

Review

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Posted Date: 7 August 2023

doi: 10.20944/preprints202308.0520.v1

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Review

# A Comprehensive Analysis of Structural and Functional Changes Induced by SARS-CoV-2 Spike Protein Mutations

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**Abstract:** The SARS-CoV-2 virus, which is responsible for the COVID-19 pandemic which emerged and spread, has sparked intense research on its spike protein, which is essential for viral entrance into host cells. Viral reproduction and transmission, host immune response regulation, receptor recognition and host cell entrance mechanisms, as well as structural and functional effects have all been linked to mutations in the spike protein. Spike protein mutations can also result in immune evasion mechanisms that impair vaccine effectiveness and escape, and they are linked to illness severity and clinical consequences. Numerous studies have been conducted to determine the effects of these mutations on the spike protein structure and how it interacts with host factors. These results have important implications for the design and development of medicines and vaccines based on spike proteins as well as for the assessment of those products' efficiency against newly discovered spike protein mutations. The paper gives a general overview of how spike protein mutations are categorized and named, as well as the genomic and phylogenetic techniques that have been used to track their genesis and dissemination. Additionally, it looks at the links between spike protein mutations and clinical outcomes, illness severity, unanswered problems, and future research prospects. Additionally, explored are the effects of these mutations on vaccine effectiveness as well as the possible therapeutic targeting of spike protein mutations.

**Keywords:** SARS-CoV-2; spike protein; mutations; viral infectivity; pathogenesis; vaccine efficacy; immune evasion; antibody neutralization; T-cell response; therapeutic targeting

## 1. Introduction

The COVID-19 pandemic has brought to light how crucial it is to comprehend the molecular and cellular processes that underlie viral pathogenesis [1]. SARS-CoV-2, the virus that causes COVID-19, has spread quickly over the world, killing millions of people while seriously disrupting economies, cultures, and healthcare systems [1,2]. The spike (S) protein, which facilitates viral entrance into host cells and interacts with the host immune system, is one of the main components of SARS-CoV-2 pathogenicity [3]. The discovery and dissemination of SARS-CoV-2 variants with S protein mutations, however, have raised questions regarding the effectiveness of available treatments and vaccines as well as the possibility that these variants could avoid immune detection and result in more severe disease [4,5].

During viral entrance and fusion with host cell membranes, the SARS-CoV-2 S protein, a highly dynamic and complex molecule, undergoes conformational changes [6]. The S protein is a crucial factor in inducing a protective immune response and is a main target for antibodies that work to neutralize pathogens [6,7]. Therefore, in order to create effective vaccines and treatments against SARS-CoV-2, it is crucial to comprehend the structural and functional effects of S protein mutations [7].

There are a number of SARS-CoV-2 variants with S protein mutations that have been linked to enhanced transmissibility, higher viral loads, and less neutralization by antibodies induced by existing vaccinations, according to recent investigations [8,9]. These results have underlined the necessity of tracking SARS-CoV-2 development and modifying current therapies and vaccines to remain effective against novel variations. Furthermore, knowing the molecular processes underlying S protein mutations and how they affect viral pathogenesis can help in the development of novel antiviral treatments as well as pandemic preparedness techniques in the future [10].

The development of viable vaccines and therapies as well as the ongoing COVID-19 pandemic are significantly impacted by the appearance of SARS-CoV-2 variants with S protein mutations [11]. In order to create measures to reduce the severity and spread of COVID-19 and get ready for upcoming pandemics, it is imperative to comprehend the structural and functional effects of these mutations [12,13].

By summarizing the current understanding of the SARS-CoV-2 spike protein, providing an overview of the evolution and classification of spike protein mutations, evaluating their impact on viral infectivity and pathogenesis, analysing their structural and functional consequences, exploring immune evasion mechanisms associated with these mutations, and discussing their implications for COVID-19 vaccines and therapeutics, the review seeks to provide a thorough analysis of the topic. By offering a useful resource for academics and clinicians engaged in COVID-19 research, the review aims to contribute to the creation of efficient strategies for managing the current COVID-19 pandemic.

2. Structural and Functional Features Of SARS-CoV-2 Spike Protein

The development of vaccines and treatments against COVID-19 must focus on the S protein of SARS-CoV-2 because it is a highly dynamic and complex molecule that is essential for viral entrance into host cells and interacts with the host immune system [14,15].

2.1. Spike Protein Structure

The SARS-CoV-2 virus's S protein, which is its most thoroughly studied component, is essential for the virus's entry and multiplication inside the host [16]. S1, S2, and a transmembrane domain make up the three components of the S protein, a complex glycosylated protein. The angiotensin-converting enzyme 2 (ACE2) receptor on host cells is bound by the receptor-binding domain (RBD) of the S1 subunit, which facilitates viral entrance (See Figure 1) [16,17]. The transmembrane domain secures the protein to the viral membrane, and the S2 subunit includes the fusion peptide that helps the viral and host cell membranes fuse [18]. During viral entry, the highly dynamic S protein changes shape from a closed conformation to an open conformation, exposing the RBD for ACE2 binding [19].

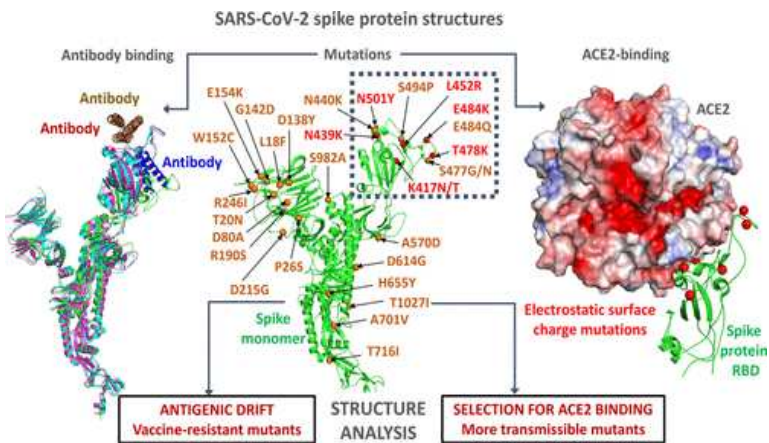


Figure 1. SARS-CoV-2 Spike protein structures  
[https://pubs.acs.org/doi/10.1021/acsinfecdis.1c00433].

Neutralizing antibodies' main target is the S protein, which also stimulates a strong immunological response. Therefore, for the development of efficient vaccines and treatments against SARS-CoV-2, understanding the structure and function of the S protein is crucial [20]. Recent research has revealed the emergence of numerous SARS-CoV-2 variants with S protein mutations, which are linked to both greater transmissibility and decreased neutralization by antibodies induced by existing vaccines [21,22]. In order to create novel vaccines and treatments against SARS-CoV-2 and to ensure that existing vaccines are still effective against new variants, more research into the structure and function of the S protein and its variants is required [23].

## *2.2. Spike Protein Function*

SARS-CoV-2's S protein is essential for the virus to enter host cells. The ACE2 receptor on the surface of the host cell is bound by the S protein, causing conformational changes that enable the fusion of the viral and host cell membranes and result in viral entrance [24]. By preventing neutralizing antibodies from recognizing the viral surface, the S protein helps the virus evade the host immune system. Strong immunological responses are triggered by the S protein, which is also the main target of neutralizing antibodies [25]. The S protein is a crucial target for the creation of potent vaccines and treatments against SARS-CoV-2 because antibodies against it can thwart viral entrance and decrease viral proliferation [26].

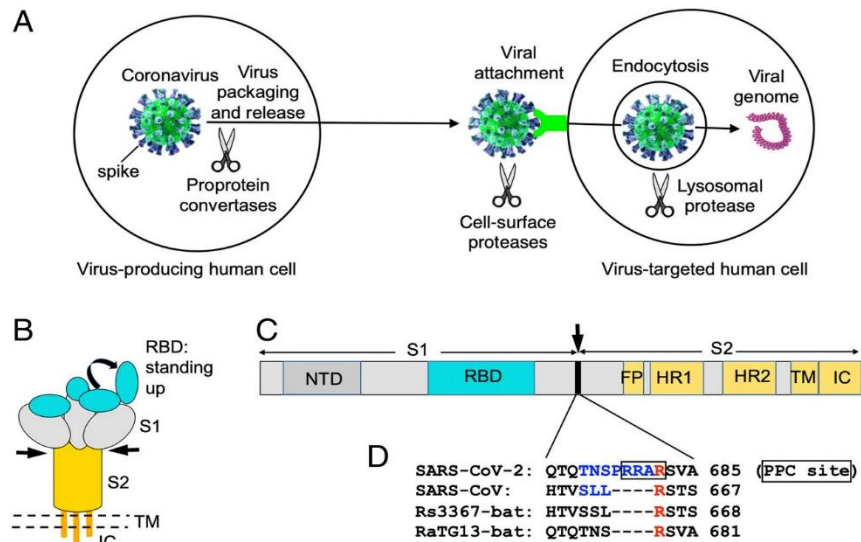
There are a number of SARS-CoV-2 variants with S protein mutations, and these alterations have been linked to both greater transmissibility and decreased neutralization by antibodies induced by existing vaccinations. Investigations are still ongoing into how these alterations affect viral pathogenesis and infectivity functionally [21,22]. For the creation of efficient SARS-CoV-2 vaccines and therapies as well as for the management of the continuing COVID-19 pandemic, it is essential to comprehend the functional characteristics of the S protein and its variations [5]. More investigation is required to determine how S protein mutations affect viral entrance, replication, and immune evasion as well as to create new methods to combat these alterations.

## *2.3. Receptor Recognition and Host Cell Entry Mechanisms*

Angiotensin-converting enzyme 2 (ACE2) receptor identification and binding by the SARS-CoV-2 spike (S) protein facilitates viral entry into host cells [27]. The S1 subunit of the S protein's receptor-binding domain (RBD) preferentially binds to the ACE2 receptor on the surface of the host cell, causing conformational changes that enable the fusion of the viral and host cell membranes and result in viral entrance (See Figure 2) [28]. The affinity of the RBD for ACE2 has been demonstrated to be a crucial factor of viral infectivity and pathogenicity. The S protein is highly selective for the ACE2 receptor [29].

The S protein of the SARS-CoV-2 virus has a stronger affinity for the ACE2 receptor than the S protein of the original SARS-CoV virus, according to structural analyses [30]. This stronger affinity could be a factor in SARS-CoV-2's higher transmissibility when compared to SARS-CoV [31,32]. Additionally, the S protein has a furin cleavage site that enables host proteases to cleave the S protein, facilitating viral entrance into host cells. During viral entry, the S protein goes through conformational changes [33,34]. It switches from a closed shape to an open conformation, exposing the RBD for ACE2 binding [34,35].



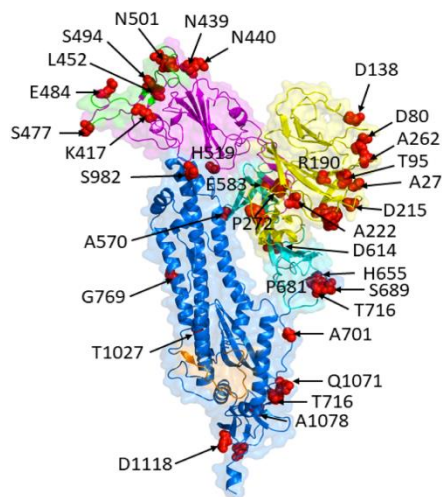


**Figure 2.** Cell entry mechanisms of SARS-CoV-2 (<https://www.pnas.org/doi/10.1073/pnas.2003138117>).

There have been a number of SARS-CoV-2 variations with changes to the S protein, and some of these changes have been linked to heightened transmissibility and diminished neutralization by antibodies induced by existing vaccinations [21]. For the creation of efficient vaccines and treatments against SARS-CoV-2, it is crucial to comprehend the effects of these mutations on receptor recognition and host cell entry mechanisms [36]. More investigation is required to determine the functional effects of these mutations on viral pathogenesis and infectivity as well as to find new ways to target the S protein and stop viral entry into host cells.

3. Evolutionary Dynamics of SARS-CoV-2 Spike Protein Mutations

As a result of the ongoing COVID-19 pandemic brought on by the SARS-CoV-2 virus, new variations with mutations in the spike [S] protein (See Figure 3), the main target for present vaccines and therapies, have emerged and spread quickly [5].



**Figure 3.** The most common SARS-CoV2 spike protein mutations (red color) (<https://www.nature.com/articles/s41598-022-04950-4>).

3.1. Emergence and Spread of Spike Protein Mutations

The appearance and propagation of SARS-CoV-2 variants with S protein mutations is extremely concerning since these changes may have an impact on the pathophysiology, immune evasion, and infectiousness of the virus [37]. Replication errors, recombination events, and selection pressure from the host immune system are only a few of the factors that can lead to mutations in the S protein [38]. It is expected that more S protein changes will occur when the virus spreads and multiplies in human populations, which could result in the creation of novel variations that are more transmissible, virulent, or resistant to current therapies and vaccines [39,40].

There have already been several SARS-CoV-2 variations with S protein mutations that have appeared. These variants have been linked to both greater transmissibility and decreased neutralization by antibodies produced by current vaccinations [21,22]. Among the most researched variants with S protein mutations are the B.1.1.7 variant, which was discovered in the UK, the B.1.351 variant, which was discovered in South Africa, and the P.1 variant, which was discovered in Brazil [41,42]. These variants have mutations in the RBD of the S protein, which have been found to decrease the S protein's affinity for the ACE2 receptor and to lessen the potency of some antibodies' neutralizing effects [43].

For an understanding of the evolutionary processes of the virus and the creation of efficient pandemic control measures, continuing surveillance and monitoring of SARS-CoV-2 variants with S protein mutations is essential.

### *3.2. Classification and Nomenclature of Spike Protein Mutations*

For monitoring and comprehending the appearance and transmission of variants with possible implications for viral infectivity, pathogenesis, and immune evasion, the classification and nomenclature of SARS-CoV-2 spike protein mutations are crucial [44,45]. The geographical origin of the variant and the mutations found in the S protein have been used by the WHO to build a methodology for the classification and designation of SARS-CoV-2 variants [46,47]. The Greek alphabet is used to name variations, beginning with Alpha for the B.1.1.7 variety that was initially discovered in the UK [48].

The reference amino acid and the variant amino acid are listed after the amino acid position in the S protein in the nomenclature of S protein mutations [49]. With instance, the S protein's D614G mutation designates a replacement of aspartic acid (D) with glycine (G) at position 614 [49,50]. This mutation, which affects the S1 subunit of the S protein, has been linked to an increase in the transmission and infectivity of viruses [49-51]. In order to track and address the current pandemic, communication and cooperation among researchers, public health professionals, and politicians must be made easier thanks to the classification and nomenclature of SARS-CoV-2 spike protein mutations.

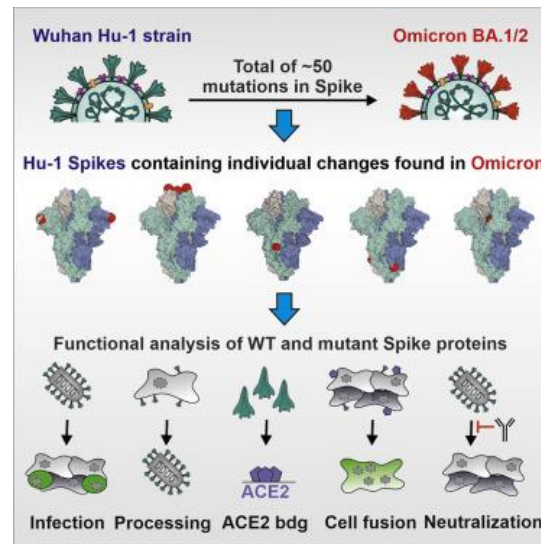
### *3.3. Genomic and Phylogenetic Analyses of Spike Protein Mutations*

To comprehend the evolution and transmission of the virus and to create efficient pandemic control measures, genomic and phylogenetic investigations of SARS-CoV-2 spike protein mutations are crucial [52]. The sequencing of the viral genome and identification of the S protein mutations are steps in the genomic study [52,53]. Based on the mutations found in the genomes of the various SARS-CoV-2 strains, a tree representing the evolutionary relationships between them is built during the phylogenetic analysis [54]. These studies can shed light on the development, dispersal, and diversity of SARS-CoV-2 variants, particularly those with S protein mutations.

Some mutations in the S protein have independently evolved in several lineages, according to genomic and phylogenetic analysis, pointing to convergent evolution or positive selection pressure [55]. For instance, the D614G mutation in the S protein, which independently appeared in several lineages and quickly rose to become the global dominant variety, may have an advantage in terms of fitness [55,56]. Other S protein mutations, however, have only been detected in certain lineages, raising the possibility of a founder effect or genetic drift [55-57]. Public health officials can implement targeted actions to stop future spread by identifying probable transmission clusters and sources of introduction for novel variants with the aid of genomic and phylogenetic investigations.

#### 4. Impact of Spike Protein Mutations on Viral Infectivity and Pathogenesis

SARS-CoV-2 spike protein mutations can significantly affect the pathogenesis and infectivity of the virus, potentially increasing the disease's severity, transmissibility, and immune evasion (See Figure 4) [58,59].



**Figure 4.** Factors affecting SARS-CoV-2's spike infectivity, processing, and neutralization (<https://www.sciencedirect.com/science/article/pii/S1931312822003523>).

##### 4.1. Effects on Viral Replication and Transmission

SARS-CoV-2 spike protein mutations can change how the virus interacts with host cells, which can impact viral reproduction and transmission [60,61]. For instance, changes to the S protein's receptor-binding domain (RBD) may alter the virus' affinity and specificity for the host cell receptor ACE2, resulting in a change in the amount of viral entry and reproduction [61-63]. In addition to affecting viral assembly and release, mutations in other parts of the S protein, such as the S2 subunit or the furin cleavage site, can also impact viral fusion and entry into host cells [18]. The S protein's stability, immunogenicity, and antigenicity can all be affected by these changes, which may have an impact on the effectiveness of treatments and vaccinations.

The B.1.1.7 variation, which was initially discovered in the UK, and the B.1.617.2 variant, which was initially discovered in India, are two SARS-CoV-2 variants having mutations in the S protein that have been linked to increased transmissibility and/or viral loads [42,64]. These variants have changes to the S protein's RBD and/or other areas, which could boost viral entrance and reproduction in host cells [14]. For the development of efficient preventive strategies, such as vaccinations, antivirals, and non-pharmaceutical treatments, it is essential to comprehend the consequences of spike protein mutations on viral reproduction and transmission.

##### 4.2. Modulation of Host Immune Responses

SARS-CoV-2 spike protein mutations can alter host immune responses, possibly resulting in immune evasion or hyperinflammatory reactions [65]. Antibodies against the S protein are essential for neutralizing the virus and avoiding infection because the S protein is a significant target of the host immune response [65,66]. However, changes to the S protein's antigenic structure may lessen the protein's ability to elicit immunological recognition and neutralizing antibodies [67]. For instance, changes to the RBD of the S protein may impact how neutralizing antibodies bind to the protein and diminish their effectiveness, increasing immune evasion [67,68]. The identification and binding of antibodies and T cells can be affected by mutations in other parts of the S protein, thereby decreasing the effectiveness of cellular immunity [69].

By causing hyperinflammatory reactions or immune system dysregulation, spike protein mutations can also have an impact on the host immunological response [65,68]. For instance, mutations in the S protein's furin cleavage site can increase the S protein's cleavage and activation, increasing viral entrance and replication and perhaps triggering hyperinflammatory reactions [70]. A dysregulation of the immune response and the potential to exacerbate inflammation and tissue damage can result from mutations in other parts of the S protein that impact the binding and activation of innate immune receptors [71].

For effective vaccines and treatments that can induce protective immunity against SARS-CoV-2 variations, it is essential to comprehend how host immune responses are modulated by spike protein mutations. It is crucial for developing targeted therapeutics to lessen inflammation and tissue damage in severe COVID-19 instances as well as for discovering potential biomarkers of disease severity.

#### *4.3. Association with Disease Severity and Clinical Outcomes*

The severity of the disease and clinical outcomes are also related to SARS-CoV-2 spike protein mutations, which may have an impact on COVID-19 disease progression [62]. The B.1.1.7 variant, originally discovered in the UK, and the B.1.351 variant, first discovered in South Africa, are two SARS-CoV-2 variants having mutations in the S protein that have been linked to increased disease severity and death [72]. Additionally, several S protein variations have been linked to an increased risk of reinfection, possibly as a result of worse immune recognition [62,72].

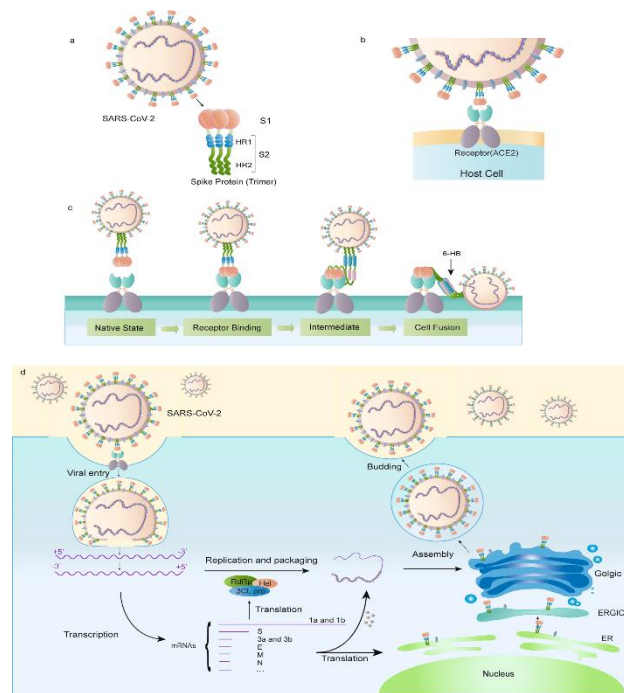
By changing the virus's pathogenicity and tropism in various tissues and organs, spike protein mutations can also have an impact on clinical outcomes [73]. For instance, changes in the S protein may alter the virus's affinity and selectivity for various cell types and tissues, which may influence the disease's severity and clinical presentation [14]. The interaction between the virus and host immune cells can also be impacted by mutations in the S protein, which could result in dysregulated immunological responses, hyperinflammation, and tissue damage [74].

Effective COVID-19 management and treatment methods require an understanding of the relationship between spike protein mutations and disease severity and clinical outcomes [75]. On the basis of the genetic makeup of the virus and the host, it can also help with the development of targeted therapies and individualized treatment plans.

### **5. Structural Consequences of Spike Protein Mutations**

Since SARS-CoV-2's spike protein is a primary target for vaccine development and treatment interventions, changes to this protein's structure may have an impact on the effectiveness of these measures (See Figure 5).





**Figure 5.** Structural and functional properties of SARS-CoV-2 spike protein (<https://www.nature.com/articles/s41401-020-0485-4>).

### 5.1. Alterations in Spike Protein Conformation and Stability

The shape and stability of the SARS-CoV-2 spike protein can change due to mutations, which may have an impact on how the virus interacts with host cells and is recognized by the immune system [76,77]. The receptor-binding domain (RBD) of the host receptor ACE2 is found in the S1 subunit of the spike protein, which is made up of two subunits, S1 and S2 [78,79]. Changes in the RBD's conformation and its binding affinity for ACE2 may result from mutations, which may have an impact on viral entry and transmission [80]. For instance, the RBD mutation (N501Y) in the B.1.1.7 variety, which was initially discovered in the UK, has been linked to higher binding affinity for ACE2 and greater transmissibility [81,82].

Mutations in the spike protein can alter its stability and antigenicity in addition to impacting receptor binding. Approximately two-thirds of the spike protein's surface is covered with glycans, indicating that it is extensively glycosylated [83,84]. The spike protein's structural flexibility and stability may be impacted by mutations in the glycosylation pattern. This may lessen the effectiveness of vaccinations and therapeutic antibodies by interfering with the recognition and binding of neutralizing antibodies [85]. For instance, the RBD and N-terminal domain (NTD) alterations in the original South African B.1.351 variation have been linked to decreased neutralization by certain monoclonal antibodies and convalescent plasma [86,87].

Creating efficient COVID-19 preventive and treatment plans requires an understanding of the structural effects of spike protein mutations. This entails creating treatments and vaccines that can target the spike protein in its many conformations and patterns of glycosylation, as well as keeping an eye on the emergence and dissemination of novel variants with mutations that can alter the effectiveness of these interventions.

### 5.2. Effects on Spike Protein Interactions with Host Factors

The interactions of the spike protein with host components like antibodies, immune cells, and other host proteins may impact the pathogenicity and infectiousness of viruses [88,89]. Mutations may result in the loss of the spike protein's epitopes, or regions of the protein that neutralizing antibodies may identify [90,91]. By reducing the potency of therapeutic antibodies and vaccinations that target specific epitopes, this may lead to breakthrough infections and decreased protection

against reinfection [59]. For instance, the E484K mutation seen in the B.1.351 and P.1 variants has been connected to decreased neutralization by certain monoclonal antibodies and convalescent plasma [21,92].

Spike protein mutations can alter not only antibody recognition but also how the protein interacts with immune cells like T cells and natural killer cells [93]. Spike protein mutations may impact how antigen-presenting cells process and display viral antigens, thereby lowering T cell activation and compromising the antiviral immune response [94,95]. The spike protein's interactions with host proteins important in viral entry and replication, such as the ACE2 receptor and host proteases, can also be impacted by mutations [18,96]. This may have an impact on the effectiveness of viral entrance and replication, thereby influencing viral pathogenesis and the severity of the disease.

For the purpose of creating efficient COVID-19 prevention and treatment techniques, it is essential to comprehend the consequences of spike protein mutations on how it interacts with host factors. In addition to creating treatments that can specifically target the spike protein and host components involved in viral entry and replication, this involves keeping an eye on the development and dissemination of novel variations with mutations that may influence viral infectivity and pathogenicity.

### *5.3. Influence on Spike Protein Cleavage and Processing*

The SARS-CoV-2 spike protein is a glycoprotein that goes through a lot of cleavage and processing when it enters host cells [97]. To allow for viral entrance, the spike protein has a polybasic cleavage site (RRAR) that is digested by host proteases such furin and TMPRSS2 [98]. The effectiveness of cleavage and processing can be changed by mutations in the spike protein, which may have an impact on the pathogenicity and infectiousness of viruses [99,100].

The D614G mutation in the spike protein, which has been found in several SARS-CoV-2 variations and is linked to increased infectivity and transmissibility, is one of the most prominent changes in the protein [32]. It has been demonstrated that the D614G mutation, which affects the receptor-binding domain (RBD) of the spike protein, makes the spike trimer more stable and makes it easier for host proteases to digest the spike protein. These modifications could be a factor in the D614G variant's heightened contagiousness and transmissibility [101].

It has also been demonstrated that other mutations in the spike protein alter cleavage and processing. For instance, the B.1.1.7 variant's P681H mutation has been linked to improved furin cleavage efficiency, which may help explain why the variety is more contagious [102,103]. On the other hand, it has been demonstrated that alterations in the polybasic cleavage site, such as the E484K mutation present in most variants of concern, impair cleavage efficiency and impact viral entrance [104].

For the purpose of creating efficient COVID-19 therapies, it is essential to comprehend the effects of spike protein mutations on cleavage and processing. Protease inhibitors are one example of a therapeutic strategy that targets these processing steps and may be useful in inhibiting viral entrance and replication. Additionally, keeping an eye on the development and dissemination of novel variants with mutations that impact cleavage and processing can reveal crucial details about the pathogenesis and evolution of SARS-CoV-2.

## **6. Functional Consequences of Spike Protein Mutations**

A SARS-CoV-2 mutation in the spike protein can have substantial functional ramifications because it is essential for viral entrance and pathogenesis [28].

### *6.1. Changes in Spike Protein Receptor Binding Affinity and Specificity*

To enable viral entrance, the SARS-CoV-2 spike protein interacts to the angiotensin-converting enzyme 2 (ACE2) receptor on host cells [24]. Alterations to the spike protein's binding affinity and specificity for ACE2 can have an impact on the pathogenicity and transmission of viruses [82]. For

instance, the RBD of the spike protein has several changes in the B.1.1.7 variation, including N501Y, which has been demonstrated to increase the binding affinity for ACE2 and improve viral infectivity [105]. Similar to the B.1.351 variant, the B.1.351 variant features many mutations in the RBD, including E484K, which decreases the binding affinity for some monoclonal antibodies and might have an impact on how well the host immune system neutralizes the virus [21,64].

Mutations in the spike protein can alter ACE2 binding as well as result in the development of novel receptor binding capacities [106]. According to research, the P681R mutation in the B.1.617 variation confers improved binding to the host protease furin, which may make it easier for the virus to enter host cells that don't express a lot of ACE2 [107]. This mutation has been found in a number of variants that are cause for concern, underscoring the possibility that the development of new receptor-binding capacities may be what propels the evolution and appearance of SARS-CoV-2 variants [108].

It is essential to comprehend how receptor binding affinity and selectivity are affected by spike protein mutations in order to create potent COVID-19 therapies. Neutralizing antibodies and ACE2 decoys are examples of therapeutic approaches that target the interaction between the spike protein and ACE2 and may be useful in limiting viral entrance and replication [109]. Additionally, keeping an eye on the appearance and spread of novel variations with mutations that impair receptor binding will shed light on the pathogenesis and evolution of SARS-CoV-2 [11].

### *6.2. Effects on Spike Protein Fusion and Membrane Fusion*

SARS-CoV-2 enters host cells by the spike protein-mediated fusion of the viral and host cell membranes, which is an important stage in the process [18,97]. This procedure can be impacted by mutations in the spike protein, changing the pathogenicity and infectivity of the virus [110]. For instance, as shown by the enhanced cleavage efficiency seen with the D614G mutation, alterations in the S1/S2 furin cleavage region of the spike protein can impact the proteolytic processing necessary for membrane fusion [111]. This mutation has been linked to increased viral transmissibility and infectivity [112].

Mutations in the spike protein can directly alter the conformational changes necessary for membrane fusion in addition to having an impact on proteolytic processing. Multiple SARS-CoV-2 variants have the E484K mutation, which has been found to stabilize the spike protein's prefusion conformation and improve the effectiveness of membrane fusion [18,113]. Additionally, this mutation has been linked to a decreased susceptibility to neutralizing antibodies and perhaps a decreased effectiveness of vaccination [114].

For the purpose of creating efficient countermeasures against COVID-19, it is essential to comprehend how spike protein mutations affect membrane fusion. Fusion inhibitors and other therapeutic approaches that focus on the fusion process may be useful in limiting viral entry and reproduction [115]. Additionally, keeping an eye on the development and dissemination of novel variations with mutations that interfere with membrane fusion can reveal vital details about the pathogenesis and evolution of SARS-CoV-2.

### *6.3. Modulation of Spike Protein Proteolytic Activation and Inactivation*

The spike protein's proteolytic activation and inactivation are crucial processes in SARS-CoV-2's entry and egress from host cells [116]. Numerous protease cleavage sites found in the spike protein are crucial for viral pathogenicity and infectivity [117]. The effectiveness of proteolytic processing and the activation and inactivation of the spike protein can both be altered by mutations in these cleavage sites [118].

The D614G mutation, which has been demonstrated to improve the efficacy of furin cleavage at the S1/S2 region of the spike protein, is one illustration of such a mutation. This results in increased viral transmissibility and infectivity [119]. The S686G mutation, among others, can disrupt cleavage at the S2' site and may have an impact on viral entrance and pathogenesis [120].

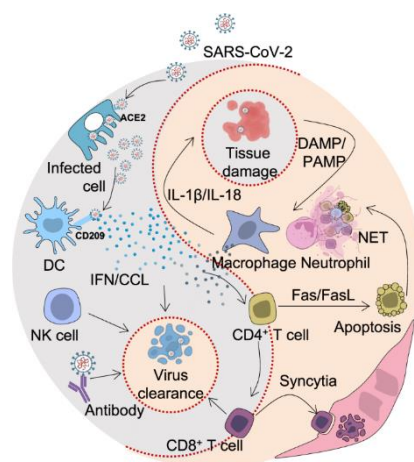
On the other hand, mutations that hinder the spike protein's activation and proteolytic processing can affect viral pathogenicity [52]. For example, changes near the furin cleavage site, like

the P681H mutation, have been linked to a reduction in furin-mediated cleavage and, possibly, a reduction in viral entrance and pathogenicity [121].

For the development of efficient therapeutic approaches against COVID-19, it is essential to comprehend the effects of spike protein mutations on proteolytic processing. It may be possible to thwart viral entrance and reproduction by using protease inhibitors that target the proteases responsible for cleaving spike proteins. Furthermore, keeping an eye on the appearance and dissemination of novel variants with mutations that alter proteolytic processing can reveal crucial details about the pathogenesis and evolution of SARS-CoV-2.

## 7. Immune Evasion Mechanisms Associated with Spike Protein Mutations

Understanding the immune evasion mechanisms linked to these changes is crucial because SARS-CoV-2 spike protein alterations can affect the virus' capacity to avoid host immunological responses and establish infection (See Figure 6) [122].



**Figure 6.** Immune response in SARS-CoV-2 (<https://www.nature.com/articles/s41418-022-01015-x>).

### 7.1. Impact on Antibody Binding and Neutralization

It is crucial to take SARS-CoV-2 spike protein mutations into account when creating efficient vaccines and treatments since they can drastically affect how well antibodies can attach to and kill the virus [123]. The portion of the spike protein's receptor-binding domain (RBD) that directly engages the host cell receptor ACE2 is the principal target of antibodies [124]. Reduced binding affinity and neutralizing potency can result from mutations in this area that change how the RBD interacts with antibodies and its structure [125]. For instance, research has revealed that the B.1.351 [South African] variety possesses RBD mutations that have been demonstrated to up to 10-fold reduce antibody binding and neutralization compared to the original strain [126].

Additionally, it has been determined that antibodies can specifically target the N-terminal domain (NTD) of the spike protein [127]. A reduction in the binding of several monoclonal antibodies has been linked to mutations in the NTD, raising worries that these mutations may aid immune evasion [128]. For instance, it has been demonstrated that a mutation in the NTD seen in the P.1 (Brazilian) variation reduces antibody binding and neutralization [129]. Antibodies can also target the S2 subunit of the spike protein in addition to the RBD and NTD [128,129]. The stability of the protein and the capacity of antibodies to recognize it can both be impacted by mutations in this area [92].

These results emphasize the significance of tracking spike protein mutations for their effect on antibody binding and neutralization, particularly in the context of developing vaccines and antibody-based treatments. To find mutations that may be able to avoid immune recognition and impair the effectiveness of vaccinations and therapeutic antibodies, it is crucial to continuously monitor developing variants. Additionally, the creation of vaccines and treatments that focus on many spike



protein areas may assist to lessen the effects of mutations in a single region and provide all-around protection from SARS-CoV-2 and its variants.

### *7.2. Influence on T-cell Responses and Immune Memory*

Mutations in the SARS-CoV-2 spike protein can also impact immunological memory and T-cell responses [130]. T-cells are essential for the adaptive immune response to viral infections because they can identify and destroy infected cells [131]. SARS-CoV-2 has the ability to subvert the host immune system, and mutations in the spike protein have been demonstrated to impact T-cell identification of infected cells [132]. According to Mengist et al., 2021, some mutations in the spike protein can impair the ability of T cells to recognize infected cells, which could result in a weakened immune response and increased viral reproduction.

The long-term immunity to SARS-CoV-2 may also be affected by spike protein mutations. By producing memory T-cells that can quickly react to re-infection with the same virus, T-cells can produce long-lasting immunity against viral infections [130]. However, memory T-cells may be less able to identify and react to the virus upon re-infection if spike protein mutations lead to modifications to the viral antigens identified by T-cells [72]. Because of this, research into the effects of spike protein mutations on T-cell responses and immunological memory is crucial, especially in light of the development of long-lasting immunity and the potential need for booster shots.

Spike protein mutations may affect immunological memory and T-cell responses, according to a number of studies. The T-cell response to various SARS-CoV-2 variants, including those with spike protein mutations, was recently examined, and it was discovered that some variants were less successfully detected by T-cells than the original Wuhan strain [133]. According to another study, people infected with variations bearing the E484K mutation in the spike protein exhibited weaker T-cell responses than people infected with versions lacking the mutation [134]. These results imply that mutations in the spike protein can influence T-cell identification of infected cells and may have consequences for the long-term immune system.

All things considered, the effect of spike protein mutations on T-cell responses and immunological memory is a significant area of research that calls for additional study. The creation of efficient vaccines and therapies for SARS-CoV-2 can be guided by an understanding of the potential effects of these mutations on the adaptive immune response.

### *7.3. Implications for Vaccine Efficacy and Escape*

The discovery of novel variations with spike protein mutations has sparked worries about the possible effects on vaccine effectiveness [4]. Vaccines that target the spike protein produce neutralizing antibodies that stop the contact between the virus and host cells, so preventing viral infection [135]. However, changes to the spike protein may lessen how well vaccine-induced antibodies work. According to numerous investigations, some variations with particular spike protein mutations, like the E484K mutation, can evade neutralization by convalescent plasma or monoclonal antibodies [136]. Additionally, in vitro research has shown that vaccine recipients' sera have less neutralizing activity against some of the variations, such as the Beta and Gamma versions [137].

The possibility of immunity conferred by vaccines escaping is a serious worry for vaccine developers. Some vaccine producers have already started making changes to their products to better fit the new versions. Moderna and Pfizer-BioNTech, for instance, have stated that their mRNA vaccines are successful against the variants tested, but they are also creating booster doses that focus on the spike protein mutations seen in the Beta and Gamma variants [138]. Additionally, Novavax has stated that it is creating a variation-specific booster because its protein-based vaccination is less effective against the Beta version [139].

It is crucial to remember that vaccines trigger a wide range of immunological responses, including T cell-mediated immunity, which may offer some amount of defense against new variations [140,141]. T cell responses have the ability to identify and eradicate virus-infected cells, which may help to reduce the severity of sickness and regulate viral reproduction [142]. According



to several research, spike protein mutations have less of an impact on T cell responses brought on by vaccination or naturally occurring infection than they do on antibody responses [143]. As a result, T cell responses brought on by vaccination may offer some degree of cross-protection against variations that include spike protein mutations.

8. Implications for Covid-19 Vaccines and Therapeutics

The outbreak of SARS-CoV-2 and the subsequent COVID-19 pandemic have had a major effect on economy and health around the world [144]. As a result, an enormous effort has been made to create efficient medicines and vaccines to combat the virus. The spike protein has been the main target for the development of vaccines and treatments because it facilitates viral entrance into host cells [145,146]. However, there are worries about the possible effects on vaccination and therapy efficacy due to the virus's continuing evolution, especially the appearance of novel spike protein mutations. Therefore, it is essential to assess how these alterations may affect the creation and application of COVID-19 vaccines and treatments (See Figure 7).

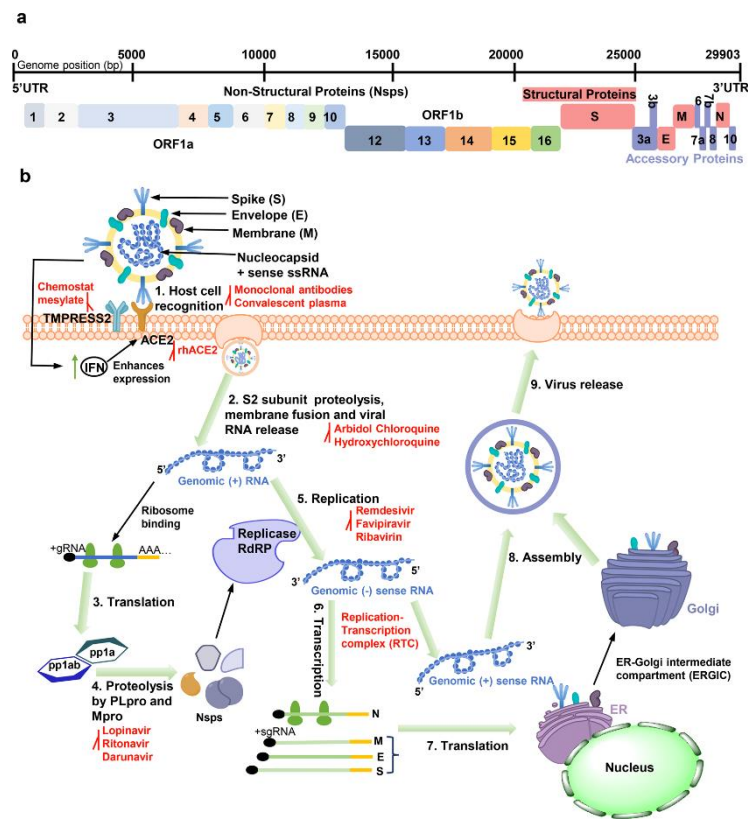


Figure 7. SARS-CoV-2 structural biology: a window for novel treatments.

(<https://www.nature.com/articles/s41392-022-00884-5>)

8.1. Design and Development of Spike Protein-Based Vaccines

Due to its crucial function in viral entrance into host cells, the spike protein of SARS-CoV-2 has been a primary target for the development of COVID-19 vaccines. Typically, entire inactivated virus, live attenuated virus, viral vectors, or mRNA technology are used in the design and development of spike protein-based vaccines [147]. The selection of a platform is influenced by a variety of elements, including cost-effectiveness, scalability, efficacy, and safety.

Utilizing virus particles that have been inactivated or have undergone attenuation and are expressing the spike protein can result in a broad immune response against several viral antigens [148]. Another strategy is to introduce the spike protein gene into host cells using viral vectors like adenovirus or vesicular stomatitis virus to start an immunological reaction against the virus [148,149].

A more recent strategy makes use of mRNA technology to transport the spike protein's genetic code to host cells, enabling those cells to manufacture the protein and trigger an immune response [150,151].

Regardless of the platform chosen, the design of a vaccine based on a spike protein must consider the possible effects of mutations on the vaccination's efficacy [152]. In order to maintain effectiveness, the vaccine's ability to trigger a protective immune response against the virus may be affected by the introduction of new spike protein mutations, necessitating design changes or booster shots [85]. For the successful development and application of spike protein-based vaccines against COVID-19, constant viral surveillance and vaccine efficacy monitoring are therefore essential.

### *8.2. Evaluation of Spike Protein Mutations on Vaccine Efficacy*

Concerns have been raised about the effect of SARS-CoV-2 spike protein mutations on the effectiveness of vaccines [153]. The impact of spike protein mutations on the capacity of vaccine-induced antibodies to neutralize the virus has been examined in a number of studies [154]. These investigations have demonstrated that specific spike protein mutations can lessen the binding of vaccine-induced neutralizing antibodies [155]. It has been discovered that changes to the spike protein's receptor-binding domain (RBD) have an impact on how well antibodies attach to and kill viruses [155,156].

Researchers have carried out tests using serum samples from vaccine recipients to evaluate the effect of spike protein mutations on vaccine effectiveness [157]. These investigations have demonstrated that vaccines in use now offer some defence against problematic variations, including those with spike protein mutations [158]. For some variations, the level of protection could be lessened. For instance, it has been demonstrated that the B.1.351 variation, which carries a number of mutations in the spike protein, decreases the effectiveness of several vaccines [21].

Updated versions of various vaccinations have been created by vaccine producers to address the potential impact of spike protein mutations on vaccine efficacy [159]. These modified vaccinations aim to boost protection against these variants by incorporating the spike protein changes discovered in variants of concern. Clinical trials are being conducted now to judge the security and effectiveness of these upgraded vaccines [160].

In addition to vaccinations, COVID-19 treatment options may also include monoclonal antibodies that target the spike protein [23]. The effectiveness of these treatments has, however, been questioned in light of the appearance of spike protein mutations [23,161]. Monoclonal antibodies' capacity to attach to the spike protein and so neutralize the virus has been demonstrated in studies to be affected by various mutations [162]. Therefore, ongoing surveillance of spike protein mutations is crucial for the development of effective vaccines and therapies to combat COVID-19.

### *8.3. Therapeutic Targeting of Spike Protein Mutations*

Research into the therapeutic targeting of SARS-CoV-2 spike protein mutations is crucial since it may lessen the severity of the disease and enhance patient outcomes [28,18]. The primary target of several monoclonal antibody treatments and currently available COVID-19 vaccines is the spike protein [158]. The usefulness of these vaccines and treatments has, however, come under scrutiny due to the appearance of novel spike protein mutations [23]. In order to create novel treatments and enhance the effectiveness of current ones, it is crucial to comprehend how these mutations affect the spike protein's structure and function [142].

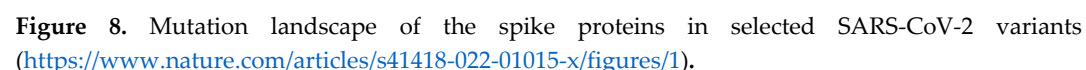
The creation of broad-spectrum antivirals that target conserved areas of the spike protein is one method to address the problems brought on by spike protein mutations [154]. A different tactic is to combine monoclonal antibodies that are directed against various spike protein epitopes [160]. Researchers are also investigating the use of mRNA-based vaccinations, which can be quickly updated to target new viral strains [82].

It's also crucial to remember that there are other factors to consider in addition to how well vaccinations and treatments work against spike protein mutations [10]. In the struggle against COVID-19, other elements including vaccination hesitancy, vaccine distribution and access, and

The current COVID-19 pandemic has brought attention to the importance of continuing research on SARS-CoV-2 and the mutations in its spike protein. It is crucial to look into the potential effects of these changes on viral infectivity, pathogenicity, and immune evasion as new virus varieties continue to appear and the world's immunization efforts change [162].

There is still a lot to learn about the SARS-CoV-2 virus and its spike protein alterations as the COVID-19 pandemic develops. Many concerns are still unsolved despite tremendous advances in understanding the structural and functional effects of these alterations. The long-term effects of spike protein mutations on viral infectivity, transmissibility, and pathogenesis are a crucial field for further study. Determining if and how new mutations will continue to appear and spread, as well as how they may affect the creation and effectiveness of vaccines and medicines, will be crucial. Further study is required to understand the mechanisms causing immune evasion and to create fresh methods of thwarting this phenomenon. There is a pressing need for continued collaboration and communication among scientists, public health officials, and policymakers to ensure that the most up-to-date information is available to guide ongoing efforts to control the spread of COVID-19.

A global health emergency has been caused by the development and spread of SARS-CoV-2. Understanding the effects of spike protein mutations is essential for the creation of potent vaccines and treatments as the virus continues to evolve. Spike protein mutations have been linked to alterations in immune evasion, pathogenesis, and receptor binding, all of which can affect the severity of a disease and the effectiveness of a vaccination. The structural and functional effects of spike protein mutations and their implications for viral pathogenesis and infectiousness have been covered in this review. We have also discussed the difficulties in developing vaccines and the possibility for therapeutic targeting of mutations in spike proteins (See Figure 8).



The rapid development of the virus, which might result in the introduction of new versions with distinct spike protein mutations, is one of the major obstacles in the fight against COVID-19. For efficient communication between scientists and medical experts, the classification and nomenclature of these mutations must be standardized. The development of tailored therapies can be aided by understanding the emergence and dissemination of these mutations using genomic and phylogenetic analysis.

Additionally, the intensity of the illness and the clinical results are significantly impacted by the effect of spike protein mutations on viral proliferation, transmission, and host immune responses. Identifying high-risk patients and creating focused therapies can be made easier by understanding the relationship between spike protein mutations and disease severity. Additionally, the effectiveness of vaccines and immunological memory may be affected by the manipulation of host immune responses by spike protein mutations.

The advent of vaccination-resistant variations emphasizes how crucial it is to assess how spike protein mutations affect vaccine effectiveness. The possibility for mutations to affect vaccination efficacy must be taken into consideration throughout the design and development of spike protein-based vaccines. A further option for the prevention and treatment of COVID-19 is the therapeutic targeting of spike protein mutations.

In conclusion, the study of spike protein mutations is crucial for understanding the evolution, pathogenesis, and control of SARS-CoV-2. Further research is needed to unravel the unresolved questions surrounding the impact of spike protein mutations on viral infectivity, immune evasion, and vaccine efficacy.

### *9.3. Concluding Remarks and Recommendations.*

The structural and functional characteristics of the SARS-CoV-2 spike protein are examined in this review paper, along with the evolutionary dynamics of spike protein mutations and their effects on viral pathogenesis, vaccination effectiveness, and viral infectivity. The article describes how changes to the spike protein's shape, receptor binding affinity and specificity, and interactions with host components can all have an impact on viral reproduction and transmission, control of host immunological responses, and immune evasion strategies. The effectiveness of vaccines and the creation and development of new treatments may both be impacted by these changes, according to the article.

The review concludes that the spike protein is a significant factor in the pathogenesis of SARS-CoV-2 and the creation of efficient vaccines and treatments. Continuous monitoring of spike protein mutations is required to spot new variants that might bypass vaccine-induced immunity and to direct the creation of fresh vaccinations and medications. The authors advise combining vaccines that target several viral components as well as continuing to create broadly protective vaccines that specifically target conserved spike protein domains. The significance and ramifications of spike protein mutations in SARS-CoV-2 pathogenesis and vaccine development are explained in detail by this review.

**Supplementary Materials:** Not applicable.

**Author Contributions:** Conceptualization, A.G.A.M. and H.K.; writing—original draft preparation, A.G.A.M.; writing—review and editing, S.C.U. and N.A.M; supervision, RK and H.M.K. All authors have read and agreed to the published version of the manuscript.

**Funding:** The APC was funded by the University of Kwazulu-Natal.

**Institutional Review Board Statement:** Not applicable.

**Informed Consent Statement:** Not applicable.

**Data Availability Statement:** Not applicable.

**Acknowledgments:** The authors acknowledge the College of Health Sciences of the University of Kwazulu-Natal for the support.

**Conflicts of Interest:** The authors declare no conflict of interest.



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