Review Article

# Invasion of host immune response by SARS-CoV-2 through mutations in the viral genome- A Review Article

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**Abstract:**

Mutations in the severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) are severe threats to the world in many aspects. Mutations in the viral genomes at small proportion will affect functional properties and may alter infectivity, disease severity or interactions with host immunity. Since late 2020, however, SARS-CoV-2 evolution has been characterized by the emergence of mutations, in the context of variants of concern that impact virus characteristics, including transmissibility and antigenicity, probably in response to the changing immune response of the human population. In this review, we summarize the literature on mutations of the SARS-CoV-2 spike protein, high transmission of virus, entry of SARS-CoV-2 in to the host cells through angiotensin-converting enzyme-2 (ACE-2) receptor and TMPRSS2, viral replication, and assembly and its invasion of host immune response. The review also explains the different Variants of Concern (VOC) of SARS-CoV-2 and how it escapes from the host immune system.

**Keywords:** *SARS-CoV-2, Variants of Concern, Mutation, ACE-2 receptor, Variants of Interest.*

## Introduction:

During December, 2019, several patients were hospitalized due to severe pneumonia without known etiology. The symptoms of the patient were same as and MERS including fever, cough and chest discomfort, and in severe cases dyspnea and bilateral lung infiltration. Most of the patients are connected to the local market and on December 31st, China reported the outbreak of unidentified cause to World Health Organisation (WHO)[1]. After isolating the virus from bronchoalveolar lavage fluid, the metagenomics RNA sequencing was carried out and found that the Virus belongs to the betacorona virus family which is not identified before. On January 22, 2020 the cause of the disease was first announced and on 10th January, the complete genome sequence was published in the virology website[2]. The transmission of virus was very rapid and within a span of one month the virus spread all over the China. On 11th February 2020, the International Committee on Taxonomy of Viruses named this virus as SARS CoV 2 and WHO coined the disease as COVID-19[3].

Corona viruses (CoV) affect the human being especially in the respiratory tract with mild to severe infections. CoV belongs to the Coronaviridae family and belongs to the order is Nidovirales. This family comprises of four genera that include α-, β-, γ-, and δ coronaviruses. In this four genera, α- and β-CoVs infect mammals, γ coronaviruses infects the avian species, and δ-coronaviruses infect both mammals and aves. Among the corona viruses family, severe acute respiratory syndrome - associated coronavirus (SARS-CoV),Middle East respiratory syndrome-associated coronavirus (MERS-CoV), SARS-CoV-2 infects the human through zoonotic transmission[4]. The outbreak of SARS-CoV and MERS-CoV was observed in the year of 2002 and 2012 respectively[5]. In late 2019, novel SARS-CoV-2 was identified in Wuhan, China and cause respiratory illness disease like other two viruses but the transmission and infection rate was very high when compared to the other corona viruses[6]. Within 5 month of the disease onset, the virus spread globally with 2 million cases and more than 150 000 deaths. WHO declared this outbreak as a pandemic on 11th March 2020[7].

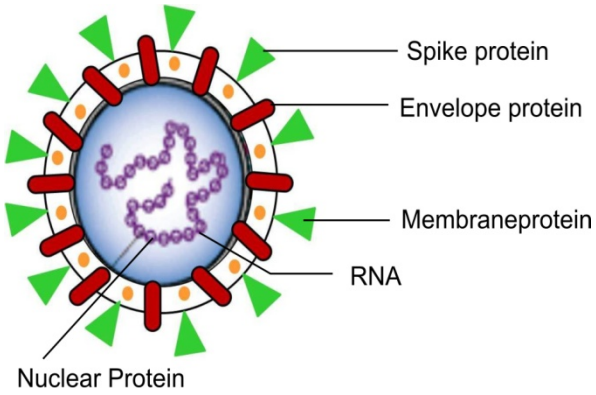


Fig 1: Structure of SARS-CoV-2

**Genomic Structure of SARS-CoV-2:**

The genomic material of SARS-CoV-2 is made up of single stranded positive sense RNA and size of about ~ 29.9 kb in size. 5' end of the RNA contains the cap and the 3' ends comprises of poly A tail. Next to the 5' cap, two ORF genes are present namely or-f1a and orf-1b. This genes involved in the polypeptide synthesis which are proteolytically processed to synthesis the 16 non-structural proteins. At the 3'end, 4 structural genes are present namely S gene, N gene, M gene and E gene which encodes for the proteins such as S (spike glycoprotein), N (nucleocapsid protein), M (membrane protein) and E (Envelope protein) respectively. The N protein binds with the viral genome and the outer envelope of the virus comprises of S, M and E protein[8]. Apart from these structural genes, the 3' end also contains nine accessory genes which encodes for the protein Orf3a, Orf3b, Orf6, Orf7a, Orf7b, Orf8, Orf9b, Orf9c, Orf10 which is mainly involved in the virulence factor and host interactions[9] (Fig 2). Spike protein plays an important role in binding with the host cell receptor. This protein is made up of 1273 amino acid residues and divided in to 2 subunits namely S1, S2. S1 involved in the binding of virus in to the angiotensin-converting enzyme-2 (ACE-2) receptor. S2 fuses the virus with the host cells[10]. Envelope protein is a group of small protein involved in the assembly and release of the virion particles. E proteins are also called as viroporins that forms protein lipid pores by assembling into the host membrane which involves in the ion transport[11]. Membrane protein (M) are 222 amino acid long structural proteins which plays an important role in the packaging RNA. This protein provides the distinct shape to the virus[12]. N protein involved in the packaging of viral RNA into ribonucleocapsid. N protein binds to the viral RNA through its 140 amino acids and appeared as a "bead on a string" manner. Another function of N protein is to protect the genomic RNA of the virus[13,14].


Fig 2: Genomic representation of SARS-CoV-2 (Naqvi et al., 2020)

**Transmission of virus:**

Similar to other corona virus, SARS-CoV-2 primarily transmits through the respiratory droplets from the infected person. The respiratory droplets once inhaled deposited on the mucous membrane and enters the cells through the angiotensin-converting enzyme-2 (ACE-2) receptor which is found mainly in the human respiratory tract epithelium, including the oropharynx and upper airway. Apart from the respiratory tract, the receptor was also found in the conjunctiva and gastrointestinal tract and it also serves as a transmission route [6]. Like SARS-CoV, SARS-CoV-2 binds to the ACE 2 receptor and cellular protease (TMPRSS2) to enter in to the cell. Though the binding site and binding affinity of SARS CoV and SARS-CoV-2 are same but the difference in infectivity and transmission is different which is mainly due to the activation of spike protein. The major change in SARS-CoV-2 is the presence of insert sequence of Ser Pro Arg Arg in the S1/S2 protease cleavage site which increases the substrate specificity of TMPRSS2 and HAT in the bronchial epithelial cells and enhances the cleavage of spike protein in to S1 and S2[15].

**Viral pathogenesis**

Once the SARS-CoV-2virus enters the host, the S protein binds to the host ACE 2 receptor. S protein comprises of 2 subunits namely S1 and S2, S1 is responsible for receptor recognition and S2 for membrane fusion. S1 subunit is further divided in to C-terminal domain (CTD) and N-terminal domain (NTD) in which CTD is mainly responsible for binding to the ACE-2 receptor. Once the viral and host interaction occurs, transmembrane protease serine 2 (TMPRSS2) enables the viral entry in to the cell. After entering in to the host cell, the viral genome is released and ORF 1a and ORF 1b will get translated to the viral replicase proteins. Then the replication, transcription and translation of the viral genome will occur. After translation, all the structural and accessory proteins are inserted into the ER–Golgi intermediate compartment (ERGIC) for virion assembly. The finally matured virus will be released into the host cells [16].

**Mutations in SARS-CoV-2**

Mutation is common in all the viruses including SARS-CoV-2 and some mutations don't exhibit any infectivity rate. But some mutations show remarkable changes in the viral properties including pathogenicity, rapid transmission of virus, response to vaccines etc. The Centers for Disease Control and Prevention (CDC) classifies the mutation into two types namely Variants of Concern (VOC) and Variants of Interest (VOI). Variants of Interest (VOI) is defined as genetic changes which is known to affect the characteristics of virus such as transmissibility, disease severity, immune escape, diagnostic or therapeutic escape. VOI causes significant community transmission or multiple SARS-CoV-2 clusters, in multiple countries with increasing relative prevalence alongside increasing number of cases over time, or other apparent epidemiological impacts to suggest an emerging risk to global public health[7]. VOC is defined as a variant with one or more

mutations in the organism which make the virus to infect the host rapidly with high transmission and low response to treatments and vaccines or failure in diagnostic methods [16]. VOI and VOC are similar but VOC have characteristics like detrimental change in disease epidemiology, clinical presentation of disease and failure in diagnostic methods or decrease in effectiveness of treatment or vaccine efficacy.

**Alpha variant:**

The first mutant variant was identified in United Kingdom, called as alpha variant and also known asplango lineage of B.1.1.7. The alpha variant showed 48% higher risk when compared to the wild variant. This variant has 23 genetic mutations of which 8 mutations were present in the genome sequence that codes for spike protein. The eight mutation in the S protein is HV 69-70 deletion, Y144 deletion, N501Y, A570D, P681H, T716I, S982A and D1118H [17]. This mutation leads to the modifications in the 17 amino acids. The mutation in the spike protein changed the phenotypic characteristics of the virus-host cell binding mechanism and thus increases the infectivity[18].

**Beta variant:**

In South Africa the variant of SARS-CoV-2 was observed and WHO named it as beta variant with the plango lineage of B.1.351. This Beta variant showed nine mutations in the spike protein and initiated the second wave of SARS-Cov-2. The nine mutations in the spike protein includes L18F, D80A, D215G, R246I, K417N, E484K, N501Y, D614G, and A701V and among which 3 mutation namely K417N, E484K, and N501Y is situated in the receptor binding domain (RBD). This three mutation in the RBD increases the affinity of host viral interactions. Antibodies elicited by SARS-CoV-2bind with the RBD region or in the N terminal domain (NTD) of the receptor. Mutations in the RBD or in the NTD further neutralize the different types of antibodies produced against the virus[19]. Most of the antibodies bind to the E484 and the substitution mutation E484Kdecreases the neutralization sensitivity to the convalescent plasma [20].

**Gamma Variant:**

The third VOC was identified in Brazil, with the plango lineage of P1and it is also called as Gamma variant. This variant has 35 mutations with 17 amino acid changes [21]. This variant have 10 remarkable mutations in the S protein including L18F, T20N, P26S, D138Y, R190S, H655Y, T1027I V1176, K417T, E484K, and N501Y. Similar to the Beta variant, L18F, K417N, E484K was present in the RBD region. This variant is 2.5 times more transmissible than the normal SARS-CoV-2 virus[22].

**Delta variant:**

The delta variant is also identified as plango lineage of B.1.617.2 strain which was first identified in India on Dec 2020. It is a highly contagious variant of corona virus with an increase in transmissibility. This variant is considered to be 40-60% more transmissible than alpha and twice transmissible than the original Wuhan strain[23]. Within the duration of three month 26% of the Indian population was infected with the delta variant and ware spread across 60 countries. This variant is the deadly variant with the high mortality rate in India. This variant was moderately resistant to vaccines especially in population with single dose of vaccine[24]. The delta variant comprises of 23 mutations when compared with the alpha strain and among the 23, 12 mutations are presented in the spike protein.

The most notable mutations are L452R, P681R, D614G and T478K and the mutation is due to the deletion of nucleotides at the position 157 and 158[25]. Like the other 2 variant including alpha and beta does not have the N501Y mutation in the spiked protein[26].

Table 1: Various VOC of SARS-CoV-2 and its country of Origin [30]

| WHO Label | Pango Lineage | Date of Designation of VOC | Country | Key Mutations |
|---|---|---|---|---|
| Alpha | B.1.1.7 and Q lineages | December 29, 2020 | United Kingdom | N501Y, P681H, H69-V70 and Y144/145 deletions |
| Beta | B.1.351 and descendent lineages | December 29, 2020 | South Africa | N501Y, K417N, E484K |
| Gamma | P.1and descendent lineages | December 29, 2020 | Brazil | N501Y, K417T, E484K |
| Delta | B.1.617.2 and AY lineages | May 11, 2021 | India | E484Q, L452R |
| Omicron | B.1.1.529 and BA lineages | 24 November 2021 | South Africa | A67V, del69-70, T951, de142-144, Y145D, del211, L212I, ins214EPE, T547K, D614G, H655Y, N679K, P681H, N764K, D796Y, N856K, Q954H, N969K, and L981F |

The mutation L452R is the important mutation which able to escape from CD8 T cells, these cell mainly responsible for killing the virus[27]. It is the substitution mutation occurs at 425$^{th}$ position with the substitution of Arginine instead of Leucine. The L425R, first reported in Denmark in the month of march 2021, and this mutation has been found more transmissible than wild type strains and also been associated with reduced antibody efficacy[28]. Next important mutation, delta variant is P681R that is substitution of arginine instead of proline at the position of 681. This mutation easily cleaves the precursor spike protein in to the active spike protein S1 and S2 that may enhance transmissibility of the virus. The P681R has been associated with chemical processes that may enhance transmissibility. Centre for disease control and Preventions (CDC) reported that variants with this mutation spread more quickly, than the previous strain. The T478K mutation was reported in around 65% of occurrences[29].

**OMICRON:**

In later 2021, the new variant was identified in many countries with various mutations in the Spike protein of SARS-CoV-2and named it as Omicron or the plango lineage B.1.1.529. This variant possesses 30 mutations in the spike protein and the transmission rate was very high when compared to other variant. In this lineage, multiple mutations occurs in various regions of the whole genome. In the envelope protein, T91 mutation was observed. In the nucleocapsid protein, P13L, E31del, R32del, S33del, R203K, G204R was present. In the spike protein particularly in the NTD region D3G, Q19E, A63T in the matrix, N211del/L212I, Y145del, Y144del, Y143del, G142D, T95I, V70del, H69del, A67V was observed. In the spike protein, RBD region

mutations including Y505H, N501Y, Q498R, G496S, Q493R, E484A, T478K, S477N, G446S, N440K, K417N, S375F, S373P, S371L, G339D was present. D796Y in the fusion peptide of the spike, L981F, N969K, Q954H in the heptad repeat 1 of the spike as well as multiple other mutations in the non-structural proteins and spike protein[30].

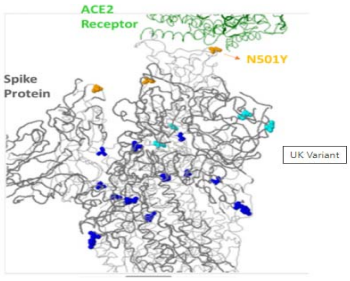


Fig 4a

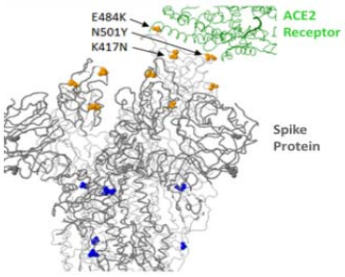


Fig 4b

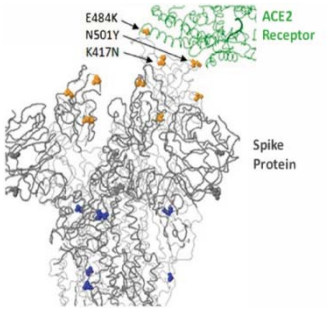


Fig 4c

Fig 4: 3D structure of the spike protein in the VOC. Mutations is given in different color (GISAID)
Fig4a: alpha variant; 4b: beta variant, 4c: delta variant

## SARS-CoV 2 VOI

In SARS-CoV 2, WHO announced eight VOI namely Epsilon (B.1.427 and B.1.429); Zeta (P.2); Eta (B.1.525); Theta (P.3); Iota (B.1.526); Kappa (B.1.617.1); Lambda(C.37) and Mu (B.1.621).

Table 2: VOI of SARS-CoV-2

| WHO Label | Pango Lineage | Designation of VOC | Country | Key Mutations | Characteristics |
|---|---|---|---|---|---|
| Epsilon | B.1.427 and B.1.429 | 2020 | U.S.A | L452R, D614G; B.1.429: S13I, W152C, L452R, D614G | Increased Transmissibility |
| Zeta | P.2 | Apirl, 2020 | Brazil | L18F; T20N; P26S; F157L; E484K; D614G; S929I; and V1176F | Escapes from the immune system by antibodies and vaccine sera neutralization |
| Eta & Lota | B.1.525 & B.1.526 | November, 2020 | Newyork | B.1.525 (A67V, Δ69/70, Δ144, E484K, D614G, Q677H, F888L) B.1.526 ((L5F*), T95I, D253G, (S477N*), (E484K*), D614G, (A701V*)) | Escapes from the immune system by antibodies and vaccine sera neutralization |
| Theta | P.3 | May 11, 2021 | Philippines and Japan | E484Q, L452R | Decreases the antibody neutralization activity by enhancing the biding of ACE receptor and Spike protein (34). |
| Mu | B.1.621 | August, 2021 | Columbia | R346K, BD-821/771, K417N, T951, YY144-145TSN [33]. | Decreases the neutralization effect of the antibodies [32]. |

## Conclusion:

This review clearly explains about the various mutations present in the VOC of SARS-CoV-2and the mutations helped in the invasion of the immune response in the SARS-CoV-2.

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