada, and Ireland. In particular, the E484K mutation of variant 484K.V2 is of interest because it appears to allow immune escape and evasion.66,67

**P.1 (gamma variant)**

SARS-CoV-2 variant P.1, also called the gamma variant, was discovered in four individuals traveling from Brazil to Haneda airport in Tokyo. It contains a cluster of mutations, especially in the S protein RBD, indicating that this variant will modulate viral entry to host cells.61 It remains to be determined how these mutations will affect the dynamics of SARS-CoV-2 pathogenicity, but the variant is predicted to have high transmissibility, like the beta variant as both share identical mutations (N501Y, E484K, and K417N/T).

**B.1.617 (delta variant)**

Underlying the more recent abrupt surge in COVID-19 cases and fatalities in India is SARS-CoV-2 variant B.1.617 (the delta variant), also now referred to as a ‘double mutant variant’.68 First detected in December 2020, this variant has pushed the healthcare resources of India to the brink of fatigue. Three diverse lineages of B.1.617 thus far, B.1.617.1, B.1.617.2, and B.1.617.3, have been reported,69 with multiple spike protein alterations linked to diverse viral characteristics. These include: E484Q (B.1.617.1 and B.1.617.3 only) which is associated with reduced neutralization by convalescent serum; L452R linked to high transmissibility and reduced neutralization by convalescent serum and therapeutic antibodies; and P681R associated with impact S1/S2 cleavage, viral entry, and infectivity.70 Cherian et al. demonstrated that E484Q and L452R mutations in the RBD suppress the affinity to REGN10933 and P2B-2F6 monoclonal antibodies,70 while Motozono et al. showed that the L452R mutation caused the evasion of HLA-associated cellular immunity, leading to enhanced viral infectivity and replication.71 However, Yadav et al. documented that the BBV152 vaccine showed promise in neutralizing the B.1.617.1 variant obtained from SARS-CoV-2 isolates of asymptomatic and mildly symptomatic patients with COVID-19 in India.70 Figure 2 depicts the
recently emerged important SARS-CoV-2 variants with their country of origin.

**Mink variant Y453F**

In the late spring and early summer of 2020, Denmark and the Netherlands reported SARS-CoV-2 outbreaks in mink farms. Further exploration revealed mink to mink, mink to human, and human to mink transmissions. Danish officials reported over 200 cases of COVID-19 linked to mink farms, and a new mutation (Y453F) in the RBD of the S glycoprotein. This mutation is thought to strengthen the affinity of SARS-CoV-2 to mink ACE2 receptors. Other mutations in this variant were also detected in the S glycoprotein, including del69_70 (deletion mutation), I692V, S1147L, and M1229I (substitution mutation), in 11 patients from Denmark who were infected with “Cluster 5” SARS-CoV-2 variants causing four changes in the S protein sequence.

**B.1.1.529 (omicron variant)**

The first confirmed case of infection by the SARS-CoV-2 B.1.1.529 variant (also known as omicron) was detected on November 9, 2021 in South Africa and reported on November 24, 2021 by the WHO. This new variant has high transmissibility and a high risk of reinfection.
compared with other earlier variants. Most of its mutations are within the RBD protein: E484A, Q493K, G496S, Q498R, N501Y, Y505H, T547K, G339D, S371L, S373P, S375F, K417N, N440K, G446S, S477N, and T478K.

Mutations in the SARS-CoV-2 genome and COVID-19 severity and progression

Genetic variations in viruses not only impact on prevention and diagnosis, but also provide a perspective for potential treatment. Mutations are regarded as the cornerstone for viral evolution because they promote viral diversity and survival within their hosts. While new mutations continue to accumulate in SARS-CoV-2, it is prudent to explore their influence on factors involved with disease severity and progression such as hospitalization, the need for intensive care unit (ICU), mechanical ventilation support, and the case fatality rate (CFR).

One key measure to delineate COVID-19 severity and progression is the CFR, which could widely vary between different studies. Various statistically significant CFRs have been reported for COVID-19. For example, one systematic quantitative analysis of 26 studies found a CFR of 0.68%, while emphasizing that this might be an underestimation, while another meta-analysis of 61 studies cited a CFR of 0.26.

Studies have reported dissimilar infection severities when age-based strata were formed, and such variations were more likely to be higher when considering the older population (≥65 years). Another study documented 90% geographical differences in disease severity based on the age dynamics of the population. This variation in virulence could reflect the distribution of diverse SARS-CoV-2 genomic variants worldwide. Earlier papers have claimed that the evolution of new strains is anticipated from comparisons with RNA viruses, although the identification of such naturally fitting SARS-CoV-2 strains is a matter of concern.

SARS-CoV-2 variant D614G and disease severity

Data ascertaining the association between SARS-CoV-2 mutations and COVID-19 severity and progression are limited. Korber et al. performed sequence analysis of GISAID data and identified SARS-CoV-2 variants across different geographic locations using bioinformatics. Interestingly, they observed that the D614G mutation, which was globally present, was simultaneously present with three other mutations: C241T, C3037T, and C14408T. Their analysis also showed a correlation between the D614G mutation and increased viral load, highlighting the high infectivity in SARS-CoV-2 strains carrying D614G. It is noteworthy that the D614G mutation was not associated with COVID-19 severity measures such as the need for hospitalization or ICU. A correlation between D614G and the CFR in various countries was reported, while Legros et al. found that D614G did not influence the neutralizing antibody activity, which could correlate with the severity of COVID-19 and might govern viral fitness and infectivity.

Omicron variant and disease severity

The omicron variant is far more infectious than previous variants, but little has been reported about its influence on COVID-19 severity. A multicenter, national, retrospective study by Wang et al. from the USA reviewed data from patients with
COVID-19, including 577,938 infected between 1 September 2021 and 24 December 2021, 14,054 infected between 15 December 2021 and 24 December 2021 (the omicron variant emergence period), and 563,884 infected between 1 September 2021 and 15 December 2021 (the delta variant predominance period). They reported significant differences in sociodemographic and clinical characteristics of omicron- and delta-affected cohorts. After constructing an artificial control group using propensity score matching for sociodemographic, comorbidities, and other key variables, the 3-day risks of emergency admission (4.55% versus 15.22%; risk ratio [RR]: 0.30, 95% confidence interval [CI] 0.28–0.33), hospitalization (1.75% versus 3.95%; RR: 0.44, 95% CI 0.38–0.52), ICU admission (0.26% versus 0.78%; RR: 0.33, 95% CI 0.23–0.48), and mechanical ventilation (0.07% versus 0.43%; RR: 0.16, 95% CI 0.08–0.32) were significantly lower than that of delta-infected patients. Likewise, the risks were also lower in pediatric COVID-19 cases (<5 years) infected with omicron compared with delta in terms of emergency admission (3.89% versus 21.01; RR: 0.19, 95% CI 0.14–0.25) and hospitalization (0.96% versus 2.65%; RR: 0.36, 95% CI 0.19–0.68). These findings were also similar for other age cohorts.

According to a recent US study by Luliano et al., the very first clinical case of omicron was identified on 1 December 2021 in the USA, and by 15 January 2022, 99.5% of sequenced specimens were omicron. To improve the understanding of the role of omicron in disease severity and its associated impact on healthcare resources, the US Centers for Disease Control and Prevention assessed data from three surveillance systems and a large healthcare database system to examine diverse predictors across three COVID-19 infection timelines (1 December 2020 to 28 February 2021, 15 July 2021 to 31 October 2021 [delta], and 19 December 2021 to 15 January 2022 [omicron]). While the average number of cases in terms of emergency admissions and hospitalization was higher during the omicron emergence, overall deaths were substantially lower compared with early timelines without omicron. It was also noted that the utilization of in-patient hospital beds during the omicron wave was 7.2 points higher than that during the delta predominance. However, ICU admissions were 1.2 percentage points lower during the omicron timeline compared with the delta timeline. Similarly, the ratio of emergency admissions to cases (87 per 1000 cases versus 167 per 1000 cases), hospital admissions (27 per 1000 cases versus 78 per 1000 cases), and mortality (9 per 1000 cases versus 13 per 1000 cases) were lower during the omicron period compared with the delta period. Mean duration of hospital and ICU stays, use of invasive mechanical ventilation, and in-hospital mortality were also lower for the omicron variant.

A South African study by Wolter et al. examined COVID-19 severity from the omicron variant via S gene target failure (SGTF) COVID-19 PCR testing, grouping patients as non-SGTF or SGTF. Genomic sequencing was used to recognize the delta variant. The study found that the SGTF cohort had a significantly reduced risk of hospital admission compared with their non-SGTF counterpart (adjusted odds ratio [aOR] 0.2, 95% CI 0.1–0.3). Both cohorts (hospitalized) showed no difference in the risk of developing severe disease (aOR 0.7, 95% CI 0.3–1.4). However, SGTF individuals had a significantly lower risk of disease severity compared with patients infected with the delta variant (aOR 0.3, 95% CI 0.2–0.5).

A recent comparative study by Zhao et al. demonstrated that replication of the omicron variant was slower than that of the delta variant in VeroE6 (VeroE6/TMPRSS2) cells expressing transmembrane
serine protease 2 (TMPRSS2), as well as in the Calu3 cell line. Additionally, the omicron variant showed weaker cell to cell fusion activity compared with the delta variant in VeroE6/TMPRSS2 cells.\(^9^0\)

The lower risk of disease severity associated with the SARS-CoV-2 omicron variant compared with earlier variants may be caused by mass vaccination which significantly diminishes disease progression and severity,\(^9^1\) its minimal virulence,\(^9^2\)–\(^9^4\) and acquired immunity from past exposure to SARS-CoV-2.\(^9^2\)–\(^9^5\) However, the reportedly high frequency of emergency admissions and hospitalizations associated with omicron can nevertheless still considerably drain healthcare resources.

**Other important mutations and disease severity**

In a genomic analysis of the GISAID database, Voss *et al.* demonstrated that the C13620T mutation in the SARS-CoV-2 genome is associated with a nearly 6-fold increased risk of severe COVID-19 manifestation through altered expression of the NSP12 protein.\(^9^6\) Because NSP12 is linked with viral RNA transcription, the C13620T mutation may result in increased virulence and disease severity.\(^9^7\) Two nonsynonymous mutations, V1176F (G25088T) and S477N (G22992A), in SARS-CoV-2 were shown to alter the S glycoprotein affinity to the ACE2 receptor, so may also play an important role in disease severity. Both mutations have been correlated with high mortality.\(^9^8\)–\(^9^9\)

Another study of the GISAID SARS-CoV-2 repository by Nagy *et al.* found that mutations in ORF8, NSP6, ORF3a, NSP4, and nucleocapsid phosphoprotein N were associated with a mild COVID-19 outcome.\(^1^0^0\) Patients were classified into mild (asymptomatic and not hospitalized), hospitalized (hospitalized, inpatient, discharged, and recovered), and severe (hospitalized, ICU, and deceased) groups. Mutations that correlated with poor outcome were found in S, RdRp, ORF3a, NSP3, ORF6, and N proteins, while those in ORF3A and NSP7 proteins were associated with severe outcomes.\(^1^0^0\)

ORF3a functions in trafficking of the S glycoprotein in a complex pathway.\(^1^0^1\)–\(^1^0^2\) The G26144T mutation leads to an ORF3a amino acid substitution, and was shown to be associated with a 4.3-fold higher risk of disease severity. This mutation results in a disorganized protein–protein interaction of ORF3a with TRAF3 and might promote the activation of inflammatory cytokines.\(^1^0^3\)–\(^1^0^4\)

The deletion mutation Δ382 of SARS-CoV-2 was detected in 29 patients with COVID-19 from Singapore.\(^1^0^5\) This study suggested that hypoxia and the requirement for supplemental oxygen was lower in patients infected with the Δ382 variant compared with those infected with wild-type SARS-CoV-2. After adjusting for confounding factors such as age and comorbidity, the Δ382 mutation was found to be associated with a reduced risk of developing hypoxia necessitating supplemental oxygen, suggesting a milder infection.

The relationship between geo-climate and COVID-19 severity has been evaluated in a study that found high mortality in European temperate countries and correlations with novel mutations.\(^2^7\) These mutations were also detected in Asian and North American SARS-CoV-2 isolates, but demonstrated a lower CFR compared with European countries. This discrepancy led to the prediction that the mutations in European isolates might be highly pathogenic. However, disease severity might be confounded by other factors such as age, host genetics, healthcare services, and adherence to preventive measures.

A study by Biswas *et al.* compared the mutation profiles of patients mildly and severely affected with COVID-19.
They identified P323L and D614G mutations as being associated with disease severity, and also observed that missense mutations 14408C > T (RdRp) and 23403 A > G (S glycoprotein) were predominant in patients severely affected with COVID-19 compared with mildly affected patients. Moreover, 241C > T and 3037C > T mutations in ORF1ab were mainly present in severely affected patients; however, these mutations did not change the amino acid sequence. These findings suggest that RdRp and S protein mutations alter the dynamics of COVID-19 severity. Recently, the WHO renamed the most common worldwide variants, as shown in Table 1.

**Implications**

Little is currently known about the clinical implications of these SARS-CoV-2 variants. However, because some variants cause higher transmission rates resulting in a higher number of cases is likely to increase the need for clinical healthcare support that worsens the burden on an already stressed healthcare system, causing more fatalities. Therefore, it is imperative to conduct persistent surveillance of the SARS-CoV-2 genome and identify cases with new variants, as well as to monitor the emergence of other novel variants. This will help direct public health action, especially when subsequent waves of COVID-19 are looming.

**Recommendation**

Notwithstanding the discussed findings having important clinical implications, the literature citing an association between novel mutations in SARS-CoV-2 and disease dynamics is extremely limited. Only countable studies (genomic analyses and observational studies) have been performed on SARS-CoV-2 sequences using the GISAID database, so the data might not be demonstrative of all circulating

| Variants of concern | PANGO lineage | Country and date of first identification | Designation date | Important mutations | Risk of severe disease (hospitalization, ICU admission, and mortality) |
|---------------------|---------------|----------------------------------------|------------------|---------------------|-----------------------------------------------------------|
| Alpha B.1.1.7       | UK (September 2020) | December 18, 2020                        | D614G, N501Y, P681H | High[^107]         |
| Beta B.1.351        | South Africa (May 2020) | December 18, 2020                        | K417N, E484K, N501Y, N501Y | Very high[^107] |
| Gamma P.1           | Brazil (November 2020) | January 11, 2021                         | N501Y, E484K      | High[^107]         |
| Delta B.1.617.2     | India (October 2020)  | May 11, 2021                             | E484Q, L452R, E484A, Q493K, G496S | Very high[^107] |
| Omicron B.1.1.529   | South Africa (November 2021) | November 26, 2021                       | E484A, Q493K, G496S | Low[^94,108] |

SARS-CoV-2, severe acute respiratory syndrome coronavirus 2; PANGO, phylogenetic assignment of named global outbreak lineages; WHO, world health organization; ICU, intensive care unit.
SARS-CoV-2 variants given the diverse demographics. This can lead to an overestimation of findings, so should be cautiously interpreted. More detailed and sound methodological studies are needed to investigate this because identifying a link between genetic variants and disease outcomes could impart a mechanistic understanding of the SARS-CoV-2 adaptability to survival.

**Conclusion**

This review highlights several SARS-CoV-2 mutations, while acknowledging that the data related to their role as key prognosticators of severe manifestations of COVID-19 are limited, especially for the omicron variant. Longitudinal studies are required to monitor the role of diverse and emerging SARS-CoV-2 mutations in affecting COVID-19 progression. Moreover, meta-analyses of available studies would assist in planning and mobilizing healthcare resources and developing management strategies at both healthcare and government levels. Finally, transcriptomic and proteomic analyses of SARS-CoV-2 should be conducted to explore its evolution in the light of disease progression, severity, and outcome.

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