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Review Article

Role of SARS-CoV-2 and ACE2 variations in COVID-19

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ABSTRACT

Coronavirus disease 2019 (COVID-19), caused by the severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), is one of the worst medical emergencies that has hit the world in almost a century. The virus has now spread to a large number of countries/territories and has caused over three million deaths. Evidently, the virus has been mutating and adapting during this period. Significant effort has been spent on identifying these variations and their impact on transmission, virulence and pathogenicity of SARS-CoV-2. Binding of the SARS-CoV-2 spike protein to the angiotensin converting enzyme 2 (ACE2) promotes cellular entry. Therefore, human ACE2 variations could also influence susceptibility or resistance to the virus. A deeper understanding of the evolution and genetic variations in SARS-CoV-2 as well as ACE2 could contribute to the development of effective treatment and preventive measures. Here, we review the literature on SARS-CoV-2 and ACE2 variations and their role in COVID-19.

In late December 2019, hospitals in Wuhan City, Hubei province of China observed a string of respiratory infections of unknown etiology. Most of the infected patients had a history of exposure to a seafood and wet animal market in Wuhan [1,2]. By January 7, 2020 a previously unrecognized virus was isolated from the throat swab of a patient by the Chinese Center for Disease Control and Prevention (CDC). Subsequently, its genome was elucidated by using next-generation sequencing [3,4]. The virus was initially named as the 2019 novel coronavirus (2019-nCoV) by the World Health Organization (WHO). On February 12, 2020 the virus was formally named as the severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) by the Coronaviridae Study Group of the International Committee on Taxonomy of Viruses and the disease associated with it as the coronavirus disease 2019 (COVID-19). The naming was based on the phylogenetic relationship of the new virus with the severe acute respiratory syndrome coronavirus (SARS-CoV). Due to the alarming spread and severity of COVID-19, on January 30, 2020, the WHO declared the SARS-CoV-2 outbreak as a Public Health Emergency of International Concern. Since the first reported

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case, the morbidity and mortality increased exponentially around the globe. This led to the declaration of COVID-19 as a global pandemic by the WHO on March 11, 2020. Indisputably, COVID-19 is one the worst medical emergencies that has hit the world in almost a century. Recently, a number of vaccines based on inactivated viruses, viral vectors, nucleic acids and viral proteins have been approved around the world for emergency use and more than 50 vaccine candidates are in clinical trials [5]. The major phenotype of this disease could include some or all of fever, cough, shortness of breath and respiratory disorders. A vast number of COVID-19 patients are either asymptomatic or mildly symptomatic but, in severe cases, infection can cause pneumonia, acute respiratory syndrome, kidney failure and possibly death [6]. COVID-19 has now spread to over two hundred countries or regions including Antarctica. In a span of around 18 months, the virus has caused more than three million deaths and confirmed cases have exceeded 178 million globally (https://coronavirus.jhu.edu).

Overview of coronaviruses

Coronaviruses (CoVs) are the largest group of viruses that belong to the *Coronaviridea* family. They cause respiratory and intestinal infections in animals and humans. Due to genomic nucleotide substitutions and recombination, CoVs are one of the most rapidly evolving viruses [7]. Until the outbreak of SARS in 2002 in China, these viruses were not considered to be highly pathogenic [8]. CoVs are relatively large, enveloped, single-stranded, positive-sense RNA viruses. They get their name from the crown-like viral protein that projects out of their envelope. Based on serological and genomic characteristics, CoVs are classified into four genera – alphacoronavirus, betacoronavirus, gammacoronavirus and deltacoronavirus [9]. They are highly contagious and cause disease in a variety of domestic and wild animals. Alphacoronaviruses and betacoronaviruses primarily infect mammals while gammacoronaviruses and deltacoronaviruses mainly infect birds [10].

Currently seven species of coronaviruses are known to infect humans. Before the outbreak of SARS, only two human CoVs (HCoVs) - the alphacoronavirus HCoV-229E and the betacoronavirus HCoV-OC43 were known to cause human disease such as mild common colds [11]. SARS was caused by a betacoronavirus eventually named as SARS-CoV. It affected over 8000 individuals and caused nearly 800 deaths [12]. Subsequent to the SARS epidemic, two more HCoVs – the alphacoronavirus HCoV-NL63 and the betacoronavirus HCoV-HKU1 - were reported that were capable of causing mild to serious lower respiratory tract infections in infants, children and adults [13]. In 2012, another novel betacoronavirus was identified and reported in the Middle East that could cause severe respiratory illness. The virus was named the Middle East respiratory syndrome coronavirus (MERS-CoV) [14]. The most recent novel betacoronavirus is the SARS-CoV-2 that causes COVID-19.

Genome organization

Coronaviruses carry relatively large RNA genomes ranging from 27 to 32 kilobases (kb). Like other coronaviruses, SARS-CoV-2 is an enveloped virus with a linear single-stranded positive-sense RNA of ~30 kb [15,16]. The SARS-CoV-2 genome consists of six major open reading frames (ORFs) and a number of accessory genes [17]. Roughly, two third of the viral RNA encodes two large overlapping ORFs namely ORF1a and ORF1b, or ORF1ab together, that produces non-structural proteins (nsps). ORF1a is translated to produce polyprotein 1a (pp1a) while ORF1ab yields polyprotein 1ab (pp1ab). These polypeptides are further cleaved by viral encoded proteases into 16 nsps (nsp1-16) that are involved in the viral replication and transcription. During the time of their infection in host cells, SARS-CoV-2 replicates its genomic RNA using nsp12 that harbors RNA-dependent RNA polymerase (RdRP). The remaining one third of the genome encodes four structural proteins namely spike (S), envelope (E), membrane (M) and nucleocapsid (N) [16]. Another interesting feature of CoVs is the synthesis of a nested set of subgenomic RNAs (sgRNAs) from genomic RNA by discontinuous transcription. These sgRNAs are translated into structural proteins (S, E, M, N) and several accessory proteins. Each viral sgRNA contains a common leader sequence derived from the 5′ end of the genomic RNA. The leader sequence plays a critical role in gene expression and the length of this sequence varies between different CoV species [16,18]. The S protein, located on the virion surface, is a trimeric class I fusion protein. It is one of the most variable and probably one of the most important part of the CoV genome. It mediates binding of the virus to the host receptor as well as membrane fusion for invading the host cell [19]. The M glycoprotein could be regarded as the central organiser since it interacts with several other structural proteins. It is the most abundant structural protein and spans the membrane bilayer three times [20]. It also plays a significant role in the budding process as well as the sorting of viral components into virions. Among the structural proteins, the E protein is the smallest and this membrane protein is essential for assembly and budding of virions. Even though they are abundantly expressed during replication, only a small number of these E proteins are incorporated into the virion envelope. The primary function of the N protein is to make up the nucleocapsid. Additionally, it is also assists with viral transcription and replication [21].

Initial analysis of the sequenced genome revealed that SARS-CoV-2 shared 80% sequence identity with SARS-CoV and 96.2% with the bat coronavirus (BatCoV) RaTG13, obtained from the bat *Rhinolophus affinis* [17,22]. As SARS-CoV-2 genome was highly similar to the BatCoV genome, it was suggested that bats may act as the primary and natural reservoir of these viruses [17]. Like SARS-CoV and MERS-CoV, this zoonotic disease was also believed to have been transmitted from an intermediate host. In both cases, bats acted as the natural reservoir while masked palm civet and the dromedary camel acted as the intermediate host [8,23]. Another interesting observation was subsequently made from coronaviruses isolated from Malayan pangolins (*Manis javanica*). Genomic and evolutionary analysis revealed that Pangolin-CoV is 91.02% and 90.55% identical to SARS-CoV-2 and BatCoV RaTG13, respectively. This makes Pangolin-CoV the second closest relative of SARS-CoV-2 behind RaTG13. This suggests that pangolin could also be considered as possible hosts of SARS-CoV-2 [24,25].

Spike protein and their receptors

The entry of SARS-CoV-2 into the host cell is mediated by the transmembrane S glycoprotein protruding from the viral surface [19]. The S protein is functionally divided into two subunits S1 and S2 that mediate attachment and membrane fusion respectively. The S1 subunit is further divided into two independent domains - the amino-terminal domain (NTD) and the carboxy-terminal domain (CTD). Depending on the type of CoV, receptor binding domain (RBD) could be in either NTD or CTD [26]. In SARS-CoV-2, the RBD is located in the S1 subunit and composed of a core and an extended insertion. Five antiparallel β-strands along with short connecting helices and loops constitute the core. It is also responsible for the formation of the S trimer. The extended insertion, that hosts the receptor binding motif (RBM), contains most of the residues that allow the SARS-CoV-2 to bind to the host receptor [27].

In the mature virus, three S1/S2 subunits assemble to form a spike-like trimer structure that protrudes from the viral envelope. The entry of virus into host cell is a complex process involving a series of steps that include the binding of the S protein to the host receptor and proteolytic cleavage of the protein to facilitate virus cell fusion. The two functional subunits (S1 and S2) of the S protein remain non-covalently bound in the prefusion state stabilized by the RBD of the S1 subunit [28–30]. Binding of S1 to the host receptor results in substantial structural rearrangement that destabilizes the trimer [31]. These conformational changes are crucial for membrane fusion since this exposes the S1/S2 cleavage site to host proteases [32,33]. Sequence analysis of SARS-CoV-2 S protein reveals an insertion of four amino acids ('PRRA') at the boundary of S1/S2 subunits. Interestingly, this insertion is not present in SARS-CoV and other SARS-related CoVs but was observed in human CoVs HCoV-OC43, HCoV-HKU1 and MERS-CoV [34,35]. The insertion introduces a "PRRARS/V" sequence, which is a furin recognition site, in SARS-CoV-2 that aids effective cleavage of S1/S2 by furin and other proteases [36]. It has been suggested that the presence of multiple arginines (multibasic) in the cleavage site could activate ubiquitously expressed proprotein convertases, like furin, that could assist the systematic spread of the virus [37]. It has been suggested that SARS-CoV-2 S protein behaves in a manner similar to that of MERS-CoV. Initially, the S protein of both viruses are cleaved by furin at the S1/S2 junction followed by plasma membrane–associated type II transmembrane serine protease (TMPRSS2) mediated membrane fusion [34]. TMPRSS2 expressing cell lines are highly susceptible to SARS-CoV-2 infection when compared to non-expressing cell lines, supporting the role of TMPRSS2 in viral infection [38].

Analysis of amino acid variation patterns of S1 proteins from SARS-CoV-2, SARS-CoV, RaTG13 and Pangolin-CoV revealed that the S1 subunit of Pangolin-CoV is more closely related to SARS-CoV-2 than RaTG13. It was also observed that RBD of both SARS-CoV-2 and Pangolin-CoV are highly conserved with just a single amino acid change (glutamine at position 500 in SARS-CoV-2 S1 and histidine in Pangolin-CoV) suggesting a similar mechanism of infection in Pangolin-CoV and SARS-CoV-2 [24].

Depending on the species, CoVs adopt different target receptor recognition patterns for binding to the host. For instance, MERS-CoV identifies and binds to the transmembrane dipeptidyl peptidase 4 (DPP4), which is also known as cluster of differentiation (CD26) [39,40]. The SARS-CoV, and other related viruses, targets angiotensin converting enzyme 2 (ACE2) for their entry into human cells [41,42]. SARS-CoV-2 has also been shown to target ACE2 for host cell entry [17,34,43]. ACE2 is an 805 amino acid long transmembrane type I glycoprotein with two functional domains – the N terminal peptidase domain and a C terminal domain homologous to glycoprotein collectrin. The peptidase domain of ACE2 has two lobes that harbors the active site [44]. The primary physiological role of ACE2 is the maturation of the peptide hormone angiotensin that controls blood pressure and vasoconstriction [45]. Additionally, it also cleaves a number of biologically active peptides like kinetensin, neurotensin, casomorphins and apelins [46]. ACE2 is expressed in type I and II alveolar epithelial lung cells, intestine, kidney, heart, adipose tissues and blood vessels, which can be correlated with high frequency of pneumonia and bronchitis in COVID-19 patients [47]. A high expression of ACE2 in the oral cavity, specifically on the tongue than buccal and gingival tissues, has also been reported. This could potentially increase the risk of SARS-CoV-2 infection as it grants easy access to the host [48]. During infection, the S1 subunit directly binds to the peptidase domain of ACE2. ACE2 is subsequently cleaved by either disintegrin and metallopeptidase domain 17 (ADAM17)/ tumor necrosis factor α-converting enzyme (TACE) or TMPRSS2. It has been shown that processing by TMPRSS2 promotes viral entry [49,50].

The association of SARS-CoV-2 and ACE2 has been reported to be stronger than SARS-CoV due to mutations in the S protein RBD. The RBD of SARS-CoV-2 S protein has evolved structural changes in the ACE2 binding ridge when compared to SARS-CoV. In SARS-CoV-2, the more compact RBD structure produced by residues between 482 and 485 (Gly-Val-Glu-Gly) forms better contacts with the N terminal helix of ACE2. It has been suggested that the replacement of Leu472 in SARS-CoV with Phe486 in SARS-CoV-2 could produce stronger van der Waals interaction with ACE2. Similarly change from Val404 to Lys417 increases the association of SARS-CoV-2 RBD and ACE2 due to the formation of a salt bridge between Lys417 of S protein and Asp30 of ACE2 [51,52]. These structural features enhance the binding affinity of SARS-CoV-2 to ACE2 when compared to SARS-CoV. In addition, S2 subunit of SARS-CoV-2 exhibits superior plasma membrane fusion capability than SARS-CoV [53]. Apart from these, crystallographic studies and biophysical assays suggest that SARS-CoV-2 S protein binds to ACE2 with 10 to 20-fold higher affinity than SARS-CoV, which could account for the higher infectivity and transmissibility [54]. In fact, it has been suggested that two S protein trimers of SARS-CoV-2 could independently bind to an ACE2 homodimer [52].

As SARS-CoV-2 targets multiple organs efficiently, work has been done to identify potential targets other than ACE2. Cluster of differentiation 147 (CD147), also known as basigin (BSG) or extracellular matrix metalloproteinase inducer (EMMPRIN), is one such receptor that has been reported to enable SARS-CoV-2 entry. Anti-CD147 humanized antibody

meplazumab, currently undergoing phase II clinical trials, has been reported to prevent SARS-CoV-2 infection by blocking CD147 [55]. Data from two studies suggest that the S1 subunit of SARS-CoV-2 could directly bind to two forms of neuropilin, a cell surface receptor, namely NRP1 and NRP2 [56,57]. Analysis of lung tissue of patients who died from COVID-19 showed an increased expression of NRP1 and NRP2 suggesting an upregulation of these genes at the site of inflammation [58]. NRP1 and NRP2 are type 1 transmembrane proteins involved in physiological processes like axon guidance, angiogenesis and control of cellular signal cascades. The extracellular region of these proteins are composed of five structured domains including two CUB (for complement C1r/C1s, Uegf, Bmp1) domains (a1 and a2), two coagulation factor domains (b1 and b2), and a MAM (meprin, A-5 protein, and receptor protein-tyrosine phosphatase mu) domain [59]. These studies have demonstrated that furin cleaved substrates of S protein, mainly the S1 subunit, is able to bind to the b1 domain of NRP1 [60]. Thus, these observations suggest that, apart from ACE2, other host cell proteins could also be targeted in a SARS-CoV-2 infection.

Genetic variations in SARS-CoV-2

Mortality due to SARS-CoV-2 infection depends on several factors including age, gender, race, quality of health care, restriction in movement, quarantine, difference in genetics and immune system response. Apart from these, genetic variations in the virus also alters its pathogenicity and virulence. The role played by these mutations and emergence of new viral strains cannot be ignored due to the rapid spread and mortality rate of the virus. The mutation rate of RNA viruses is dramatically high as error rates in RdRP are generally high due to the lack of a proofreading mechanism [61]. Additionally, viruses could also exploit genetic variations to improve their virulence and evolve to evade the immune response [62]. Soon after the emergence of SARS-CoV-2 in late December 2019, the viral genome was sequenced and made publicly accessible [63]. The reference genome of SARS-CoV-2 is available from the Global Initiative on Sharing All Influenza Data (GISAID; https://www.gisaid.org; hCoV-19/Wuhan-Hu-1/2019, EPI_ISL_402125) as well as NCBI (Accession: NC_045512). Since then, extensive sequencing of viral genomes was done in a number of countries and made available to the public through the GISAID initiative and NCBI.

A number of studies have now looked at the genomic data to identify new variants and their association with disease severity. An analysis of 95 SARS-CoV-2 whole genome sequences identified 116 unique variants, most of which were missense and synonymous variations. However, this study did not consider the association of these variations with severity of disease [64]. Another analysis of 86 genomic sequences obtained from GISAID detected 3 deletions and 93 variations in genomes of viral isolates from Japan, USA and Australia. Most of the variations were observed in the ORF1ab and RBD of S protein [65]. As S protein plays a critical role in binding to the host receptor, it was suggested that some of these variations could induce conformational changes that alter the virulence of the virus. Genome diversity analysis of 7666 publicly available genome assemblies identified 198 recurrent mutations in the SARS-CoV-2 genome of which nearly 80% were missense variations. Most of the recurrent mutations were observed in the ORF1ab region coding for nsp6, nsp11, and nsp13, and the S protein. Since majority of these variations are in protein coding regions, it was suggested that the SARS CoV-2 is possibly adapting [66]. Another analysis of mutation data from four geographic areas - Asia, Oceania, Europe, North America - using 220 SARS-CoV-2 genomes identified 8 novel mutation hot spots: 5 of these were predominantly observed in European samples while 3 were exclusively present in North American samples. Mutations at positions 3036, 14408 and 23403 (nsp3, RdRP and S protein) were recurrent in the European region. The mutation at position 14,408 causes an amino acid substitution at position 323 (Pro323Leu) in the RdRP protein, a key component of the replication and transcription machinery. As RdRP works with several other viral cofactors involved in proofreading activity (ExoN, nsp7 and nsp8) this mutation could have altered the binding capability or impaired the proofreading. It was proposed that this could have led to an increase in mutations in the European region since February 2020 [67]. To further understand the effects of RdRP mutations on viral transmission, their association with mutations identified in the M and E glycoproteins was also investigated. Ten mutations were frequently observed in the RdRP region and mutations found at positions 14,408, 14,805, 15,324, and 13,730 were observed to increase the risk of mutations in M/E glycoproteins. Among these, the 14408C > T mutation was found to be associated with and increased likelihood of M/E mutations. Thus, these findings suggest that RdRP significantly contributes to the evolution of SARS-CoV-2 [68] [Table 1].

A phylogenetic network analysis using 160 complete viral genomes attempted to reconstruct the evolutionary path of SARS-CoV-2 from Wuhan to Europe and North America. Based on the analysis, three distinct strains of the virus were identified and clustered into three related lineages designated as Type A, Type B, and Type C. Type A represents the root of the outbreak; Type B was derived from A separated by two mutations (T8782C and C28144T) and Type C is derived from B separated by a single mutation (G26144T). Foster and colleagues found that Type A closely resemble the virus that was found in bats and pangolins. Initially, Type A was found in patients from China and later in patients from the United States and Australia. Even though Type A was found in Wuhan, it was not the predominant strain found in the city. Type B was the major viral strain found in Wuhan and East Asian regions and this strain didn't travel much beyond these regions. This suggests an environmental or immunological adaptation of the viral strain that limited it to the Asian region. Type C was observed across much of Europe, most notably among the early patients from France, Italy, Sweden, and the United Kingdom. This network provides a detailed picture of evolutionary paths of COVID-19 during the early stages of the pandemic [69].

SARS-CoV-2 genes exhibits varying levels of selective pressure. While M and E integral proteins tend to evolve slowly, proteins that are involved in viral replication, transmission and pathogenesis, such as viral replicase, S and N proteins, are more prone to mutations due to host adaptation

Table 1 Summary of mutations reported in SARS-CoV-2 genome.

Gene	Mutation	Amino acid change	Type of mutation	References
ORF1ab	8782C > T	–	Synonymous	[64]
ORF8	28,144 T > C	L84S	Non synonymous	
N	29095C > T	–	Synonymous	
ORF1ab	11083G > T	L3606F	Non synonymous	[66]
ORF1ab	16887C > T	–	Synonymous	
S	21575C > T	L5F	Non synonymous	
RdRp	14408C > T	P323L	Non synonymous	[67,68,71]
S	23403A > G	D614G	Non synonymous	
N	28881G > A	R203K	Non synonymous	
RdRp	14805C > T	–	Synonymous	
RdRp	15324C > T	–	Synonymous	
RdRp	13730C > T	A4489V	Non synonymous	
ORF1ab	8782C > T	–	Synonymous	[69]
ORF3a	26144G > T	G251V	Non synonymous	
S	22879C > A	N439K	Non synonymous	[74,75]
S	22988G > A	G476S	Non synonymous	
S	22899G > A	G446V	Non synonymous	
S	22919A > T	Y453F	Non synonymous	[76]
S	23636G > T	I692V	Non synonymous	
S	25249G > T	M1229I	Non synonymous	
S	25002C > T	S1147L	Non synonymous	
S	23603A > T	N501Y	Non synonymous	[77,78]
S	23271C > A	A570D	Non synonymous	
S	23604C > T	P681H	Non synonymous	
S	23709C > T	T716I	Non synonymous	
S	24506T > G	S982A	Non synonymous	
S	24914G > C	D1118H	Non synonymous	
S	23012G > A	E484K	Non synonymous	[79–81]
S	22813G > T	K417N	Non synonymous	
S	21614C > T	L18F	Non synonymous	
S	21801A > C	D80A	Non synonymous	
S	22206A > G	D215G	Non synonymous	
S	22299G > T	R246I	Non synonymous	
S	23664C > T	A701V	Non synonymous	

[70]. As the pandemic spread, several mutations specific to spike proteins have emerged. These variations alter viral infectivity and transmission as well as the efficiency of neutralizing antibodies that play an important role in the host defense mechanism.

Phylogenetic analysis of the S gene from 144 SARS-CoV-2 genomes from several countries confirmed the presence of a novel missense mutation 23403A > G. This non-conservative mutation causes a change in amino acid from an aspartate to a glycine (Asp614Gly; D614G). This change, from a negatively charged aspartate to a achiral glycine without a side chain and greater flexibility, is located near the furin recognition site at the S1–S2 junction. Asp614 was the predominant form during the early days of the epidemic. In the course of its expansion to Europe, the mutant form Gly614 gained foothold before spreading to other regions. Furthermore, it was observed that both forms (Asp614 and Gly614) were circulating in most sampled countries. By the end of March 2020, the mutated Gly614 was the major variant in most reported samples. The Asp614Gly mutation could have altered the infectivity of virus by improving receptor binding, fusion activation or antibody dependent enhancement elicitation [71]. Zhang et al. reported that D614G mutation increases viral infectivity and virion spike density. This mutation may also provide a fitness advantage to the virus by providing greater flexibility and stability to the S protein for efficient transmission [Fig. 1]. The mutated variant was reported to infect ACE2 expressing cells more efficiently thereby promoting viral infection and transmission [72]. However, this contradicts another study that suggested that Asp614Gly mutation is not associated with increased viral transmissibility. In this analysis 15,000 SARS-CoV-2 viral genomes from 75 countries were assessed for the transmissibility of recurrent mutations. Results suggest that the observed mutations do not increase the transmissibility of the virus and most of the mutations are either detrimental or neutral to the virus. Additionally, most of the recurrent mutations, including Asp614Gly, was suggested to occur due to the interaction with the human immune system [73]. An analysis of 2147 viral genomes from 50 countries identified 23-point mutations in the RBD of the S protein. Among these, 9 mutations exhibited increased affinity and binding to ACE2 while 14 mutations showed lower affinity and stability. Additionally, an analysis of 1150 S protein variants reported by CDC found 76 missense variants in the RBD region (Thr333-Cys525). Out of these, five variants had enhanced binding affinity while three increased the stability of virus–receptor complex [74].

Emerging viral variants that can surpass human immune system could pose a challenge to researchers. Immune escape will make people more susceptible to reinfection and less

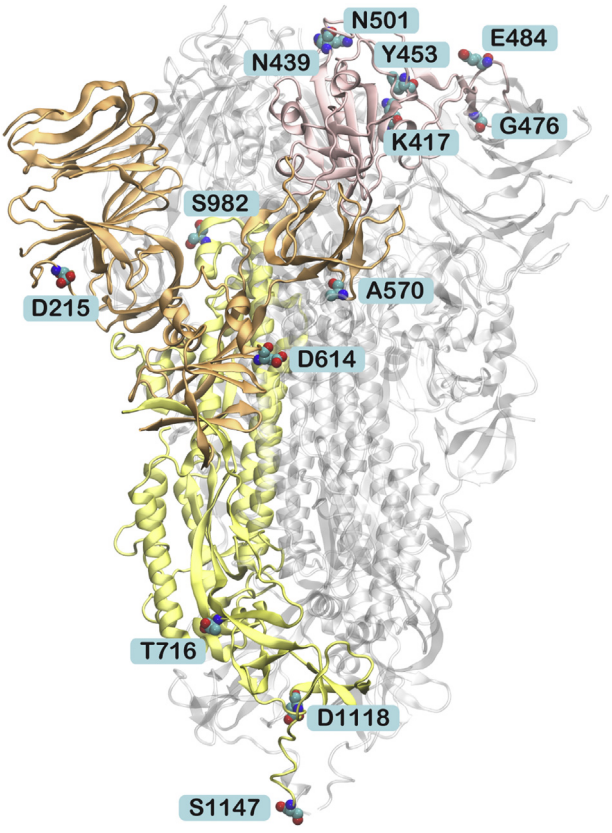

Fig. 1 Rendering of the SARS-CoV-2 spike glycoprotein trimer based on an X-ray crystal structure (Protein Data Bank ID: 6XLU) [83]. For clarity, only one subunit is shown in color while the other two are shown in translucent white cartoon representation. In the trimer, the S1 subunit is shown in orange and the RBD harbored within the S1 subunit is shown in pink. The S2 subunit is shown in yellow. Locations with mutations reported in the literature are shown in van der Waals representation. The image was rendered using Visual Molecular Dynamics 1.9.3 [84].

responsive to vaccines. The human immune system fights against viral invasion by producing neutralizing antibodies against RBD region to disrupt the viral binding to the ACE2 receptor. However, in an effort to survive, the virus could mutate to evade the host immune system. Several mutations have been reported in the spike region and one of the most dominant mutation is the D614G. This mutation, combined with others, could make the virus more infectious. Another important mutation reported in the RBD region of SARS-CoV-2 is Asn439Lys (N439K) [Fig. 1]. This mutation results in a stronger association with human ACE2 (hACE2) due to the formation of a new salt bridge in the RBD-hACE2 interface. Apart from this, additional hydrogen bonds and van der Waals interactions were also observed in the mutated virus-hACE2 complex. Consequently, the stronger binding of this mutated strain may be the reason for it being more infectious than the original strain [75]. Furthermore, this strain has been reported to be resistant to neutralizing antibodies and capable of immune escape from a panel of neutralizing monoclonal antibodies [82]. Thus, this variant has raised concerns about reduced antibody production and vaccine efficacy. In addition to this, in early November 2020, Denmark reported several COVID-19 cases caused by SARS-CoV-2 variants from farmed minks. Viral variants identified from these infections were grouped into five closely related clusters. Initial observations and clinical data suggest that the severity and transmission of these variants are closely related to other SARS-CoV-2 strains except for the cluster 5 variant. This variant had four substitutions and two deletions in the spike protein region including Y453F, I692V, M1229I, S1147L and 69-70Δ HV. In early experiments the cluster 5 variant showed moderately decreased sensitivity towards neutralizing antibodies suggesting that this variant could be less responsive to vaccines or antibodies. By taking proper preventive measures and mass culling, Danish authorities have declared that the variant is under control [76].

A rapidly spreading SARS-CoV-2 variant (SARS-CoV-2 VUI 202012/01) was first reported in the United Kingdom (UK) in December 2020 as the country faced rapid increase in COVID-19 cases. Genomic sequencing of the variant identified multiple spike protein mutations (N501Y, A570D, D614G, P681H, T716I, S982A, D1118H, Δ69–70, Δ144) and other genomic variations. Preliminary data indicated that the newly reported strain was more transmissible than previously reported variants with up to 70% higher transmissibility rate [77]. Among the mutations in the spike region, N501Y is one of the six key amino acids involved in the RBD-hACE2 complex formation. This novel mutation could therefore facilitate a stronger association between the spike protein and hACE2. Additionally, this strain also harbors the D614G mutation which help the virus to spread rapidly. In early January 2021, the National Institute of Infectious Diseases (NIID) of Japan reported a new variant isolate of SARS-CoV-2. The new variant had previously reported mutations in the spike protein including N501Y and E484K. The E484K mutation has been identified as a neutralizing antibody escape mutation, which suggest a change in antigenicity [85]. On December 18, 2020, the South African government announced the emergence of a new SARS-CoV-2 strain (501Y.V2) harboring eight mutations in the spike protein. In addition to D614G, three mutations were found in key residues in the RBD (N501Y, E484K and K417N), three at the N terminal domain (L18F, D80A and D215G) and one in the loop 2 region (A701V). Initial reports suggested that the new variant was highly transmissible and exhibited complete immune escape from neutralizing antibodies [79,80]. Furthermore, recent genomic studies reported the emergence of two B.1.1.28 sub-clades designated as P.1 (B.1.1.28.1) and P.2 (B.1.1.28.2) in Brazil. These emerging variants harbor several mutations in the spike protein including N501Y, E484K and K417N [Fig. 1] [81]. These rapidly spreading strains, with reduced antibody neutralization, were found in the United Kingdom, South Africa and Brazil and has now been classified as Variants of Concern (VOCs) [78].

Thus, it is evident that over the course of time, this virus is expected to mutate and evolve resulting in the emergence of novel viral variants. Persistent monitoring is imperative during its evolution for efficient vaccine development. Studies have also reported that introduction of vaccines could cause selection pressure in S protein which may change the T cell and B cell epitopes with immune escape property. These

strategic mutations could hamper the ability of host immune system in recognizing and combating the virus [86,87].

Genome-wide association studies have also been performed to identify whether genetic variations are linked to the severity of COVID-19. In one such study, 152 complete viral genomes were analyzed from symptomatic or asymptomatic patients. Variations at the position 11,083, located in the coding region of nsp6, were found to be associated with disease severity. Two variations were observed at the site, a thymine variant (11083T) was often found in asymptomatic patients and a guanine variant (11803G) in symptomatic infections. It was suggested that interaction between viral RNA and host microRNAs play a crucial role in the development of viral infections. Three microRNAs - miR-485-3p, miR-539-3p, and miR-3149 – were found to target the two variants differentially. It was suggested that targeting the 11083G variant by miRNAs could be a way to alter the severity of the disease [88].

ACE2 variants and SARS-CoV-2

As SARS-CoV-2 primarily depends on ACE2 for fusion and entry, specific genetic variants of ACE2 could potentially alter the binding affinity and susceptibility to infection. Several studies have reported the association between ACE2 variants and diseases like cardiovascular disorders, hypertension and diabetes [89,90]. Structural studies have documented the key residues that are involved in the binding interface of ACE2 [19,52,53,55]. Crucial modifications occurred in several CoV S protein RBDs, including Bat CoVs and other suspected intermediate host CoVs like pangolin and palm civet, that facilitated SARS-CoV and SARS-CoV-2 infection of humans [52,53]. It has been demonstrated, by mutagenesis in rat ACE2, that variations in ACE2 alter the binding affinity of S protein of SARS-CoV. Mutating Lys353, a residue involved in S protein binding, to histidine partially inhibited the S protein mediated viral entry. Similarly, by modifying the glycosylation site (Asn90) in rat ACE2 altered the ACE2 conformation which in turn improved S protein mediated SARS-CoV infection [91]. Thus, it is likely that genetic variations in ACE2 could affect the expression level as well as protein conformation and stability. This could, in turn, also alter SARS-CoV-2 S protein affinity making individuals more resistant or susceptible to an infection. However, more work is required to ascertain this.

An analysis of ACE2 expression profiles in normal lung cells showed that Asian males have a higher expression of ACE2 compared to white and African populations suggesting that they could be more susceptible to a viral infection [92]. Mortality data, suggests that men succumbed to COVID-19 more often than women. Multiple reasons have been proposed, including pregnancy, sex chromosomes, estrogen signalling, and ACE2 receptor levels [93]. However, contradicting this, in another study no significant difference was observed in ACE2 expression among Asians and Caucasians, among different age groups (>60 vs < 60) and between males and females. This study also compared ACE2 expression in smokers and non-smokers and it was found that smokers were more prone to COVID-19 [94]. The differing conclusions in these studies may have occurred due to a difference in sample size or purity of ACE2 expressing cells.

Recent studies have also looked at the relationship between ACE2 variants and susceptibility to SARS-CoV-2 infection. Thirty-two ACE2 variants that included 7 hotspot variants (Lys26Arg, Ile468Val, Ala627Val, Asn638Ser, Ser692Pro, Asn720Asp, and Leu731Ile/Leu731Phe) have been identified in data from the 1000 genomes project and the China Metabolic Analytics Project. Thus, genetic variants among different populations could also affect overall ACE2 function [95]. A similar result was reported in another study involving Europeans, Africans, Asians and Americans that identified 31 variants of ACE2. Among these ACE2 variants, 13 were found to bind more efficiently to SARS-CoV-2 while 18 variants were characterized as interaction inhibitors. Among the 13 that improved binding, Ser19Pro, Ile21Thr, Lys26Arg, Thr27Ala, Asn64Lys, and His378Arg were found abundantly in all population groups [96]. Lending further support, a comprehensive analysis of 290,000 genomic samples representing more than 400 population groups identified multiple ACE2 variants. The data suggested that ACE2 variants Ser19Pro, Ile21Val, Glu23Lys, Lys26Arg, Thr27Ala, Asn64Lys, Thr92Ile, Gln102Pro and His378Arg found in the binding region increased disease susceptibility whereas the variants Lys31Arg, Asn33Ile, His34Arg, Glu35Lys, Glu37Lys, Asp38Val, Tyr50Phe, Asn51Ser, Met62Val, Lys68Glu, Phe72Val, Tyr83His, Gly326Glu, Gly352Val, Asp355Asn, Gln388Leu and Asp509Tyr exhibited lower binding to SARS-CoV-2 S protein [97]. Analysis of the whole exome sequence of 6930 Italians also identified 30 ACE2 variants. Of these, three commonly found missense variants (Lys26Arg, Gly211Arg, and Asn720Asp) were reported to affect protein structure and stability. Lys26 is located near the N terminal region and forms a hydrogen bond with Asn90. The variant Lys26Arg is likely to cause a destabilization in secondary structure of ACE2 protein. Gly211 is located in the turning point of a loop and its neighbouring Val212 forms strong hydrophobic interaction with Leu91 that stabilizes ACE2 structure. A change from an achiral and flexible glycine to charged and polar arginine is not favorable for the loop and it also introduces a hydrophilic group that weakens the Val212-Leu91 interaction and possibly destabilizing the ACE2 structure. The Asn720Asp variant is located close to the TMPRSS2 cleavage sequence and could affect virion intake [98]. ACE2 variants from Genome Aggregation Database identified 12 deleterious missense variants - Ser19Pro, Asp206Gly, Gly211Arg, Arg219His, Arg219Cys, Lys341Arg, His378Arg, Ser547Cys, Ile468Val, Arg697Gly, Ser692Pro, Leu731Phe. Based on structural analysis, nine of these variants could disrupt protein structure or its interaction with the RBD of SARS-CoV-2 [99]. Since the impact of a CoV infection varies between countries, age and race, it is possible that an individual's ACE2 could also have an effect on susceptibility or resistance to the virus. Identifying the frequently found variants will aid in developing preventive measures against COVID-19. However, much more work needs to be done to establish a strong association between ACE2 variants and COVID-19.

Conclusions

The rapid spread and mortality rate of COVID-19 has undoubtedly grabbed global attention and has been a significant threat to public health systems. In this review, we have

focused on the current state of knowledge of genetic adaptations that may have occurred in SARS-CoV-2 genome as well as its host receptor ACE2. Adaptive mutations in SARS-CoV-2 have the potential to empower it to attain increased virulence, high transmissibility and immune escape. Similarly, natural genetic variants of host receptors could also confer increased susceptibility or resistance against evolving pathogenic SARS-CoV-2. Gaining a deeper understanding of these variations is crucial for developing effective measures to develop solutions and to manage future outbreaks of SARS-CoV-2.

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Conflicts of interest

The authors declare no conflicts of interest.

REFERENCES

[1] Tian HY. 2019-nCoV: new challenges from coronavirus. Zhonghua Yufang Yixue Zazhi 2020;54:E001.
[2] Carlos WG, Dela Cruz CS, Cao B, Pasnick S, Jamil S. Novel Wuhan (2019-nCoV) coronavirus. Am J Respir Crit Care Med 2020;201:P7–8.
[3] Chan JFW, Yuan S, Kok KH, To KKW, Chu H, Yang J, et al. A familial cluster of pneumonia associated with the 2019 novel coronavirus indicating person-to-person transmission: a study of a family cluster. Lancet Lond Engl 2020;395:514–23.
[4] Wang C, Horby PW, Hayden FG, Gao GF. A novel coronavirus outbreak of global health concern. Lancet 2020;395:470–3.
[5] Dong Y, Dai T, Wei Y, Zhang L, Zheng M, Zhou F. A systematic review of SARS-CoV-2 vaccine candidates. Signal Transduct Target Ther 2020;5:237.
[6] Huang C, Wang Y, Li X, Ren L, Zhao J, Hu Y, et al. Clinical features of patients infected with 2019 novel coronavirus in Wuhan, China. Lancet Lond Engl 2020;395:497–506.
[7] Lim YX, Ng YL, Tam JP, Liu DX. Human coronaviruses: a review of virus-host interactions. Diseases 2016;4:26.
[8] Cui J, Li F, Shi ZL. Origin and evolution of pathogenic coronaviruses. Nat Rev Microbiol 2019;17:181–92.
[9] Fehr AR, Perlman S. Coronaviruses: an overview of their replication and pathogenesis. Methods Mol Biol 2015;1282:1–23.
[10] Fan Y, Zhao K, Shi ZL, Zhou P. Bat coronaviruses in China. Viruses 2019;11:210.
[11] Pyrc K, Berkhout B, van der Hoek L. Identification of new human coronaviruses. Expert Rev Anti Infect Ther 2007;5:245–53.
[12] Cheng VC, Lau SK, Woo PC, Yuen KY. Severe acute respiratory syndrome coronavirus as an agent of emerging and reemerging infection. Clin Microbiol Rev 2007;20:660–94.
[13] Woo PC, Lau SK, Chu CM, Chan KH, Tsoi HW, Huang Y, et al. Characterization and complete genome sequence of a novel coronavirus, coronavirus HKU1, from patients with pneumonia. J Virol 2005;79:884–95.
[14] Graham RL, Donaldson EF, Baric RS. A decade after SARS: strategies for controlling emerging coronaviruses. Nat Rev Microbiol 2013;11:836–48.
[15] Chen L, Liu W, Zhang Q, Xu K, Ye G, Wu W, et al. RNA based mNGS approach identifies a novel human coronavirus from two individual pneumonia cases in 2019 Wuhan outbreak. Emerg Microb Infect 2020;9:313–9.
[16] Kim D, Lee JY, Yang JS, Kim JW, Kim VN, Chang H. The architecture of SARS-CoV-2 transcriptome. Cell 2020;181:914–21. e10.
[17] Zhou P, Yang XL, Wang XG, Hu B, Zhang L, Zhang W, et al. A pneumonia outbreak associated with a new coronavirus of probable bat origin. Nature 2020;579:270–3.
[18] Wu HY, Brian DA. Subgenomic messenger RNA amplification in coronaviruses. Proc Natl Acad Sci U S A 2010;107:12257–62.
[19] Walls AC, Park YJ, Tortorici MA, Wall A, McGuire AT, Veesler D. Structure, function, and antigenicity of the SARS-CoV-2 spike glycoprotein. Cell 2020;181:281–92. e6.
[20] Mousavizadeh L, Ghasemi S. Genotype and phenotype of COVID-19: their roles in pathogenesis. J Microbiol Immunol Infect = Wei Mian Yu Gan Ran Za Zhi 2021;54:159–63.
[21] Schoeman D, Fielding BC. Coronavirus envelope protein: current knowledge. Virol J 2019;16:69.
[22] Andersen KG, Rambaut A, Lipkin WI, Holmes EC, Garry RF. The proximal origin of SARS-CoV-2. Nat Med 2020;26:450–2.
[23] Guan Y, Zheng BJ, He YQ, Liu XL, Zhuang ZX, Cheung CL, et al. Isolation and characterization of viruses related to the SARS coronavirus from animals in southern China. Science 2003;302:276–8.
[24] Zhang T, Wu Q, Zhang Z. Probable pangolin origin of SARS-CoV-2 associated with the COVID-19 outbreak. Curr Biol 2020;30:1346–51.e2.
[25] Lam TT, Jia N, Zhang YW, Shum MH, Jiang JF, Zhu HC, et al. Identifying SARS-CoV-2-related coronaviruses in Malayan pangolins. Nature 2020;583:282–5.
[26] Ou X, Liu Y, Lei X, Li P, Mi D, Ren L, et al. Characterization of spike glycoprotein of SARS-CoV-2 on virus entry and its immune cross-reactivity with SARS-CoV. Nat Commun 2020;11:1620.
[27] Lan J, Ge J, Yu J, Shan S, Zhou H, Fan S, et al. Structure of the SARS-CoV-2 spike receptor-binding domain bound to the ACE2 receptor. Nature 2020;581:215–20.
[28] Gui M, Song W, Zhou H, Xu J, Chen S, Xiang Y, et al. Cryo-electron microscopy structures of the SARS-CoV spike glycoprotein reveal a prerequisite conformational state for receptor binding. Cell Res 2017;27:119–29.
[29] Yuan Y, Cao D, Zhang Y, Ma J, Qi J, Wang Q, et al. Cryo-EM structures of MERS-CoV and SARS-CoV spike glycoproteins reveal the dynamic receptor binding domains. Nat Commun 2017;8:15092.
[30] Kirchdoerfer RN, Cottrell CA, Wang N, Pallesen J, Yassine HM, Turner HL, et al. Pre-fusion structure of a human coronavirus spike protein. Nature 2016;531:118–21.
[31] Walls AC, Tortorici MA, Snijder J, Xiong X, Bosch BJ, Rey FA, et al. Tectonic conformational changes of a coronavirus spike glycoprotein promote membrane fusion. Proc Natl Acad Sci U S A 2017;114:11157–62.
[32] Belouzard S, Chu VC, Whittaker GR. Activation of the SARS coronavirus spike protein via sequential proteolytic cleavage at two distinct sites. Proc Natl Acad Sci U S A 2009;106:5871–6.
[33] Millet JK, Whittaker GR. Host cell proteases: critical determinants of coronavirus tropism and pathogenesis. Virus Res 2015;202:120–34.
[34] Hoffmann M, Kleine-Weber H, Schroeder S, Krüger N, Herrler T, Erichsen S, et al. SARS-CoV-2 cell entry depends on ACE2 and TMPRSS2 and is blocked by a clinically proven protease inhibitor. Cell 2020;181:271–80. e8.

[35] Jaimes JA, André NM, Chappie JS, Millet JK, Whittaker GR. Phylogenetic analysis and structural modeling of SARS-CoV-2 spike protein reveals an evolutionary distinct and proteolytically sensitive activation loop. J Mol Biol 2020;432:3309–25.
[36] Coutard B, Valle C, de Lamballerie X, Canard B, Seidah NG, Decroly E. The spike glycoprotein of the new coronavirus 2019-nCoV contains a furin-like cleavage site absent in CoV of the same clade. Antiviral Res 2020;176:104742.
[37] Hoffmann M, Kleine-Weber H, Pöhlmann S. A multibasic cleavage site in the spike protein of SARS-CoV-2 is essential for infection of human lung cells. Mol Cell 2020;78:779–84. e5.
[38] Matsuyama S, Nao N, Shirato K, Kawase M, Saito S, Takayama I, et al. Enhanced isolation of SARS-CoV-2 by TMPRSS2-expressing cells. Proc Natl Acad Sci U S A 2020;117:7001–3.
[39] Wang N, Shi X, Jiang L, Zhang S, Wang D, Tong P, et al. Structure of MERS-CoV spike receptor-binding domain complexed with human receptor DPP4. Cell Res 2013;23:986–93.
[40] Lu G, Hu Y, Wang Q, Qi J, Gao F, Li Y, et al. Molecular basis of binding between novel human coronavirus MERS-CoV and its receptor CD26. Nature 2013;500:227–31.
[41] Li F, Li W, Farzan M, Harrison SC. Structure of SARS coronavirus spike receptor-binding domain complexed with receptor. Science 2005;309:1864–8.
[42] Li W, Moore MJ, Vasilieva N, Sui J, Wong SK, Berne MA, et al. Angiotensin-converting enzyme 2 is a functional receptor for the SARS coronavirus. Nature 2003;426:450–4.
[43] Letko M, Marzi A, Munster V. Functional assessment of cell entry and receptor usage for SARS-CoV-2 and other lineage B betacoronaviruses. Nat Microbiol 2020;5:562–9.
[44] Towler P, Staker B, Prasad SG, Menon S, Tang J, Parsons T, et al. ACE2 X-ray structures reveal a large hinge-bending motion important for inhibitor binding and catalysis. J Biol Chem 2004;279:17996–8007.
[45] Donoghue M, Hsieh F, Baronas E, Godbout K, Gosselin M, Stagliano N, et al. A novel angiotensin-converting enzyme-related carboxypeptidase (ACE2) converts angiotensin I to angiotensin 1-9. Circ Res 2000;87:E1–9.
[46] Vickers C, Hales P, Kaushik V, Dick L, Gavin J, Tang J, et al. Hydrolysis of biological peptides by human angiotensin-converting enzyme-related carboxypeptidase. J Biol Chem 2002;277:14838–43.
[47] Li MY, Li L, Zhang Y, Wang XS. Expression of the SARS-CoV-2 cell receptor gene ACE2 in a wide variety of human tissues. Infect Dis Poverty 2020;9:45.
[48] Xu H, Zhong L, Deng J, Peng J, Dan H, Zeng X, et al. High expression of ACE2 receptor of 2019-nCoV on the epithelial cells of oral mucosa. Int J Oral Sci 2020;12:8.
[49] Gheblawi M, Wang K, Viveiros A, Nguyen Q, Zhong JC, Turner AJ, et al. Angiotensin-converting enzyme 2: SARS-CoV-2 receptor and regulator of the renin-angiotensin system: celebrating the 20th anniversary of the discovery of ACE2. Circ Res 2020;126:1456–74.
[50] Heurich A, Hofmann-Winkler H, Gierer S, Liepold T, Jahn O, Pöhlmann S. TMPRSS2 and ADAM17 cleave ACE2 differentially and only proteolysis by TMPRSS2 augments entry driven by the severe acute respiratory syndrome coronavirus spike protein. J Virol 2014;88:1293–307.
[51] Shang J, Ye G, Shi K, Wan Y, Luo C, Aihara H, et al. Structural basis of receptor recognition by SARS-CoV-2. Nature 2020;581:221–4.
[52] Yan R, Zhang Y, Li Y, Xia L, Guo Y, Zhou Q. Structural basis for the recognition of SARS-CoV-2 by full-length human ACE2. Science 2020;367:1444–8.
[53] Xia S, Liu M, Wang C, Xu W, Lan Q, Feng S, et al. Inhibition of SARS-CoV-2 (previously 2019-nCoV) infection by a highly potent pan-coronavirus fusion inhibitor targeting its spike protein that harbors a high capacity to mediate membrane fusion. Cell Res 2020;30:343–55.
[54] Wrapp D, Wang N, Corbett KS, Goldsmith JA, Hsieh CL, Abiona O, et al. Cryo-EM structure of the 2019-nCoV spike in the prefusion conformation. Science 2020;367:1260–3.
[55] Ulrich H, Pillat MM. CD147 as a target for COVID-19 treatment: suggested effects of azithromycin and stem cell engagement. Stem Cell Rev Rep 2020;16:434–40.
[56] Daly JL, Simonetti B, Klein K, Chen KE, Williamson MK, Antón-Plágaro C, et al. Neuropilin-1 is a host factor for SARS-CoV-2 infection. Science 2020;370:861–5.
[57] Cantuti-Castelvetri L, Ojha R, Pedro LD, Djannatian M, Franz J, Kuivanen S, et al. Neuropilin-1 facilitates SARS-CoV-2 cell entry and provides a possible pathway into the central nervous system. Science 2020;370:856–60.
[58] Ackermann M, Verleden SE, Kuehnel M, Haverich A, Welte T, Laenger F, et al. Pulmonary vascular endothelialitis, thrombosis, and angiogenesis in covid-19. N Engl J Med 2020;383:120–8.
[59] Guo HF, Vander Kooi CW. Neuropilin functions as an essential cell surface receptor. J Biol Chem 2015;290:29120–6.
[60] Jobe A, Vijayan R. Neuropilins: C-end rule peptides and their association with nociception and COVID-19. Comput Struct Biotechnol J 2021;19:1889–95.
[61] Lauring AS, Andino R. Quasispecies theory and the behavior of RNA viruses. PLoS Pathog 2010;6:e1001005.
[62] Domingo E, Holland JJ. RNA virus mutations and fitness for survival. Annu Rev Microbiol 1997;51:151–78.
[63] Wu F, Zhao S, Yu B, Chen YM, Wang W, Song ZG, et al. A new coronavirus associated with human respiratory disease in China. Nature 2020;579:265–9.
[64] Khailany RA, Safdar M, Ozaslan M. Genomic characterization of a novel SARS-CoV-2. Gene Rep 2020;19:100682.
[65] Phan T. Genetic diversity and evolution of SARS-CoV-2. Infect Genet Evol 2020;81:104260.
[66] van Dorp L, Acman M, Richard D, Shaw LP, Ford CE, Ormond L, et al. Emergence of genomic diversity and recurrent mutations in SARS-CoV-2. Infect Genet Evol 2020;83:104351.
[67] Pachetti M, Marini B, Benedetti F, Giudici F, Mauro E, Storici P, et al. Emerging SARS-CoV-2 mutation hot spots include a novel RNA-dependent-RNA polymerase variant. J Transl Med 2020;18:179.
[68] Eskier D, Karakülah G, Suner A, Oktay Y. RdRp mutations are associated with SARS-CoV-2 genome evolution. PeerJ 2020;8:e9587.
[69] Forster P, Forster L, Renfrew C, Forster M. Phylogenetic network analysis of SARS-CoV-2 genomes. Proc Natl Acad Sci U S A 2020;117:9241–3.
[70] Dilucca M, Forcelloni S, Georgakilas AG, Giansanti A, Pavlopoulou A. Codon usage and phenotypic divergences of SARS-CoV-2 genes. Viruses 2020;12:498.
[71] Korber B, Fischer WM, Gnanakaran S, Yoon H, Theiler J, Abfalterer W, et al. Tracking changes in SARS-CoV-2 spike: evidence that D614G increases infectivity of the COVID-19 virus. Cell 2020;182:812–27.e19.
[72] Zhang L, Jackson CB, Mou H, Ojha A, Peng H, Quinlan BD, et al. SARS-CoV-2 spike-protein D614G mutation increases virion spike density and infectivity. Nat Commun 2020;11:6013.
[73] van Dorp L, Richard D, Tan CCS, Shaw LP, Acman M, Balloux F. No evidence for increased transmissibility from recurrent mutations in SARS-CoV-2. Nat Commun 2020;11:5986.

[74] Xue X, Shi J, Xu H, Qin Y, Yang Z, Feng S, et al. Dynamics of binding ability prediction between spike protein and human ACE2 reveals the adaptive strategy of SARS-CoV-2 in humans. Sci Rep 2021;11:3187.
[75] Zhou W, Xu C, Wang P, Luo M, Xu Z, Cheng R, et al. N439K variant in spike protein may alter the infection efficiency and antigenicity of SARS-CoV-2 based on molecular dynamics simulation. BioRxiv 2020. 11.21.392407. [Preprint] 2020 [cited 2021 January 26]. Available from: https://www.biorxiv.org/content/10.1101/2020.11.21.392407v1.
[76] Larsen CS, Paludan SR. Corona's new coat: SARS-CoV-2 in Danish minks and implications for travel medicine. Travel Med Infect Dis 2020;38:101922.
[77] Tang JW, Tambyah PA, Hui DS. Emergence of a new SARS-CoV-2 variant in the UK. J Infect 2021;82:e27–8.
[78] European Centre for Disease Prevention and Control. Risk related to spread of new SARS-CoV-2 variants of concern in the EU/EEA, first update – 21 January 2021. ECDC: Stockholm; 2021.
[79] Wibmer CK, Ayres F, Hermanus T, Madzivhandila M, Kgagudi P, Oosthuysen B, et al. SARS-CoV-2 501Y.V2 escapes neutralization by South African COVID-19 donor plasma. Nat Med 2021;27:622–5.
[80] Tegally H, Wilkinson E, Giovanetti M, Iranzadeh A, Fonseca V, Giandhari J, et al. Detection of a SARS-CoV-2 variant of concern in South Africa. Nature 2021;592:438–43.
[81] Faria NR, Mellan TA, Whittaker C, Claro IM, Candido D, da S, Mishra S, et al. Genomics and epidemiology of the P.1 SARS-CoV-2 lineage in Manaus, Brazil. Science 2021;372:815–21.
[82] Thomson EC, Rosen LE, Shepherd JG, Spreafico R, da Silva Filipe A, Wojcechowskyj JA, et al. Circulating SARS-CoV-2 spike variant N439K maintains fitness while evading antibody-mediated immunity. Cell 2021;184:1171–87.e20.
[83] Zhou T, Tsybovsky Y, Gorman J, Rapp M, Cerutti G, Chuang GY, et al. Cryo-EM structures of SARS-CoV-2 spike without and with ACE2 reveal a pH-dependent switch to mediate endosomal positioning of receptor-binding domains. Cell Host Microbe 2020;28:867–79. e5.
[84] Humphrey W, Dalke A, Schulten K. VMD: Visual molecular dynamics. J Mol Graph 1996;14:33–8.
[85] Greaney AJ, Loes AN, Crawford KHD, Starr TN, Malone KD, Chu HY, et al. Comprehensive mapping of mutations to the SARS-CoV-2 receptor-binding domain that affect recognition by polyclonal human serum antibodies. Cell Host Microbe 2021;29:463–76.e6.
[86] Yuan X, li liangping. The influence of major S protein mutations of SARS-CoV-2 on the potential B cell epitopes. BioRxiv 2020. 08.24.264895 [Preprint] 2020 [cited 2021 January 26]. Available from: https://www.biorxiv.org/content/10.1101/2020.08.24.264895v1.
[87] Agerer B, Koblischke M, Gudipati V, Smyth M, Popa A, Genger JW, et al. SARS-CoV-2 escapes CD8 T cell surveillance via mutations in MHC-I restricted epitopes. Sci Immunol 2021;6:eabg6461.
[88] Aiewsakun P, Wongtrakoongate P, Thawornwattana Y, Hongeng S, Thitithanyanont A. SARS-CoV-2 genetic variations associated with COVID-19 severity. MedRxiv 2020. 05.27.20114546. [Preprint] 2020 [cited 2020 August 13]. Available from: https://www.medrxiv.org/content/10.1101/2020.05.27.20114546v1.
[89] Luo Y, Liu C, Guan T, Li Y, Lai Y, Li F, et al. Association of ACE2 genetic polymorphisms with hypertension-related target organ damages in south Xinjiang. Hypertens Res 2019;42:681–9.
[90] Meng N, Zhang Y, Ma J, Li H, Zhou F, Qu Y. Association of polymorphisms of angiotensin I converting enzyme 2 with retinopathy in type 2 diabetes mellitus among Chinese individuals. Eye Lond Engl 2015;29:266–71.
[91] Li W, Zhang C, Sui J, Kuhn JH, Moore MJ, Luo S, et al. Receptor and viral determinants of SARS-coronavirus adaptation to human ACE2. EMBO J 2005;24:1634–43.
[92] Zhao Y, Zhao Z, Wang Y, Zhou Y, Ma Y, Zuo W. Single-cell RNA expression profiling of ACE2, the receptor of SARS-CoV-2. Am J Respir Crit Care Med 2020;202:756–9.
[93] Stelzig KE, Canepa-Escaro F, Schiliro M, Berdnikovs S, Prakash YS, Chiarella SE. Estrogen regulates the expression of SARS-CoV-2 receptor ACE2 in differentiated airway epithelial cells. Am J Physiol Lung Cell Mol Physiol 2020;318:L1280–1.
[94] Cai G. Bulk and single-cell transcriptomics identify tobacco-use disparity in lung gene expression of ACE2, the receptor of 2019-nCov. MedRxiv 2020. 02.05.20020107 [Preprint] 2020 [cited 2020 August 13]. Available from: https://www.biorxiv.org/content/10.1101/2020.04.07.024752v1.
[95] Cao Y, Li L, Feng Z, Wan S, Huang P, Sun X, et al. Comparative genetic analysis of the novel coronavirus (2019-nCoV/SARS-CoV-2) receptor ACE2 in different populations. Cell Discov 2020;6:11.
[96] Darbani B. The expression and polymorphism of entry machinery for COVID-19 in human: juxtaposing population groups, gender, and different tissues. Int J Environ Res Public Health 2020;17:3433.
[97] Suryamohan K, Diwanji D, Stawiski EW, Gupta R, Miersch S, Liu J, et al. Human ACE2 receptor polymorphisms and altered susceptibility to SARS-CoV-2. Commun Biol 2021;4:475.
[98] Benetti E, Tita R, Spiga O, Ciolfi A, Birolo G, Bruselles A, et al. ACE2 gene variants may underlie interindividual variability and susceptibility to COVID-19 in the Italian population. Eur J Hum Genet 2020;28:1602–14.
[99] Guo X, Chen Z, Xia Y, Lin W, Li H. Investigation of the genetic variation in ACE2 on the structural recognition by the novel coronavirus (SARS-CoV-2). J Transl Med 2020;18:321.