Review Article

The Spike Protein Mutations and its Effect on SARS-CoV-2 Pathogenesis

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Abstract

The severe acute respiratory syndrome coronavirus 2 spike (S) glycoprotein facilitates receptor binding to initiate cell entry that is the critical initial step in the infection cycle. Due to S glycoprotein's pivotal role, in this review, we pointed to show potential functional and structural consequences of S glycoprotein and its variants, which has been related to increased viral load in humans with SARS-CoV-2 infection.

Keywords: Spike glycoprotein; Mutation, Macromolecule, COVID-19, SARS-CoV-2

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Introduction

The novel coronavirus outbreak named COVID-19 was reported for the first time in December 2019 in Wuhan, in the Hubei province of China. Due to the rapid spread of infections, the World Health Organization (WHO) announced COVID-19 as a global health emergency and a pandemic on January 30, 2020 (1-4). Elderly and people of any age with medical problems are at higher risk for severe illness with COVID-19 and even death (5). According to results findings in China from 81% of patients with COVID-19, approximately 80% of deaths happened in adults aged 60 and higher, and only one death in teens around 19 (6). Animals such as birds and mammals, including humans, are the coronavirus's primary source; and are transmitted through aerosols or the fecal-oral route (7).

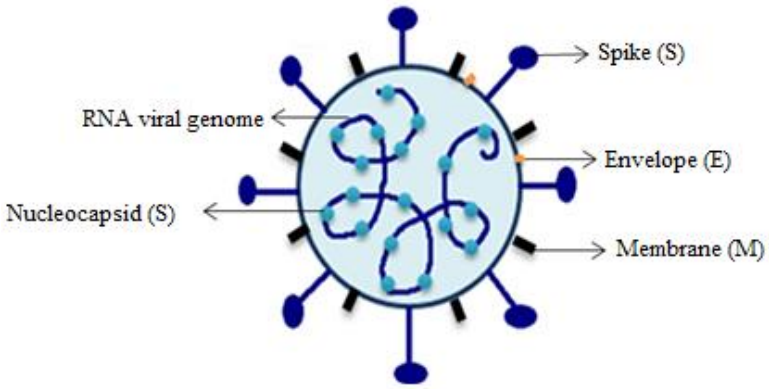

Figure 1. Schematic structure of Coronaviruses.

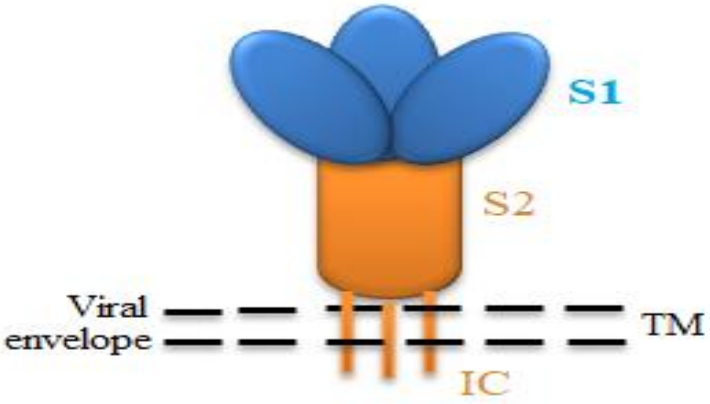

Figure 2. Structure of coronavirus spikes. S1: receptor-binding subunit, S2: membrane-fusion subunit, (TM): transmembrane anchor, (IC): intracellular tail, and the viral envelope.

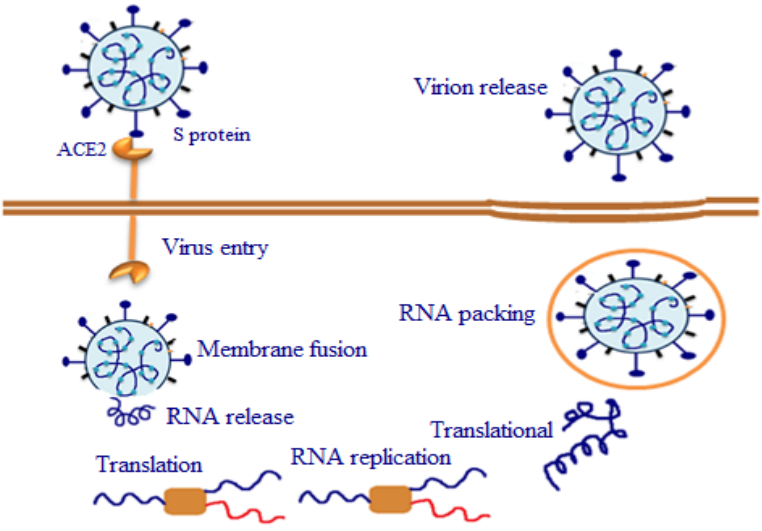

Figure 3. The virus entry mechanisms and functions.

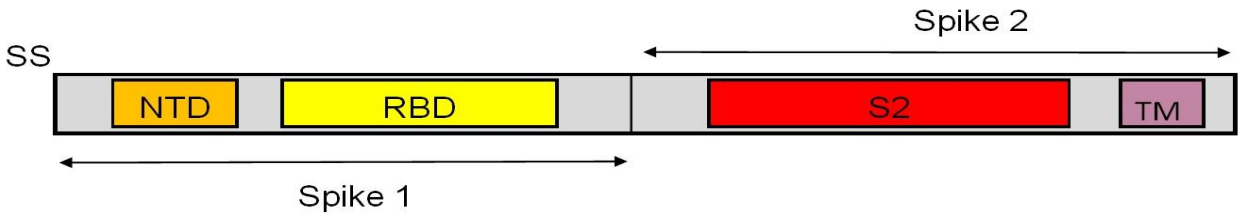

Figure 4. Schematic representation of spike protein.

Table 1: Mutations reported in spike protein S1 domain of SARS-CoV-2.

Domain	Region	Mutation
S1	N- Terminal	L5F
		R214L
		R408I
	C- Terminal	G476S
		V483A
		H519Q

COVID-19 is the seventh member of the family of coronaviruses that infect humans, after MERS-CoV and SARS-CoV. Generally, coronaviruses in humans cause mild respiratory infections similar to those seen in the common cold; although, recently some coronavirus infections in humans such as the SARS (originated from Southern China), MERS (Middle East Respiratory Syndrome, Saudi Arabia in 2012), and COVID-19 have led to lethal endemics (7). Researchers worldwide are studying to identify the novel virus, severe acute respiratory syndrome coronavirus 2; SARS-CoV-2 (the cause of COVID-19 disease), to prevent its dissemination and reduce its complications.

Structure of coronavirus: Coronavirus is an enveloped spherical or pleomorphic single-stranded RNA virus that ranges from 60 to 220 nm in size. It belongs to the Orthocoronavirinae subfamily and falls into the genus beta-coronavirus. The virus has variable G + C contents (~32% - 43%) and about 26.4–31.7 kb among all known RNA viruses that encode some structural and unstructured proteins. The nucleocapsid (N), Membrane (M), Envelop (E), and Spike (S) are four main structural proteins for producing a structurally complete viral particle (Fig. 1) (7, 8).

The Nucleocapsid coats the viral RNA genome which has a major function in its replication and transcription and promotes the virus attachment to human cells to produce virus factories (9). The M-protein is the most abundant glycoprotein in the viral surface that has three domains including a short N terminal ectodomain, a triple-spanning transmembrane domain, and a C-terminal endodomain. E-protein is a small membrane protein with approximately 76 to 109 amino-acid and a minor component of the virus particle. Both E and M proteins have essential functions such as viral assembly, budding, and virus particle replication as well as a crucial role in augmenting the immune response against SARS-CoV (10, 11). The S-protein is integrated over the virus's surface and is responsible for host receptor binding and fusion of the viral and host cell membranes (12).

Structural and functional properties of S glycoprotein: The spike (S) glycoprotein of the coronavirus, located on the virion's outer envelope, is a class I viral fusion protein that binds to receptors on coronavirus-susceptible cells induces cell fusion (13).

It comprises three segments, including a large ectodomain, a single-pass transmembrane anchor (TM), and a short intracellular tail (IC). The S precursor protein of SARS-CoV-2 consisting of ~1,300 amino acids is proteolytically divided into two subunits; S1 (~700 amino acids) and S2 (~600 amino acids) (Fig. 2).

The S1 subunit contains a receptor-binding domain (RBD), and the function of S1 is to bind the host cell receptors for viral attachment. S2 subunit contains a hydrophobic fusion peptide (FP) and two heptad repeat (HR) regions that fuse the host and viral membranes, allowing viral genomes to enter host cells. S1 and S2 share about 70% and 99% identity with that of human SARS-CoVs, respectively (14-16). On the other hand, for many coronaviruses and even SARS-CoV-2, S glycoprotein features two distinct protease cleavage sites between the S1 and S2 subunits, which remain noncovalently bound in the prefusion conformation (12, 17).

Role of spike protein in infection: The initial and critical stages in the coronavirus infection cycle are receptor binding and membrane fusion. S proteins cover the surface of SARS-CoV-2. During viral entry, S protein mediates attachment of viral particle to angiotensin-converting enzyme 2 (ACE2) as a cellular receptor. Then, entry of the virus into the host cell is promoted by a type 2 TM serine protease (located on the host cell membrane). When the virus enters the cell, the viral genetic material, a single-stranded RNA, is released, and the RNA genome is translated to generate poly-proteins. In the next step, the viral RNA genome replication and transcription processes occur via protein cleavage and assembly of the replication/transcription complex (RTC). The viral genome is replicated and translated into proteins, finally assembled, and packaged in the host cell, then viral particles are released out of host cells. In the last novel, virions are exported from infected cells that can infect other cells. (Fig. 3) (18-20).

Mutations in spike protein: According to the mutation on spike glycoprotein of SARS-CoV-2, D614G, G476S, and V483A are the main mutations. Other mutations with low frequency in SARS-CoV-2 spike glycoprotein include L5F, R214L, R408I, H519Q, and T572I (Table1). The N-Terminal Domain (NTD) of the S1 domain harbors L5F and R214L mutations while R408I, H519Q, T572I mutations occur at the C-Terminal Domain (CTD) of the S1 domain (Fig. 4) (21).

In early February 2020, an aspartic acid-to-glycine substitution at amino acid position 614 (D614G) in the S gene was observed in Europe. D614G mutation as a non-synonymous mutation of spike protein became the dominant variant of SARS-CoV-2 during the COVID-19 pandemic (22). Zhang et al. characterized and compared both variants of S^{D614} and S^{G614}; their analysis revealed that retroviruses pseudotyped with S^{G614} are more efficient than those with S^{D614} in infecting hACE2-expressing cells (23).

Korber et al. showed that D614G could increase viral loads in patients with COVID-19 (24). Recently in one study, Plante JA et al. reported that SARS-CoV-2 replication is increased in cell culture by D614G through enhances virion infectivity; however, it also improves fitness and transmission of SARS-CoV-2 in vitro and in vivo (25). According to clinical findings, a study showed that there is not a significant relationship between D614G mutation and severity and mortality of COVID-19 disease (24); however, more research about this issue is needed. Hydrophobicity of the SS may enhance via L5F as another mutation; it may facilitate protein folding and assembly of virion through S glycoproteins mediate entry into ER (26).

The dominant D614G variant has little effect on the epitope and its antigenicity. While the epitopes and antigenicity of some mutants with a low frequency significantly change (27). Because of mutations on S glycoprotein, it is considered a key target for SARS-CoV-2 development vaccines, therapeutic drugs against SARS-CoV-2 and can be crucial in diagnostics of the virus; among mentioned mutations, D614G and L5F are more critical.

According to WHO reports, different vaccine technology platforms are used to develop an effective vaccine against COVID-19. As of 02 October 2020, 42 candidate vaccines are in phase III clinical trials evaluation. However, there are also 151 COVID-19 candidate vaccines in preclinical evaluating. Formulation of both clinical and preclinical COVID-19 candidate vaccines is designed for intramuscular administration (28).

Some studies showed that S protein-based vaccines against SARS-CoV-2 could induce significant cellular and humoral immune responses in animal models and clinical trials (29). Similarly, SARS-CoV, S gene of SARS-CoV-2 considers as a critical target for COVID-19 vaccines (30). The receptor-binding domain (RBD) of the spike (S) protein can elicit neutralizing antibodies and T-cell immune responses (31). Ravichandran S et al. reported that RBD immunogen induced high antibody titer with higher affinity antibodies than other spike antigens (32). Immune cells, such as T cells and RBD-specific antibodies, were detected in the sera of discharge COVID-19 cases (31). Thus, RBD is considered a target for the development of vaccines against SARS-CoV-2.

Conclusion

At the moment, the world is amid a COVID-19 pandemic, the mutation rate of SARS-CoV-2 as an RNA virus is high. Studying mutation as the first step of SARS-CoV-2 evolution is significant for developing effective vaccines. The SARS-CoV-2 dominant variant in the spike protein "D614G" impacts the transmission and infectivity of the virus. However, there are some low-frequency mutations in spike protein. Nevertheless, more researches are needed on the impact of mutations in the SARS-CoV-2 proteins, especially in spike protein.

Acknowledgment

None.

Conflicts of Interest

The authors declare that there are no conflicts of interest.

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