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Key Substitutions in the Spike Protein of SARS-CoV-2 Delta and Omicron Variants

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Abstract. The delta and omicron variants of SARS-CoV-2 (PANGO B.1.617.2. and B.1.1.529) outcompeted the earlier variations shortly after they were discovered due to their quick global dissemination. While the majority of these changes are extremely unlikely to alter the spike protein's composition or function, some might increase the virus's susceptibility to infection or make it less susceptible to vaccination, in which case they are referred to as "variants of concern" (VOC). According to previous research, the severity and transmissibility of mutant spike proteins might change if certain critical changes were present. Based on the verified mutations of spike protein, this article will discuss the specific amino acid mutations' effects on the change in its transmissibility and severity. The main mechanisms affected by the mutation are the cleavage of S1 subunit1/S2 subunit2 and conformation alteration of the receptor binding domain (RBD). In conclusion, mutations in delta drastically increased its transmissibility, and mutations in omicron increased its transmissibility by becoming more stable in the environment with compromised cell fusion ability, leading to lower severity. These results emphasize the intricate relationships between alterations happened in the spike protein which influence virus' stability, neutralization, and cell entering.

Keywords: spike protein, delta, omicron, transmissibility, fusogenicity

1. Introduction

The world is currently in a situation where the Covid-19 virus is rampant with extremely high infectivity and the virus of concern (VOC) has evolved to even increased infectivity or pathogenicity are of great concern. Four existing variants of concern are alpha, beta, delta and omicron, and they replaced the previous wave in sequence. Recorded by 29 August 2022, the Omicron variant of concern (VOC) was accounting for 99.6% of sequences reported globally in the past 30 days.

Most of the mutation sites that used to have a role in infectivity and pathogenicity are in the spike protein. The S protein, a class I viral membrane fusion protein, coats the surface of the coronavirus. This protein is necessary for the virus to connect to and enter human cells, and it is surrounded by glycans that prevent the virus from being recognized by the body's immune system. Starting from the S protein, SARS-CoV-2 has shown to be superior to other coronaviruses in terms of invasion technology. Take the influenza virus for an example, its external fusion protein is relatively rigid. In contrast, the S protein of SARS-CoV-2 is very flexible and can articulate with three sites, which makes it easier to attach to human cells. The S protein is broadly divided into three parts: the S2 subunit primarily aids in the virus's

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merging with the cellular surface, whereas the S1 subunit is connected to the RBD and is in charge of attaching to the receptor on the cell membrane.

This article will analyze the mutant loci in the spike protein of two currently virulent strains, delta and omicron, and determine which sites are mutated and associated with enhanced infectivity and whether their mutated sites overlap or differ. The significance of this study is to indicate which mutations are worth tracking and to note dangerous recombinant mutations might occur in the future.

Table 1. Compilation of major spike proteins mutations in Alpha, Beta, Delta, and Omicron.

Variant:	B.1	B.1.1.7 (alpha)	B.1.351 (beta)	B.1.617.2 (delta)	B.1.1.529 (omicron)
S protein mutations:	D614G	D614G N501Y A570D P681H T716I S982A D1118H Δ*69-70, 144	D614G L18F D80A D215G R246I K417N E484K N501Y A701V	D614G T19R† G142D R158G L452R T478K P681R D950N Δ156-157	69-70del, K417N, N501Y, N679K, P681H, T478K, G142D/143-145del, T95I, +R346K

2. Delta

According to WHO, B.1.617.2 (Delta), which possesses P681R, L452R, and T478K mutations in the spike's RBD, has been classified as a VOC. This is the first time that L452R and T478K (Delta) mutations have been discovered to coexist for Delta variants, as well as the first time that P681R has been noticed. The L452R mutation has increased transmissibility and decreased susceptibility to antibodies produced by vaccination ([1, 2]). The structural investigation of the RBD mutations L452R, T478K, and E484Q found that they may likely boost ACE2 binding, whilst P681R in the furin cleavage site may accelerate the percentage of S1-S2 cleavage, improving transmissibility.

The two RBD mutations L452R and E484Q demonstrated reduced binding to certain monoclonal antibodies (mAbs), which might influence the mAbs' capacity to neutralize. A comparison from a U.K.-based study supports this. There is 88% effectiveness observed after two doses of a previously designed vaccine targeting the alpha variant when it works on Delta, but only 30.7% efficacy after one dose, which is less than the efficacy threshold for COVID-19 vaccines [3].

When the RBD mutations L452R and E484Q were structurally analyzed to see how they affected ACE2 binding, it was found that there were less intramolecular and intermolecular contacts than in the wild type. Since the hydrophobic L452 residue was changed to the hydrophilic 452R, the mutant complex had a lower minimum energy, which suggests that improving interactions with water molecules and overall complex stability may be possible. The increased stability of the RBD-ACE2 complex provided additional evidence of the impact of the mutations L452R and T478K on ACE2 binding.[4]. More virus particles were able to remain in the atmosphere as a result. According to a Chinese research, virus loads in infections generated by the 19A/19B strains during the first 2020 epidemic wave were 1,000 times greater than those in infections caused by the Delta strain.[5].

According to a recent study[4], L452R, which alone enhanced infectivity by 3.5-fold and when combined with E484Q, raised it by 3 fold, was responsible for the B.1.617 spike protein's increased infectiousness. Additionally, observations of B.1.617.2's increased transmissibility have been made [4]. To fully comprehend the phenotypic impact of these mutant strains, more in vitro and/or in vivo research

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would be needed to validate these results. A recent investigation showed that variations, which include the L452R mutation, can evade humoral immune responses and HLA-restricted responses to the body's defense system[6]. Additional research showed that the L452R transformation had a lower affinity for the vaccination than the original variety[7].

The P681R mutation, another important change at the furin cleavage site, increased the basicity and may have opened up more opportunities for S1-S2 cleavage by allowing furin to make extra connections.[7, 8]. The potential for P681R to hasten membrane fusion, internalization, and subsequently increase transmissibility suggests the significance of P681R for the powerful fusogenic activity of SP from the Delta form. According to a collection of earlier investigations, the rapid spread of these variations may be facilitated by greater human and mouse ACE2 receptor activation, higher spike cleavage, and decreased susceptibility to neutralizing antibodies Delta.

3. Omicron

The actual name for the omicron variety is B.1.1.529. The main reason Omicron raised so many questions when it was first gathered and examined was because it had roughly 50 genetic mutations, including 32 that damaged the spike protein, whereas Delta only had 13–17 alterations.

The modifications that made omicron different from delta were especially notable for improving stability and reducing viral fusogenicity. One of its traits is its ability to be more stable in the environment and compact. This significantly improves the ability of transmission in domestic environments. Omicron has a stronger transmissibility than delta. According to South African data, compared to 98.7 days for beta and 50.5 days for delta, Omicron only requires 14 days to affect half the population[9]. The risk of omicron transmissibility was rated as very high by WHO. Omicron has extremely high Rt and R0 (effective reproduction numbers and basic reproduction numbers).

In comparison to the Delta variation and parental virus, the Omicron variant decreased the cleavage of the S protein. This reduces the fusogenicity while boosting the transmissibility of the virus. Spike (S) protein cleavage at the S1/S2 site is required for efficient syncytia formation.[10]. The virus-infected cells aggregating the nearby through a process called syncytia to create multinucleated cells. Syncytia may aid in virus virulence, immunological evasion, and transmission. Syncytia development is associated with illness severity, and 87 percent of SARS-CoV-2 patients who passed away had infected syncytial pneumocytes, according to reports. Since the fusogenicity is correlated with the severity of the sickness, this is also the reason why omicron generates relatively mild symptoms in comparison to other types.

However, the possibility of viral fusion could be significantly strengthened if a mutation like L452R develops in the Omicron variant. Due to the increased fusogenicity associated with the omicron form, the synthetic L452R version has already been determined to be more transmissible. The variant would become more hazardous if functional mutations were to arise in natural environments, which is something we should continue to monitor to keep it under control.

12% of people in South Africa have HIV, which weakens the immune system and slows the clearance of SARS-CoV-2 if it is present. This leads to a chronic infection status, which provides the virus more time to replicate in more turns, increasing the possibility of erroneous replication and, consequently, the possibility that the virus may mutate into a new variant.

In addition to already present accumulating mutations like K417N, S477N, T478K, E484A, and N501Y, these also include three novel clusters: (1) S371L, (2) N440K and G446S, and (3) Q493, G496, Q498 and Y505H, which are likely to impart raised resistance to counteracting antibodies and humoral immunity triggered by vaccination.[11-13]. As a result, Omicron can evade some of the pre-existing antibodies. Especially new Omicron subvariants BA.4/5, which pose less of an antigenic threat. And one current expectation is that these variants will outcompete BA.2.12.1 [14].

4. Comparative analysis

B stands for the version that was gathered in Wuhan in December. Early in 2020, B.1 was collected in the northern region of Italy. A single missense mutation that converted the amino acid aspartate to

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glycine at position 614 (D614G) of the spike protein was one of the first changes to show up. This viral variety quickly overtook others worldwide. D614G research has revealed that, while the mutation increases transmissibility, it does not increase severity. Consequently, Gly is present at position 614 in all later variations that descend from this new basal "B.1" lineage. In the intervening period, other VOCs from the B.1 lineage have appeared, including B.1.1.7, B.1.617.2, B.1.351 variants.

According to earlier research, distal S regions like the furin cleavage loop and D614G can have allosteric effects on the RBD open/down disposition[15]. Currently, the delta variant, which has a R0 of roughly 5 (compared to 2.5) and patient viral loads that can be 1,000 times higher than those of the alpha variant (B.1.1.7), is of particular concern. In the sequenced viruses that were in circulation in the UK between June 2 and June 9, 2021, it was discovered in 77% of the viruses. Thus, keeping the virus under control requires monitoring the formation of variations and comprehending their characteristics.

Every week, more viral variants are discovered. The mutations vary a lot based on this parental variant. The mutations which cause a sharp increase in transmissibility in spike protein are different in these VOCs but all function by changing the conformational characteristics of spike protein.

5. Conclusion

The S protein is the protein which grabs the receptor protein on the human cell membrane for invasion. This paper mainly discusses the mutations in spike protein corresponding to the changes in transmissibility and fusogenecity of SARS-CoV-2 variants delta and omicron. Key substitutions in spike protein were mutated majorly in S1 and S2 binding sites and RBD sites in these two variants of concern. These mutations contributed to the higher transmissibility and the ability of immune evasions to make these two variants spread over the previous waves.

Overall, delta and omicron variants all share common mutations from the parental B.1 lineage such as, D614G, which results in increasing transmissibility. Whilst the transmissibility was improved by mutation to increase the cleavage at the furin site in the delta variant, the cleavage of the S protein in the Omicron variant was reduced. This difference contributed to the relatively mild symptoms in the Omicron variant since this results in decreased syncytia formation.

Though they were not initially intended to be an in-depth surveillance study in particular geographic regions, the sample collection bias for surveillance data collected for transmissibility evaluation cannot be excluded. They still strongly demonstrated the threatening speed of transmission in new variants and the possibility of naturally occurring new recombination of variants.

Variable mutations in the spike protein make it easier to invade human cells while evading the vaccine and the human immune system. This results in tracking the variants' appearance with new mutations in spike protein and understanding their properties being essential in finding an effective vaccine to keep the virus under control.

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