Review

Variants in ACE2; potential influences on virus infection and COVID-19 severity

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ABSTRACT

The third pandemic of coronavirus infection, called COVID-19 disease, was first detected in November 2019th. Various determinants of disease progression such as age, sex, virus mutations, comorbidity, lifestyle, host immune response, and genetic background variation have caused clinical variability of COVID-19. The causative agent of COVID-19 is an enveloped coronavirus named severe acute respiratory syndrome coronavirus-2 (SARS-CoV-2) that invades host cells using an endocytic pathway. The SARS-CoV-2 spike protein is the main viral protein that contributes to the fusion of the virus particle to the host cell through angiotensin-converting enzyme 2 (ACE2). The highly conserved expression of ACE2 is found in various animals, which indicates its pivotal physiological function. The ACE2 has a crucial role in vascular, renal, and myocardial physiology. Genetic factors contributing to the outcome of SARS-CoV-2 infection are unknown; however, variants in the specific sites of ACE2 gene could be regarded as a main genetic risk factor for COVID-19. Given that ACE2 is the main site for virus landing on host cells, the effect of amino acid sequences of ACE2 on host susceptibility to COVID-19 seems reasonable. It would likely have a substantial role in the occurrence of a wide range of clinical symptoms. Several ACE2 variants can affect the protein stability, influencing the interaction between spike protein and ACE2 through imposing conformational changes while some other variants are known to cause a decrease or an increase in the ligand-receptor affinity. The other variations are located at the proteolytic cleavage site, which can influence virus infection; because soluble ACE2 can act as a decoy receptor for virus and decrease virus intake by cell surface ACE2. Notably, polymorphisms of regulatory and non-coding regions such as promoter in ACE2, can play crucial role in different expression levels of ACE2 among different individuals. Many studies should be performed to investigate the involvement of ACE2 polymorphism with susceptibility to COVID-19. Herein, we discuss some reported associations between variants of ACE2 and COVID-19 in details. In addition, the mode of action of ACE2 and its role in SARS-CoV-2 infection are highlighted which is followed by addressing the effects of several ACE2 variants on its protein stability, viral tropism or ligand-receptor affinity, secondary and tertiary structure or protein conformation, proteolytic cleavage site, and finally inter-individual clinical variability in COVID-19. The polymorphisms of regulatory regions of ACE2 and their effect on expression levels of ACE2 are also provided in this review. Such studies can improve the prediction of the affinity of mutant ACE2 variations with spike protein, and help the biopharmaceutical industry to design effective approaches for recombinant hACE2 therapy and vaccination of COVID-19 disease.
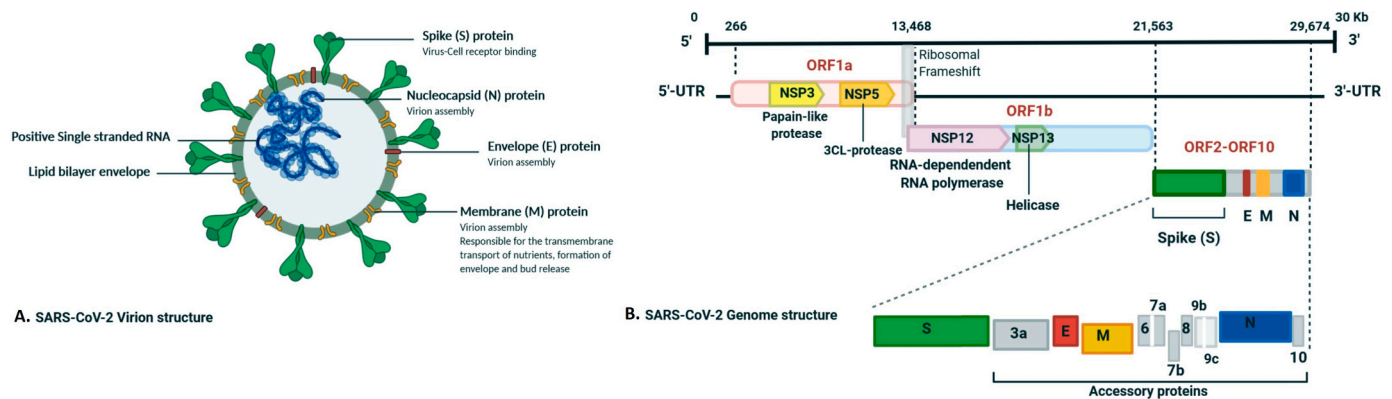
Fig. 1. (A) Schematic illustration of SARS-CoV-2 structure and its major structural proteins (B) the Schematic representation of the genomic organization of SARS-CoV-2. The distinct genomic locations, including ORFs, are represented. SARS-CoV-2 is comprised of four structural proteins, containing the E and M proteins responsible for the viral envelope, the N protein binding to the viral genome, and the spike protein capable of binding to the hACE2 receptor. Besides, translation of ORF 1a and ORF 1b to non-structural proteins (nsp) and accessory proteins, containing 3a, 6, 7a, 7b, 8, 9b, 9c, and 10 are indicated. Kb, kilobase pair, 3′-UTR 5′-UTR, untranslated regions at the 3′ and 5′ regions, respectively.

1. COVID-19 disease

Severe acute respiratory syndrome coronavirus-2 (SARS-CoV-2) is a new coronavirus which was firstly reported on 2019. This virus causes Coronavirus Disease 2019 (COVID-19) The virus has infected about 80 million cases in various countries contributing to approximate 2 million deaths during one year (Chan et al., 2020; Huang et al., 2020; Organization., W.H, 2020; Zhu et al., 2020).

Prior to this outbreak, in 2003 and 2012, MERS-CoV and SARS-CoV, which caused acute respiratory disease, have spread in China and Saudi Arabia, respectively (Rabaan et al., 2020). Analyzing the entire length of the virus genome showed that the SARS-CoV-2 genome is about 70–80% and 50% similar to SARS-CoV and MERS-CoV, respectively (Chen et al., 2020b). Although SARS and MERS have far higher mortality rates than the SARS-CoV-2, this novel coronavirus spreads more easily among people, leading to an incomparable number of infections (Rabaan et al., 2020).

The results of different studies on the severity of the infection with SARS-CoV-2 reveals that about 81% of cases are mild, ~14% severe, and ~ 5% critical (Nikpouraghdam et al., 2020; Novel, 2020; Zhang et al., 2020b). Moderate cases suffering from COVID-19 usually show fever, dry cough, and headache (Zhong et al., 2003), but in the severe cases, it can affect multiple organs, including the cardiovascular system, lungs, gut, kidney, liver, and central nervous system (Chen et al., 2020b; Zou et al., 2020). The incubation period was estimated to range from 3 to 10 days (Poutanen et al., 2003). Comorbidities such as diabetes, chronic kidney diseases, chronic respiratory diseases, cardiovascular diseases, hypertension, and cancer affect the severity of the infection leading to an increased risk of death (Nikpouraghdam et al., 2020; Zhang et al., 2020b). In the early stage, case fatality rate (CFR), had been estimated 11% (11 of 99 confirmed cases) (Xu et al., 2020a), and nearly one month later 2.3% (1023 of 44,672 confirmed cases) (Zhang et al., 2020b). WHO Situation Report–134 on June 02, 2020, illustrates that the global CFR is 6.07 (WHO, 2020). CFR is different in countries depending on the quality of the healthcare system and the situation of the outbreak. The minimum amount of CFR has been reported by the Korea Centers for Disease Control and Prevention, which was 0.9% total (Choe, 2020). The group of patients more than 80 years old had the highest mortality rate that was 19.27% (Nikpouraghdam et al., 2020), 14.8% (Zhang et al., 2020b), 8.5% (Choe, 2020).

2. SARS-CoV-2

Some scientists suggested that bats are natural hosts of SARS-CoV-2 because a comparison of SARS-CoV-2 sequences and bat SARS

coronavirus (SARS-CoV-RaTG13) revealed 96% similarity. Pangolins, camels and snakes are another potential intermediate hosts (Bravo et al., 2014; Nikpouraghdam et al., 2020; Xu et al., 2020a).

SARS-CoV-2, as a member of the beta coronaviruses family, is composed of positive-stranded RNA as genetic material, capsid proteins, and an outer envelope. The viral envelope consists of the membrane (M), spike glycoprotein (S), nucleocapsid (N), envelope protein (E), and hemagglutinin esterase (HE) as structural proteins. The spike protein is cleaved by furin-like protease into S1 and S2 functional domains (Fig. 1) (De Groot et al., 1987; Spaan et al., 1988). The coronavirus enters the host cell by endocytosis or direct merger of the viral envelope with the cell membrane (Magrone et al., 2020).

SARS-CoV basic reproductive number (R0) has been estimated 2.7 by Riley et al. (Riley et al., 2003) and about three by Lipsitch et al. (Lipsitch et al., 2003). In many studies, the range of R0 has been estimated between 2.2 and 3.8 (Xu et al., 2020b),(Li et al., 2020). Another study using the exponential growth model method indicated the mean R0 range from 2.24 to 3.58 (Wu et al., 2020).

Although genetic evidence suggests that SARS-CoV-2 may have originated in animals, it is not yet clear exactly where the virus came from and what its primary origin was. Addressing this highly controversial issue requires further research around the world.

2.1. Cytokine storm in COVID-19

According to the previous investigations, increasing numbers of pro-inflammatory cytokines including interleukin 1B, IL-6, IL-12, and Interferon γ and chemokines, including CCL2 and CXCL10 are correlated to inflammation of lungs and extensive pulmonary involvement in MERS-CoV and SARS-CoV infected patients (Channappanavar and Perlman, 2017). Similar to other coronavirus pathogenesis, in COVID-19 patients, increased levels of chemokines and pro-inflammatory cytokines, and higher activation of T-helper-1 (Th1) cells induced by elevated levels of CCL2, CXCL10, IFN γ, and IL-1B are also observed. Prominently, cytokine storm plays a crucial role in the promotion of a more severe clinical state. Rapid replication of SARS-CoV-2 induced activation and then differentiation of T helper cell to Th1 cells which is responsible for releasing pro-inflammatory cytokines such as IL-6, GM-CSF, and IFN-γ. Accordingly, the clinical observations in patients admitted to intensive care unit (ICU) with SARS-CoV-2 demonstrated a higher concentration of TNFα, CCL2, and CXCL10 than other cases with less severe conditions (Coperchini et al., 2020). It should be noted that, in SARS-CoV infections, higher secretion of IL-10 and IL-4 as Th2-immune-oriented cytokines has been observed, which plays a prominent role in the inflammatory response suppression (Zhang et al.,

2020a). In SARS cases, acute respiratory distress syndrome (ARDS) is the eventual consequence of cytokine storm. In this situation, massive amounts of chemokines (CCL3, CCL3, CCL, CXCL8, CXCL9, CXCL10) and pro-inflammatory cytokines (TNFα, IFNγ, IFNα, IL-12, IL-1β, IL-6, IL-33, IL-18, TGFβ) are released by immune effector cells, and subsequent precipitation drive to the excessive systemic inflammatory response (Cameron et al., 2008; Channappanavar and Perlman, 2017; Huang et al., 2020; Williams and Chambers, 2014).

The ARDS seen with SARS-CoV-2 infection is a cytokine release syndrome (CRS), which is a disorder induced by cytokine storms. Indeed, ARDS is a severe immune-pathological lung condition causing serious hypoxemia in SARS-CoV, MERS-CoV, and SARS-CoV-2 infections (Huang et al., 2020). Recent studies revealed that ARDS accounts for a significant number of deaths in COVID-19 patients, defined by severe hypoxemia and bilateral lung infiltrates (Zou et al., 2020). Increased lung permeability and the enhancement of the high protein content of edema fluid in the airway of the lungs lead to inadequate respiration, which is the main consequence of ARDS inflammatory injury (Bhatia et al., 2012). Overall, ARDS contributes to the cytokine storm that is a life-threatening condition characterized by a severe systemic inflammatory response induced by massive amounts of chemokines and pro-inflammatory cytokines.

Upon entry of SARS-CoV-2 into respiratory epithelial cells, the production of inflammatory cytokines combined with a weak interferon response induces an immune response mediated by membrane-bound immune receptors (e.g., Fc and Toll-like receptors). Ultimately, the infiltration of macrophages and neutrophils into lung tissue leads to a cytokine storm. One suggested mechanism for cytokine storms is that SARS-CoV-2 infection in the respiratory system can activate NF-κB via pattern recognition receptors (PPR). STAT3 is required for full activation of the NF-κB pathway. Both NF-κB and STAT3 are capable of activating the IL-6 amplifier (IL-6 Amp), which is a mechanism for the hyper-activation of NF-κB by STAT3. It in turn induces a variety of proinflammatory cytokines, including IL-6, TNFα, and chemokines (Hu et al., 2021).

Ultimately, the cytokine storm causes ARDS and organs failure, and final death in at least the most severe COVID-19 cases (Huang et al., 2020).

2.2. Innate immune evasion of coronavirus

Get the main antivirus response, innate immune cells as professional guards recognize the invasion of the virus by binding immunogenic antigens such as RNA coronavirus. Innate immunity is initiated by a molecular pattern related to pathogens, evolutionary conservation of special molecular structures for pathogens acting as a ligand for PRR. Ligand binding triggers signaling paths that include transcription factors including NF-κB, IRF3, and AP-1, which synergistically promotes type I interferon (IFN-I) production. Interferon-stimulated genes are an important component of innate antivirus defense, acting to limit virus entries and limit viral replication after the virus enters the host cell. The expression of the interferon stimulated gene was driven mainly by the activation of JAK/STAT pathway STAT mediated by IFNAR, which resulted in the binding of homodimer STAT1 and STAT1/2 heterodimer to the ISGS promoter area (Taefehshokr et al., 2020). Overproduction of IFN-I increases the penetration of neutrophils, macrophages, and inflammatory factors into the lung, which is then followed by cytokine storm syndrome, as well. In this situation, the numbers of Th1, Th17, M1 macrophage, NK cells and their secreted inflammatory cytokines increase (Rahmani-Kukia et al., 2020). An increase in the content of activated immune cells at the site of infection and their released inflammatory cytokines triggers cytokine storm. Subsequently, serious pathological complications induced in the patients' lung can worsen the illness from COVID-19 and even terminate the life. On the other hand, the virus provides ways to escape the immune system.

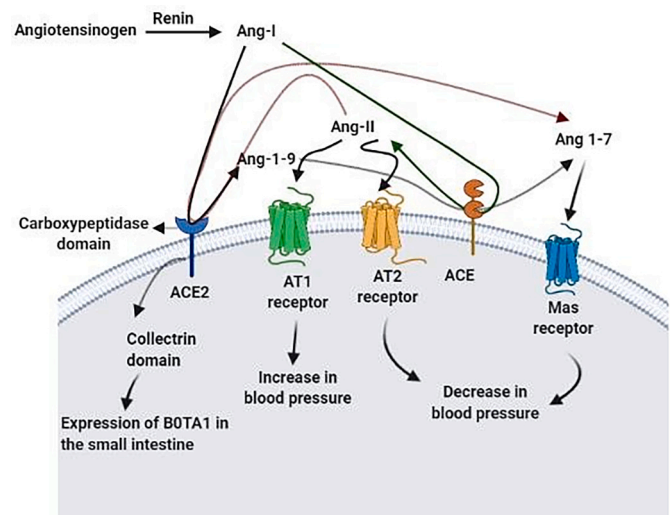Generally, coronaviruses have multiple strategies to avoid the

Fig. 2. Renin hydrolyzes Angiotensin to Ang l. ACE2 carboxypeptidase domain catalyzes the conversion of Ang l to Ang 1–9 and Ang II to Ang 1–7. ACE, as another enzyme in the RAS transforms Ang l to Ang II and Ang 1–9 to Ang 1–7. Ang II signals through AT1 and AT2 receptors, which leads to an increase and decrease in blood pressure, respectively. Mas receptor is also a receptor for Ang 1–7 and causes a decrease in blood pressure. These altogether controls blood pressure homeostasis. ACE2 collectrin domain also plays a critical role in expressing the Hartnup amino acid transporter B0AT1 in the small intestine.

immune cells leading them to better survival and infection of the host cells (Guo et al., 2020c). The coronaviruses can form double-membrane vesicles (DMV) at the extracellular space as an avoidance strategy. The formation of DMV shields dsRNA, produced as an intermediate of viral replication, from recognition by intracellular cytosolic PRRs, including RIG-I and MDA5 (Kikkert, 2020). As another avoiding immune strategy, they can suppress interferons with the aid of their eight specific viral proteins (Guo et al., 2020c). The lack of a 5` cap in the genome of RNA viruses could facilitate the recognition of the viral RNA. However, the virus mimicry strategy of the host capping combats with immune system activation. The virus recruits two nonstructural proteins (nsp), nsp 14 and nsp 16, leading to cap formation and modification of viral RNA, respectively, following its similarity to host cell RNA and evading PRRs recognition (Chen et al., 2009; Daffis et al., 2010; Totura and Baric, 2012). Moreover, nsp3 is another nonstructural coronavirus protein capable of immune response prevention. The virus also encodes two functional proteins, including PLpro, to cleave nsps and macro domains. Studies in mice concluded that according to the loss of deubiquitinating enzyme activity in MERS-CoV, another possible role of PLpro is to contribute coronavirus to evade immune response through antagonizing the IFN response (Knaap et al., 2019; Nelemans and Kikkert, 2019). Besides, other protein accessories can be utilized by SARS-CoV as an alternative to the immune evasion strategy. An instance of antagonizing the IFN signaling pathways by the gene located on ORF3b of this virus leads to the inhibition of effector cell activation cascade, which is essential for inhibiting the virus replication (Freundt et al., 2009). In contrast, the results of SARS-CoV-2 investigations indicate that the SARS-CoV-2 is more susceptible to IFN-I pretreatment and shows STAT1 phosphorylation and higher ISG proteins in the context of IFN-I induction. Considering the homology of interferon antagonists among viral proteins of SARS-CoV and SARS-CoV-2, the absence of ORF3b and dramatic difference to ORF6 suggesting the differences in IFN-I response susceptibility between the members of coronaviruses (Lokugamage et al., 2020). Overall, the production of IFN-I plays an important role in the secretion of antiviral proteins, promoting phagocytosis by macrophages, and facilitating the protection of infected cells. Coronavirus accessory proteins can interfere with the activation of these pathways during virus replication. This interference facilitates the persistence of
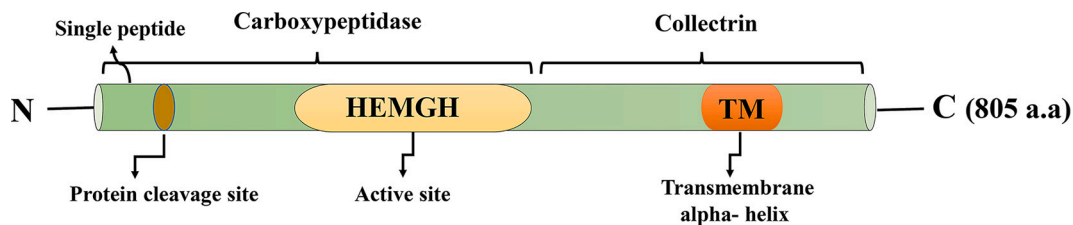
Fig. 3. ACE2, an enzyme with 805 amino acid residues, consists of two domains: the collectrin domain locates at the C-terminal and includes a transmembrane alpha-helix, the carboxypeptidase domain, is at the N-terminal and has one active site. There is also a signal peptide at the end of the N-terminal, which the protein cleavage site is located next to it.

the virus and causes the innate immune response to be dysregulated (García-Salido, 2020).

3. Angiotensin-converting enzyme (ACE)

The renin-angiotensin system (RAS) serves as the primary volume regulator in mammals and has an essential role in regulating body homeostasis, including blood pressure, electrolyte balance, and vascular tone (Rice et al., 2004; Verma et al., 2019). In the RAS, angiotensinogen is transformed into angiotensin (Ang) I by renin, followed by hydrolysis of Ang I to Ang II by angiotensin-converting enzyme (ACE) (Bernstein et al., 2018a). As a pivotal enzyme in the RAS, ACE is expressed in most of the tissues in the human body(Donoghue et al., 2000a). ACE is a zinc-dependent di-carboxypeptidase that catalyzes the alternation of Ang I to Ang II and Ang (1–9) to Ang (1–7) (Tikellis et al., 2003a). It is a membrane-bound enzyme and exists in secretory form (Bernstein et al., 2018b). ACE has two different isoforms, including somatic ACE and testicular ACE (tACE). Somatic ACE possesses two homologous domains for active sites, while tACE has just one (Fig. 2) (Guy et al., 2003a).

4. Angiotensin-Converting enzyme 2 (ACE2)

In 2000, ACE2 was discovered as the first homolog of ACE (Donoghue et al., 2000b; Tipnis et al., 2000), although some years later, ACE3, another homolog of ACE, was identified, which is expressed in sperm (Inoue et al., 2010). ACE2 was identified in a study conducted on the genes contributing to heart failure and also in independent research, reported as angiotensin-converting enzyme homolog (ACEH) (Tipnis et al., 2000). ACE2 consists of 805 amino acids, and its molecular weight is about 120 kDa (Alenina and Bader, 2019). ACE2 has one zinc-binding motif His-Glu-Xaa-Xaa-His as an active site (residues 374–378) (Tipnis et al., 2000), in which two zinc ions coordinate with histidine residues (Guy et al., 2003a). This enzyme is anchored in the membrane from the C-terminal, and 17 residues at the N-terminal act as a signal peptide (Fig. 3) (Tipnis et al., 2000).

It is believed that ACE, ACE2, neurolysin, and PfuCP have a common ancestral protein (Donoghue et al., 2000a) and were evolved from the same clan (Guy et al., 2003a). ACE2 is a bio-chimeric protein; the fusion between ACE gene and the collectrin gene during evolution resulted in the ACE2 gene (Turner and Hooper, 2002). In ACE2, the N-terminal domain sequence is 42% similar to the catalytic domains of endothelial ACE (Donoghue et al., 2000a), and its C-terminal sequence is 48% identical to collectrin (Alenina and Bader, 2019). Its global alignment also shows a 33% identity to human tACE (Donoghue et al., 2000a).

When ACE2 was characterized, its expression in endothelial cells of the heart, kidney, and testis was identified (Donoghue et al., 2000a; Tipnis et al., 2000). However, further studies showed that this enzyme also exists in the gastrointestinal tract, lungs, and lower brain (Alenina and Bader, 2019; Gu et al., 2005; Komatsu et al., 2002). Based on research, ACE2 has the highest expression levels in the small intestine, duodenum, gall bladder, kidney, and heart (Fagerberg et al., 2014). According to an investigation on the expression of ACE2 in 31 human tissues, the kidneys, testis, heart, small intestine, thyroid, and adipose

tissue have significant levels of ACE2 expression, and the bladder, lungs, liver, large intestine, and adrenal gland express to a lesser extent (Chen et al., 2020b). ACE2 expression also occurs in the oral cavity mucosa and mainly exists in the tongue epithelial cells. Overall, ACE2 does not exhibit a tissue-specific expression pattern (Zhao et al., 2020).

4.1. ACE2 orthologues in other species

ACE2 expression is found in a wide variety of animals and highly conserved, especially in mammals. This conservation indicates the pivotal physiological function of ACE2. Some scientists have constructed the phylogenic tree of mammalian ACE2 using the Maximum Likelihood method (Luan et al., 2020). Species, which express ACE2, could be infected by SARS-CoV or SARS-CoV-2. Many different studies have supported this subject. Although receptor recognition by spike protein of the virus mainly determines the host range (Li, 2013), other steps in the infectious cycle of the virus, including genome replication, translation, virion assembly, and budding, must be done correctly for the host infection (Zhao et al., 2020). It has been reported that murine and rat ACE2 has less affinity for attachment to the S1 domain of SARS-CoV in comparison with human ACE2. SARS-CoV can infect some primates, cats, hamsters, bats, raccoon dogs, and ferrets (Li et al., 2004; Li et al., 2006). In a study on SARS-CoV-2 hosts, ACE2 of 14 mammalian species have been investigated, and ACE2 of human and rhesus monkey showed the highest receptor activity (Zhao et al., 2020). ACE2 in Bovidae, Cetacea, Cricetidae, and Primates species could recognize the spike protein of SARS-CoV-2, in contrast to turtle and snake ACE2 (Luan et al., 2020).

4.2. Enzymatic activity of ACE2

ACE2 is a type l transmembrane enzyme (Donoghue et al., 2000b), and its domain on the cell surface has carboxypeptidase activity (Donoghue et al., 2000b; Vickers et al., 2002) and hydrolyzes circulating peptides. Its ectodomain catalyzes the transformation of Ang l to Ang (1–9) (via cleavage of the C-terminal Leu) (Donoghue et al., 2000b) and Ang II to Ang 1–7 (Vickers et al., 2002). Ang l, Ang II, Ang (1–7), and Ang (1–9) are the RAS peptide hormones. Ang II is a vasoconstrictor, while Ang (1–7) is a vasodilator hormone. Ang II type (AT) 1 and AT2 receptors are two receptors for Ang II, which have different roles. Ang (1–7) signaling also occurs via the Mas receptor (Fournier et al., 2012). Attachment of Ang II to AT1 receptors leads to the increase of the blood pressure, and the stimulation of the Mas receptor by Ang (1–7) eventuates in nitric oxide release followed by prevention of neurogenic hypertension (Xia et al., 2013). ACE2 opposes the increase in blood pressure by transforming Ang II to Ang (1–7). It also turns Ang l, a substrate converted to Ang II by ACE, to Ang (1–9), decreasing Ang II levels. Furthermore, ACE hydrolyzes Ang (1–9) and other vasoactive peptides and produces Ang (1–7). These all lead to a reduction in blood pressure (Donoghue et al., 2000b; Rice et al., 2004; Tikellis et al., 2003b; Xia et al., 2013).

In the small intestine, the expression of B0AT1 on the surface of enterocytes depends on ACE2 (Camargo et al., 2009). Collectrin is

necessary for the intake of amino acid by kidneys and the action of Hartnup amino acid transporter B0AT1, which is encoded by the SLC6A19 gene, in renal epithelial cells (Danilczyk et al., 2006). Similarly, the function of B0AT1 in the small intestine is controlled by the cytoplasmic domain of ACE2, a homologous of collectrin (Camargo et al., 2009).

The active site sequence and structure in ACE is almost conserved in ACE2, except one alternation in ACE2 active site residues (Guy et al., 2003b). Despite the high similarity in ACE and ACE2, they differ in substrate specificity. ACE acts as a peptidyl dipeptidase; however, ACE2 omits just an amino acid residue from its substrate's C-terminal. The differences between these two enzymes in ligand-binding pockets, which results in different substrate specificity, were studied (Guy et al., 2003b). Optimum pH for ACE2 activity is ~6.5, and like ACE, monovalent anions such as Cl^- and F^- improve its function up to ~10-fold (Vickers et al., 2002). Another study stated that pH 7.0 with 300 mM NaCl is the optimum condition for ACE2 activity, and Cl^- rises Ang I hydrolysis but declines Ang II cleavage (Guy et al., 2003b). ACE2 has relatively high specificity and cannot cleave unrelated hormonal peptides (Donoghue et al., 2000b). In a study on the possible substrates of ACE2, elevens of the peptides were hydrolyzed, especially Ang II, APLN13 (the fragment of apelin consists of thirteen residues), dynorphin A (1–13) and des-Arg(9)-bradykinin with higher efficiency (Tipnis et al., 2000; Vickers et al., 2002). Neurotensin and kinesin are other substrates that ACE2 acts on them (Donoghue et al., 2000b). However, unlike ACE, it has not any effect on bradykinin (Donoghue et al., 2000b; Vickers et al., 2002). It also hydrolyzes Ang I, but ~400-fold less efficient in comparison with Ang II (Rice et al., 2004; Vickers et al., 2002). So it is suggested that its primary role is in the metabolism of Ang II to Ang (1–7) (Rice et al., 2004). Pro-Xaa (1–3)-Pro-hydrophobic was recognized as a consensus sequence in all substrates of ACE2, and the peptide bond between proline and hydrophobic residues as the cleavage site (Vickers et al., 2002). However, in another study, by analyzing ACE2 different substrates, Pro-Xaa-Pro-hydrophobic/basic was known as its protease specificity (Guy et al., 2003b). Although ACE2 and ACE have similar catalytic domains (Guy et al., 2003b), ACE inhibitors that block the conversion of Ang II to Ang I, have no effect on ACE2 activity (Donoghue et al., 2000b; Tipnis et al., 2000). ACE inhibitors, including lisinopril, enalaprilat, and captopril which bind in the S1′ and S2′ pockets of ACE, cannot bind to ACE2. This is probably because the S2′ pocket of the active site in ACE2 is smaller than that of ACE. The active site model was able to help design new ACE2 inhibitors, for example the presence of Pro-Phe in the concentration of 180 μM could inhibit ACE2 activity (Guy et al., 2003b). ACE2 cleaves the peptide bond between Pro and Phe residues at the Ang II C-terminal. Dipeptide Pro-Phe can itself be a substrate for this enzyme and also act as an inhibitor for ACE2 while hydrolyzing Ang II (Guy et al., 2003b).

Ethylenediamine-tetraacetic acid (EDTA), which inhibits metalloprotease by the chelation of the metal ion, is another inhibitor for ACE2 (Tipnis et al., 2000). In a study by Anguiano et al., circulating ACE2 and ACE activity were measured in EDTA-plasma; EDTA inhibited ACE2 and ACE activity. When zinc chloride was added to plasma samples to prevent the effect of EDTA chelation, enzymatic activity returned. The results showed that in the presence of EDTA, endogenous and exogenous ACE2 activities are inhibited (Anguiano et al., 2015).

4.3. Physiological roles of ACE2

ACE2 has a crucial role in vascular, renal, and myocardial physiology (Crackower et al., 2002; Donoghue et al., 2000b; Tikellis et al., 2003b). In ACE2 knocked out mice, heart dysfunction, memory, and cognition problems were observed (Guy et al., 2003b; Wang et al., 2016). ACE2 has a profound antihypertensive and sympatholytic activity in the hypothalamus (Alenina and Bader, 2019) and is necessary for protecting against metabolic and cardiovascular disorders like hypertension, diabetes, and Ang II-mediated vascular inflammation (Sahara et al., 2014;

Verma et al., 2019). Its brain action helps the human body regulate the stress response, cognition, brain damage, and neurogenesis (Alenina and Bader, 2019). ACE2 modulates the RAS negatively (Imai et al., 2005), and its functions contradict ACE activity. Diabetes is also associated with a decrease in renal ACE2, which could be the target of treatment (Tikellis et al., 2003b). In acute lung injury, an increase in Ang ll resulting from ACE activity improves the damage through the AT1 receptor. ACE2 and the AT2 receptor could prevent lung failure, but they are downregulated (Imai et al., 2005). Despite ACE2 function in reducing the blood pressure, its expression decreases in neurogenic hypertension. A disintegrin and metalloproteinase 17 (ADAM17 or TNFα convertase) cleaves ACE2 ectodomain and releases it in a soluble form. The brain's RAS activation improves ADAM17 activity and reduces membrane-bound ACE2 in the brain, inhibiting its role in compensating hypertension, although ACE2 soluble form preserves its carboxypeptidase activity (Xia et al., 2013).

Dietary tryptophan uptake almost occurs through the transport pathway mediated by B0AT1 and ACE2 in the small intestine. ACE2 inactivation causes a decrease in B0AT1 expression. Because the absorption of the neutral amino acids is regulated by B0AT, this would result in low concentrations of Thr, Val, Tyr, and essential amino acid Trp in the serum. ACE2 deficiency leads to deterioration of local Trp hemostasis that is the cause of reducing regenerative responses and so susceptibility to intestinal damage. So inactivation of ACE2 can lead to colitis and intestinal injury. It also alters the intestinal microbiome (Hashimoto et al., 2012). Trp is a precursor of serotonin, so its deficiency reduces the concentration of serotonin in the blood and the brain (Klempin et al., 2018).

4.4. ACE2 as a receptor for pathologic viruses

Enveloped viruses almost enter the host cells by attaching to the cell surface proteins. ACE2, as a cell surface protein, acts as a receptor for some coronaviruses, including SARS-CoV (Li et al., 2003), HCoV-NL63 (Hofmann et al., 2005), and the recently emerged novel coronavirus SARS-CoV-2 (Lu et al., 2020).

The spike proteins of a coronavirus consist of two domains: S1 domain, the N-terminal domain that attaches to the receptor and S2 domain, the membrane fusion domain (Gallagher, 2001). As an enveloped virus, SARS-CoV enters the target cell by fusing the host cell membrane (Simmons et al., 2004). Indeed, direct membrane fusion and receptor-mediated endocytosis are two possible mechanisms for virus entry (Peiris et al., 2004b). As mentioned earlier, ACE2 is the receptor for the virus and attaches the S1 domain of SARS-CoV with excellent specificity and affinity (affinity of 1.7 nM) (Sui et al., 2004). Spike protein is the only protein of a virus that contributes to the fusion of the virus particle with the host cell, and it is not cleaved after the fusion (Li et al., 2003). Nevertheless, the enzymatic activity of ACE2 does not play a role in virus entry (Spiga et al., 2003), and after attachment to S1 domain, its catalytic activity is not affected (Kuhn et al., 2004). A small region of the S1 domain, residues 318 to 510, attaches to ACE2, and two amino acid Glu and Asp at the position of 452 and 454, respectively, play a critical role in this attachment (Wong et al., 2004). Different approaches for preventing the infection with this virus have been studied, like using soluble ACE2, an antibody against ACE2, or the ACE2-binding domain of the virus spike protein (Sui et al., 2004). Some researchers have identified CD209L, another human cellular glycoprotein, as a second receptor for SARS-CoV, which associates with spike protein less efficiently in comparison with ACE2 (Jeffers et al., 2004).

Human coronavirus NL63 (HCoV-NL63) is the fourth identified human coronavirus, which, unlike SARS-CoV, causes mild respiratory disease in infants or immune-deficient adults with severe respiratory tract infections (Van Der Hoek et al., 2004). At the time of discovery, it was not an emerging virus and existed before, but it had not been identified (Van Der Hoek et al., 2006). HCoV-NL63 infection is observed globally but mostly in winters (Arden et al., 2005). This virus stands

Table 1

Viruses that employ ACE2 as a receptor.

| Virus	Group Of Coronavirus	Year Of Emergence	Disease	Symptoms	References
SARS-CoV	Group 2	2003	SARS	fever, dry cough, dyspnea, headache, hypoxemia	Li et al. (2003)
HCoV-NL63	Group 1	Much before discovery	Mild/moderate respiratory disease	fever, cough, sore throat, rhinitis	Hofmann et al. (2005)
SARS-CoV-2	Group 2	2019	COVID-19	cough, fever, myalgia, fatigue, dyspnea, pneumonia	Coperchini et al. (2020)

under group 1 coronaviruses in the classification, and its genome has a 65% sequence identity to HCoV-229E, another human coronavirus (Van Der Hoek et al., 2004). Due to this similarity, it was expected that HCoV-NL63 occupies the same receptor for entering the cell as HCoV-229E, which employs CD13 (Yeager et al., 1992). However, the lack of homology in critical regions of the S protein changes the HCoV-NL63 receptor to ACE2. Like SARS-CoV, the S2 domain binds ACE2, but in HCoV-NL63, with less affinity. So, SARS-CoV and HCoV-NL63 have the same target cells, although HCoV-NL63 is a weaker pathogen. It could be because of the lack of pathogenicity factors or lower affinity to ACE2 in this virus. The spike proteins of these two viruses are just 14% similar, and it could be concluded that they have different ways of interacting with ACE2 (Hofmann et al., 2005). A similar receptor for these two viruses could lead to recombination between them and the evolution of more pathogenic variants in the future (Van Der Hoek et al., 2006). ACE2 also contributes to HCoV-NL63 replication in the cell lines (Hofmann et al., 2005). The recently emerged novel coronavirus SARS-CoV-2 also enters the cell via ACE2 (Lu et al., 2020). The viruses which employ ACE2 as a receptor are summarized in Table 1.

Recently a study demonstrated that the comparison of ACE2 WES data between the cohorts of 131 patients and 258 controls allowed identifying higher allelic variability statistically in control group compared to patients in the Italian population (Benetti et al., 2020b).

This opens the possibility that some people can be less or more susceptible to SARS-CoV-2 infection than others.

5. The pathogenic mechanism of SARS-CoV-2 in human

Clinical presentations of COVID-19 patients are familiar to symptoms of other coronavirus infections, including SARS-CoV and MERS-CoV (Huang et al., 2020; Peiris et al., 2004a). According to the poor understanding of the new coronavirus pathogenesis, more investigations need to be elucidated. However, the similar pathogenesis of MERS-coronavirus and SARS-coronavirus can help understand SARS-CoV-2 infections better. The pathogenesis of coronavirus begins with the binding to host airway epithelial cells using ACE2 receptors, leading to viral entry and its replication (Liu et al., 2010; Mason, 2020). The viral entry to host cells is mediated by direct membrane fusion between the host cell membrane receptor (ACE2) and the virus (spike protein), leading to the release of the viral ssRNA genome into the host cell. Following the viral entry, ACE2 is down-regulated, causing excessive activity of ACE/Ang II and increasing the permeability of lung vascular and consequent lung damage (Magrone et al., 2020). It has been reported that coronaviruses show dual entry pathways including, early and late pathways. Subsequent to the binding of the virus to the cellular receptor, it can be accomplished by plasma membranes or endosomes.
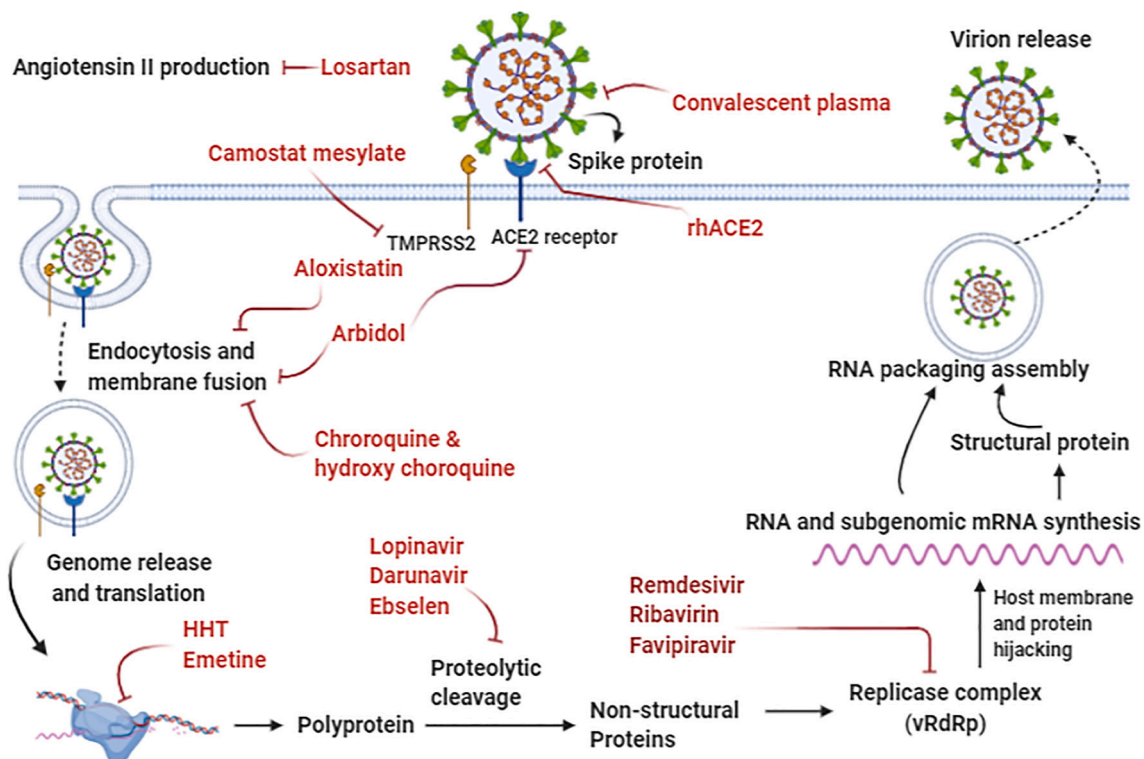
Fig. 4. Cell entry and viral replication mechanisms of SARS-CoV-2 and potential drugs targeting different virus life cycle stages. Abbreviations: ACE2; Angiotensin-Converting Enzyme 2, HHT; Homoharringtonine, rhACE2; Recombinant Human Angiotensin-converting Enzyme 2, TMPRSS2; Transmembrane protease serine 2, vRdRp; viral RNA-dependent RNA polymerase.

Table 2

The ongoing clinical trials being tested on COVID-19 subjects identified at Clinicaltrials (https://clinicaltrials.gov) using the listed keywords: SARS-CoV-2, 2019-nCoV, COVID-19, SARS-Coronavirus-2 along with the name of each administered drug.

Drug	Mechanism of Action	Medical Condition	Sponsor	Trial Phase	Ref.
Arbidol (Umifenovir)	Blocking SARS-Coronavirus-2 binding to receptor and prevention of intracellular vesicle trafficking	380 participants with COVID-19 pneumonia	Jieming QU, Ruijin Hospital	4	(Wang et al., 2020)
Bromhexine Hydrochloride	Inhibiting the viral entry by interfering with TMPRSS2	140 participants with Increased Risk of SARS-CoV-2 Infection	Federal State Budgetary Institution	4	(Habtemariam et al., 2020)
Camostat Mesilate	TMPRSS2 inhibitor	580 participants with COVID-19	University of Aarhus	1 and 2	(Uno, 2020)
Chloroquine	Preventing the Endosomal maturation	Preventive treatment for COVID-19 with 10,000 participants	University of Oxford	N/A	(Duan et al., 2020)
Danoprevir - Ritonavir	HCV NS3 protease inhibitor	11 participants with COVID-19	The Ninth Hospital of Nanchang	4	(Chen et al., 2020a)
Darunavir-Cobicistat	Inhibitor of HIV protease / blocking Darunavir metabolism by the enzyme CYP3A	30 participants with COVID-19 pneumonia	Shanghai Public Health Clinical Center	3	(De Meyer et al., 2020)
Favipiravir	Inhibitor of RNA-dependent RNA polymerase	210 participants with COVID-19	Peking University First Hospital	N/A	(Shannon et al., 2020)
Hydroxychloroquine	Less-toxic derivative of chloroquine, Inhibition of Endosome maturation	30 participants with COVID-19 pneumonia	Shanghai Public Health Clinical Center	3	(Chowdhury et al., 2020)
Lopinavir-Ritonavir	Acting as a protease inhibitor, interfering with the action of 3CL^{pro}, inhibiting the viral replication and viral release from host cells	1220 participants in a preventive treatment study for COVID-19	St. Paul's Hospital	3	(Dorward and Gbinigie, 2020)
Nitric Oxide Gas	Blocking the synthesis of viral protein and RNA	240 participants with COVID-19	Massachusetts General Hospital	2	(Gianni et al., 2020)
Interferon A2β	Initiating the JAK-STAT signaling cascades	328 participants infected by 2019-nCoV	Tongji Hospital	1	(Mantlo et al., 2020)
Remdesivir	Inhibition of SARS-CoV-2 RNA synthesis	452 participants with COVID-19	Capital Medical University	3	(Saha et al., 2020)
Baricitinib	JAK/STAT inhibitor	80 participants with COVID-19	University of Colorado, Denver	2 and 3	(Lo Caputo et al., 2020)
Bevacizumab	Monoclonal antibody against VEGF	130 participants with COVID-19 pneumonia	Assistance Publique - Hôpitaux de Paris	2	(Tu et al., 2020)
Clazakizumab	Humanized monoclonal anti-IL-6 antibody	60 participants with COVID-19	Cedars-Sinai Medical Center	2	(Vaidya et al., 2020)
Colchicine	Blockage of the NLRP3 inflammasome assembly	6000 adult and older participants with COVID-19	Montreal Heart Institute	3	(Shah, 2020)
Convalescent Plasma	Plasma with the specific antibody	100 participants with COVID-19	Thomas Jefferson University	2	(Rojas et al., 2020)
Eculizumab	Humanized anti-C5 monoclonal Ab	120 participants with COVID-19	Alexion Pharmaceuticals	2	(Diurno et al., 2020)
Fingolimod	Sphingosine-1-phosphate receptor regulator	30 individuals with COVID-19	First Affiliated Hospital of Wenzhou Medical University	2	(Foerch et al., 2020)
IVIG	Block FcR activation	50 participants with COVID-19 pneumonia	Dow University of Health Sciences	1 and 2	(Xie et al., 2020)
Kineret	Human Interleukin-1(IL-1) receptor Antagonist	240 individuals with acute pneumonia caused by 2019-nCoV	University Hospital, Tours	3	(Huet et al., 2020)
Methylprednisolone	Inhibition of several cytokines (e.g. IL-6, IL-1, IL-2, IFN-δ and TNF-β) gene expression	80 individuals with COVID-19	Peking Union Medical College Hospital	2 and 3	(Xie et al., 2020)
Naproxen	Antiviral function against COX-2 of influenza A virus nucleoprotein	584 participants with COVID-19	Assistance Publique - Hôpitaux de Paris	3	(Alizadeh-Navaei et al., 2020)
Pirfenidone	inhibition of IL-1β and IL-4	294 individuals with severe COVID-1 pneumonia	Huilan Zhang	3	(George et al., 2020)
Ruxolitinib	Inhibitor of JAK-1 and 2	100 participants with COVID-19	Marcelo Iastrebner	2	(Mukherjee et al.)
Sarilumab	Monoclonal recombinant human anti-IL6 receptor antibody	120 participants with COVID-19	Westyn Branch Elliman	2	(Gremese et al., 2020)
Siltuximab	chimeric monoclonal Anti-IL-6 antibody	220 participants with COVID-19	A.O. Ospedale Papa Giovanni XXIII	4	(Palanques-Pastor et al., 2020)
Stem Cells Therapy	Anti-inflammation and immunoregulation– restoring immune balance and homeostasis and induction of immunological tolerance in autoimmune T cells	ARDS caused by SARS-Coronavirus-2 with 24 participants	University of Miami	1 and 2	(Golchin et al., 2020)
Thalidomide	anti-angiogenesis, anti-fibrotic, anti-inflammatory, and immune regulatory effect	100 individuals with COVID-19 pneumonia	First Affiliated Hospital of Wenzhou Medical University	2	(Zhao et al., 2020)
Tocilizumab	Monoclonal humanized Recombinant anti-interleukin-6 antibody	100 participants with SARS-CoV-2	University Hospital	2	(Luo et al., 2020)

Table 2 (*continued*)

Drug	Mechanism of Action	Medical Condition	Sponsor	Trial Phase	Ref.
Vitamin C	Antioxidant properties	20 participants with COVID-19	Inselspital, Berne Hunter Holmes Mcguire Veteran Affairs Medical Center	1 and 2	(Erol, 2020)
Rhace2	Blocking the Angiotensin II receptor	2000 participants with COVID-19	Apeiron Biologics	2	(Guo et al., 2020a)
Carrimycin	Macrolide antibiotic	520 participants with 2019-nCoV	Beijing YouAn Hospital	4	(Cortegiani et al., 2020)
Heparin	Anticoagulant	3000 individuals with COVID-19 pneumonia	University of Manitoba	2 and 3	(Liu et al., 2020)
Losartan	Blocking the Angiotensin II receptor	200 participants with 2019-nCoV	University of Minnesota	2	(Gurwitz, 2020)

SARS-CoV-2, severe acute respiratory syndrome coronavirus 2; TMPRSS2, transmembrane serine protease 2; HCV, hepatitis C virus; HIV, Human immunodeficiency viruses; 3CL ^{pro}, 3-chymotrypsin-like protease; VEGF, vascular endothelial growth factor; IL-6, Interleukin 6; NLRP3, NLR family pyrin domain containing 3; FcR, Fc Receptor; TNFα, tumor necrosis factor α; COX-2, cyclooxygenase-2; RHACE, Recombinant human angiotensin-converting enzyme.

The presence of trypsin and TMPRSS2 as membrane-bound exogenous proteases leads to the entry of viruses through early fusion pathways or by the endocytosis pathway. On the other hand, the investigations on the MERS-CoV have been reported that the cleavage of spike protein at S1/S2 by furin during its biosynthesis and the presence of aforementioned membrane bound exogenous proteases mediates the early entry pathway. Likewise, spike protein cleavage at the S1/S2 site leading to endocytosis of the virus. In both entry pathways, the low acidic pH within the endosomes activates another cleavage site at the spike protein, leading to the fusion pathways and releasing of the virus genome into the host cell cytoplasm. Indeed, current studies indicate that the entry pathways of SARS-CoV-2 are accomplished by both endosomal cathepsin L or membrane-bound TMPRSS2 and direct fusion of virus membrane with host cell membrane without formation of endosome. Moreover, calcium and cholesterol are other influencing factors on viral entry (Straus et al., 2020).

While SARS-CoV-2 enters the cell, the positive sense single-stranded RNA genome of the virus is entered into the host cell cytoplasm. Both ORF1a and ORF1ab are readily translated into pp1a and pp1ab polyproteins, respectively, which are then readily undergo the proteolytic cleavage process by the transcription complex (RTC) proteases. During the replication, RTC contributes to the synthesis of full length (−) RNA copies of the viral genome, which is utilized as templates for the full-length synthesis of positive RNA genomes. At the time of transcription, a set of sub-genomic RNAs (sgRNAs) is synthesized using fragmented transcription (Perlman and Netland, 2009). Despite the presence of several open reading frames (ORFs) in these sgRNAs, the closest ORF to the 5′ end will be exclusively translated. Afterward, the recently translated envelope glycoprotein is then inserted into the ER and Golgi membranes, respectively, and the viral nucleocapsid is constituted from the assembling of nucleocapsid protein and new synthesized genomic RNA. Afterward, new virions start budding into the lumen of ER-Golgi intermediate compartments. The vesicles carrying the virions fuse to the cytoplasmic membrane, and the newly synthesized viruses can be released by exocytosis and infect other surrounding host cells (Alanagreh et al., 2020).

Studies suggest that the high transmissibility and higher infectivity of SARS-CoV-2 are related to its characteristics, including the longer spike protein and the higher affinity of receptor-binding domain (RBD) to ACE2. In addition, the existence of the furin-like cleavage site facilitates the priming of the viral spike protein (Wrapp et al., 2020).

The roles of some biochemical inhibitors on SARS-CoV-2 entry and infection are presented in Fig. 4. At the beginning of viral entry into the cell, Losartan, as an Ang II receptor blocker, can alleviate the vasocontraction induced by the viral infection. Indeed, Camostat mesylate can inhibit the entry of the viral spike protein into the host cell via

inhibition of TMPRSS2. Furthermore, rhACE2 functionally binds to the virus and sequesters circulating viruses, and contributes to the prevention of further spike protein interactions with ACE2 on the host cell. Moreover, convalescent plasma can contribute to passive immunization by neutralizing antibodies against the virus. In the cytoplasm, E64d, the cysteine proteinase inhibitor, can inhibit the viral RNA synthesis and protein processing. As a potent antiviral drug, Arbidol can inhibit viral entry through the induction of structural rigidity in the virus glycoprotein leading to disruption of membrane attachment and counteract clathrin-dependent trafficking. Moreover, Chloroquine and Hydroxychloroquine prevent viral entry and endocytosis, and also play the role of potential immunomodulatory agents. Homoharringtonine (HHT) and Emetine as protein synthesis inhibitors can prevent viral replication. The anti-retroviral drugs, including Lopinavir and darunavir, act as a potential protease inhibitor, probably can inhibit the 3C-like protease 3CLpro of SARS-CoV-2. Additionally, Ebselen and N3 can effectively inhibit the viral main protease (Mpro), leading to viral replication inhibition. Nucleoside analogs, including Remdesivir, Favipiravir, and Ribavirin, act as interfering with the viral RNA-dependent RNA polymerase (RdRp), consequently leading to the termination of viral protein synthesis.

Following the entry of SARS-CoV-2 into the cells, the specific viral antigen will be processed and presented to the antigen-presenting cells (APC), thereby stimulating the body's antiviral immunity to both CD4-positive T cells, and CD8-positive T cells. Viral Antigenic peptides can be detected by the virus-specific CTL, mediated by their presentation by MHC (major histocompatibility complex) on the surface of the infected cells. As a result, a comprehensive perception of the SARS-CoV-2 antigen presentation will help us understanding COVID-19 pathogenesis better. Regretfully, there is not still sufficient report about it, and the previous investigation on MERS-CoV and SARS-CoV can be beneficial to get some information. The previously published reports about SARS-CoV indicates the importance of both MHCI and MHCII molecules in its antigen presentation *(Liu et al., 2010)*. Moreover, it has been reported that numerous HLA polymorphisms are associated with the SARS-CoV susceptibility including HLA-Cw*0801, HLA-DR B1*1202, HLA-B*4601, and HLA-B*0703 (Chen et al., 2006), whilst the HLA-A*0201, HLA-DR030, and HLA-Cw1502 related to the prevention from being infected by SARS-CoV (Wang et al., 2011). It has been also demonstrated that in MERS-CoV infected patients, MHC II molecules, including HLA-DQB1*02:0 and HLA-DRB1*11:01, are correlated with the higher MERS-coronavirus infection susceptibility (Hajeer et al., 2016). Furthermore, the association of Mannose-binding lectin (MBL) gene polymorphisms with antigen polymorphisms shows correlations with a higher rate of SARS-CoV infections (Tu et al., 2015). Aforementioned studies will contribute to beneficial clues for SARS-CoV-2 prevention,

Table 3

List of potential missense mutations of ACE2 from gnomAD and their possible alteration in SARS-CoV-2 infection.

Wild aa	Mutant aa	Position	Function of wild residue	Effect of the mutation
His	Arg	378	By forming hydrogen bond and π-interaction stabilize the structure of the catalytic center and metal atom	This mutation weakens ACE2 peptidase activity by break the chelation network to Zn atoms.
Ser	Pro	19	Locating at the beginning of helix Ser19-Ile54 - forming hydrogen bonds and hydrophilic interactions to stabilize the helical structure.	This mutation would destabilize the helix structure since Proline has poor helix-forming tendencies.
Gly	Arg	211	Locating at the turn point of a loop and stabilizing the secondary structure of ACE2 by its strong hydrophobic interaction	It would weaken the hydrophobic interaction as arginine is not suitable for the loop turning and because of its hydrophilic feature.
Asp	Gly	206	This asparagine is on a helix to stabilize secondary structures by a hydrogen bond	This mutation influences on the ACE2 inhibitor binding site and disturbs the binding site of the catalytic zinc atom.
Arg	Cys/His	219	This arginine has a strong salt-bridge, hydrogen bond, and charge interaction to stabilize protein	Its mutation disrupts the strong interactions and destabilizes the protein structure.
Lys	Arg	341	Stabilizing the loop structure by a strong hydrogen bond	Weakening hydrogen bond and so destabilizing the loop structure
Ile	Val	468	This isoleucine located at a loop to stabilize two helical structures by hydrophobic and π-stacking interaction	Because valine has a shorter side-chain and weaker hydrophobic interaction so this mutation destabilizes the protein structure.
Ser	Cys	547	This serin stabilizes local helix by hydrogen bonds	This mutation weakens the hydrogen bond so destabilizes the helical structure.

treatment, and pathogenesis. Indeed, various clinical trials evaluate the effectiveness of different therapeutic strategies to treat individuals with COVID-19 (Table 2).

6. The role of ACE2 variants in SARS-CoV-2 infection and severity of COVID-19 disease

Clinical variability of COVID-19 is because of various known and unknown determinants of disease progression such as age, sex, host immune response, virus mutations, comorbidity, lifestyle, and host genetic background (Qiu et al., 2020). Genetic factors contributing to the outcome of SARS-CoV-2 infection are unknown; however, variants in the specific sites of the ACE2 gene could be regarded as a main genetic risk factor for COVID-19 (Hou et al., 2020b). Due to the central role of ACE2 as the primary cellular receptor for COVID-19, many researches have investigated the association of ACE2 variants and COVID-19 susceptibility. The relationship between genetic variations in ACE2 and their effects on ACE2 structure and function, which may also affect the binding of the virus and subsequent infection severity, has been described previously (Guo et al., 2020c).

By bioinformatics and docking studies, the interacting residues of ACE2 at the interface between ACE2 and spike protein have been identified. Some residues have been suggested to map to the interface

site of viral ligand and human receptors, such as residues 19, 35, 38, 206, 211,219, 341, 353, 378, 468, and 547 (Benetti et al., 2020a; Lippi et al., 2020a). In another in silico study using molecular docking, six ACE2 missense variants, Ile21Thr, Ala25Thr, Lys26Arg, Glu37Lys, Thr55Ala, and Glu75Gly with higher affinity to spike protein and 11 variants, Ile21Val, Glu23Lys, Lys26Asp, Thr27Ala, Glu35Lys, Ser43Arg, Tyr50Phe, Asn51Asp, Asn58His, Lys68Asp, Met82Ile with lower affinity were computationally predicted (Calcagnile et al., 2021).

Missense mutations in ACE2 may perturb the receptor–ligand interactions. Some important missense mutations and their effects on the function and structure of ACE2 are demonstrated in Table 3.

Replaced amino acids can lead to various outcomes depend to replaced amino acids that is more impact potential variant would be appearing when the exchanged amino acids have different specifications; for instance, in p. Ser19Pro and p. Arg206Gly mutations, a polar amino acid (Ser or Arg) replaced with a nonpolar amino acid (Pro or Gly) or inversely, in p. Gly211Arg mutation, a nonpolar amino acid (Gly) replaced with a polar amino acid (Arg) (Guo et al., 2020b). As mentioned in Benetti et al. study, some of the ACE2 variants have direct effect on SARS-CoV-2 spike protein binding such as p. Leu351Val and p. Pro389His which interfere with the internalization process of virus to the host cell in Italian population.

A study reported ACE2 variants in two populations, Caucasians and Asians, and their possible effect on SARS-CoV-2 infection Rojas et al. (2020). Their results showed that two mutations have different frequencies in these two races, affecting ACE2 binding to spike protein. In another study on ACE2 variants in different ethnicities, Lys26Arg mutation in Ashkenazi Jews reduced ACE2 affinity, in contrast to mutations in other populations, including East Asian (Ile468Val), South Asian (Arg219Cys), African and African American (Lys341Arg), European (Asp206Gly), European and South Asian (Gly211Arg) (Ali et al., 2020). In an independent study, ACE2 genotypes in Africa and Eastern Mediterranean population were considered protective against COVID-19 (Karahalil and Elkama, 2020). In a cohort of Russian patients infected by COVID-19, three rare variants (rs146598386, rs755766792, rs73195521) may affect SARS-CoV-2 infection outcome were found (Shikov et al., 2020).

Hussain et al. examined multiple ACE2 gene variants and the binding efficiencies of various encoded proteins to SARS-CoV-2 spike protein, each varying in the polymorphisms of the RBD binding sequence (Hussain et al., 2020). Exchange of amino acids leads to alteration in chemical characteristics, peptidase activity of ACE2, ligand-receptor affinity, stability, conformational changes, hydrogen bonds and hydrophilic interactions, secondary and tertiary structures of ACE2, and subsequently, its interaction with RBD of spike protein and behavior with other involved molecules in ACE2 functions.

Although most ACE2 gene variants showed a high percentage of structural homologies, remarkable differences were also determined in central binding residues' spatial orientation. Two particular ACE2 alleles, including rs143936383 and rs73635825, demonstrate a relatively reduced binding affinity for the viral spike protein, which likely indicate a reduced likelihood of SARS-CoV-2 attachment and possible lowered susceptibility to infection. In a subsequent study, Stawiski and colleagues assessed the probability that several ACE2 variants, particularly those variants that estimated to interact with SARS-CoV-2 spike proteins, might be correlated to different host-virus interactions, leading to the alteration of pathogenicity and the SARS-CoV-2 virulence (Stawiski et al., 2020a). In brief, the authors performed a broader genomic analysis to construct a map involving synthetic mutants of ACE2, using samples from over 400 populations and 290,000 cases. Remarkably, they reported a list of ACE2 variants from existing genomic datasets utilizing structural modeling to characterize and identify ACE2 variants that were predicted to be protective against viral entry since they exhibited a lower binding affinity to SARS-CoV-2 spike protein such as S19P, I21V, E23K, K26R, T27A, N64K, T92I, Q102P, H378R. While ACE2 gene variants including K31R, N33I, H34R, E35K, E37K, D38V,
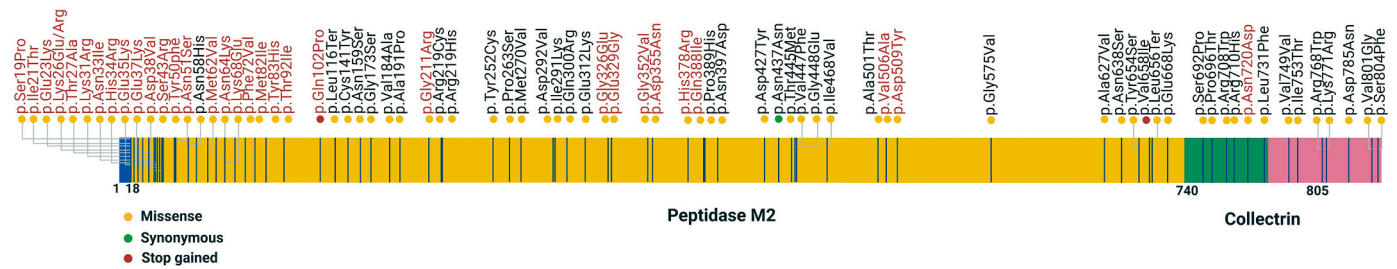
Fig. 5. Schematic representation of different ACE2 gene polymorphisms in regulatory, non-coding, and coding regions with possible effects on SARS-CoV-2 binding, internalization, and ACE2 expression levels, respectively. The human ACE2 gene includes 5′UTR, 3′UTR, intronic, 18 exogenic regions (E1-E18), and five novel exogenic regions (N1-N5) participating in various alternative splicing. The polymorphisms contribute to ACE2 variations. Solid and dashed lines illustrate introns and possible splicing patterns, respectively. Abbreviations: ACE2; Angiotensin I converting enzyme 2, E; Exon, N; Novel exon, NTS; nucleotides, TF; Transcription factor, UTR; Untranslated Region.

Table 4
Reported variants of ACE2 and their possible functional roles.

Cohort/ Database	Variants in ACE2 Protein (accession number)	Possible Effect	Ref.
Italian Cohort	V506A (rs775181355)	Destabilizing effect on spike protein and ACE2 interaction	(Benetti et al., 2020a)
	N720D (rs41303171), K26R (rs4646116), and G211R (rs148771870)	Effect on the interaction between spike protein and ACE2	
GnomAD database (Canadian group)	E37K (rs146676783), T27A (rs781255386), K329G (rs143936283), and K26E (rs1299103394)	Increase the binding affinity between S protein and ACE2	(Gibson et al., 2020)
	N720D (rs41303171), S43R (rs1447927937), G326E (rs759579097), M82I (rs766996587), K26R (rs4646116)	Decrease the binding affinity between S protein and ACE2	
GnomAD database (UK group)	G326E (rs759579097)	Enhance ACE2 binding with spike protein	(MacGowan and Barton, 2020)
	E37K (rs146676783), G352V (rs370610075), and D355N (rs961360700)	Weaken ACE2 binding with spike protein	
Other large genomic datasets	S19P (rs73635825), I21V (rs778030746), E23K (rs756231991), K26R (rs4646116), T27A (rs781255386), N64K (rs1199100713), T92I (rs763395248), Q102P (rs1395878099), H378R (rs142984500)	Enhanced susceptibility to viral attachment	(Procko, 2020; Stawiski et al., 2020b)
	K31R (rs758278442), N33I, H34R, E35K (rs1348114695), E37K (rs146676783), D38V, Y50F (rs1192192618), N51S (rs1569243690), M62V (rs1325542104), K68E (rs755691167), F72V (rs1256007252), Y83H (rs759134032), G326E (rs759579097), G352V (rs370610075), D355 N (rs961360700), Q388L (rs751572714), D509Y	Decrease attachment propensity to spike protein	

Y50F, N51S, M62V, K68E, F72V, Y83H, G326E, G352V, D355N, Q388L, D509Y were predicted an enhanced binding propensity to the viral binding based on the interactions with the spike glycoprotein. Another

study reported that some of ACE2 gene variants were not capable of biding to the spike protein, while other variants showed higher binding affinity (Procko, 2020). Renieri et al. mined whole-exome sequencing data obtained from about 7000 Italian individuals to identify 33 ACE2 gene variations, explaining various SARS-CoV-2 spike protein binding, affinity, internalization, and processing (Benetti et al., 2020a). Particularly, variant N720D was closely located to TMPRSS2 cleavage-dependent viral intake site. However, several mutations (P389H, L351V, W69C) were predicted to modulate conformational changes that modify interactions with the RBD of the spike protein due to their location in the proximity of sequences is fundamental for SARS-CoV-2 spike protein binding. In part, this finding elucidated the greater rate of case fatality in the Italian population than in china (Lippi et al., 2020c). Significantly, other particular ACE2 gene variants were identified in mentioned investigation, some of which are probably able to establish conformational variation in the RBD binding sequence, which can alter the SARS-CoV-2 spike protein binding affinity. Consequently, it is possible that some of the ACE2 variants can affect the stability of ACE2, and imposing conformational changes can impact its interaction with RBD of spike protein and decrease or increase the ligand-receptor affinity.

Besides, some variations of ACE2 are located at its proteolytic cleavage site (C terminal domain of ACE2), which can influence virus infection (Benetti et al., 2020a); because it was suggested that soluble ACE2 could act as a decoy receptor for virus and decrease virus intake by cell surface ACE2.

Notably, ACE2 level of expression, which differs among people, could correlate with susceptibility to the infection (Devaux et al., 2020). Polymorphisms of regulatory and non-coding regions such as promoters or 3'-UTR in ACE2 can have a role in the different expression levels of ACE2 among individuals (Chaudhary, 2020; Lippi et al., 2020b). Association between some mutation of ACE2 (affecting its expression) with the SARS-CoV intake was also strengthened the possible effect of ACE2 variant on SARS-CoV-2 entrance (Lippi et al., 2020a). In one study, 6 SNPs (rs4240157, rs6632680, rs1548474, rs4830965, rs1476524, and rs2048683) out of 61 evaluated ones in the interionic sequence of ACE2 showed significant association with the expression level of ACE2 and hospitalization in COVID-19 patients (Wooster et al., 2020). Therefore, polymorphisms of both coding and non-coding regions of ACE2 have influencing potential on virus susceptibility and COVID-19 severity (Fig. 5).

Several studies investigated coding variants of ACE2 in multiple global databases. They estimated an increase or decrease of affinity between ligand and receptor by calculating the changes in the binding energy of those variants. As shown in Table 4, the predicted function for some of the variants of ACE2, as mentioned above, was in contrast with the results of Gibson et al., which worked on the same database (Gibson et al., 2020).

On the other hand, an evaluation of exome sequence data in the UK
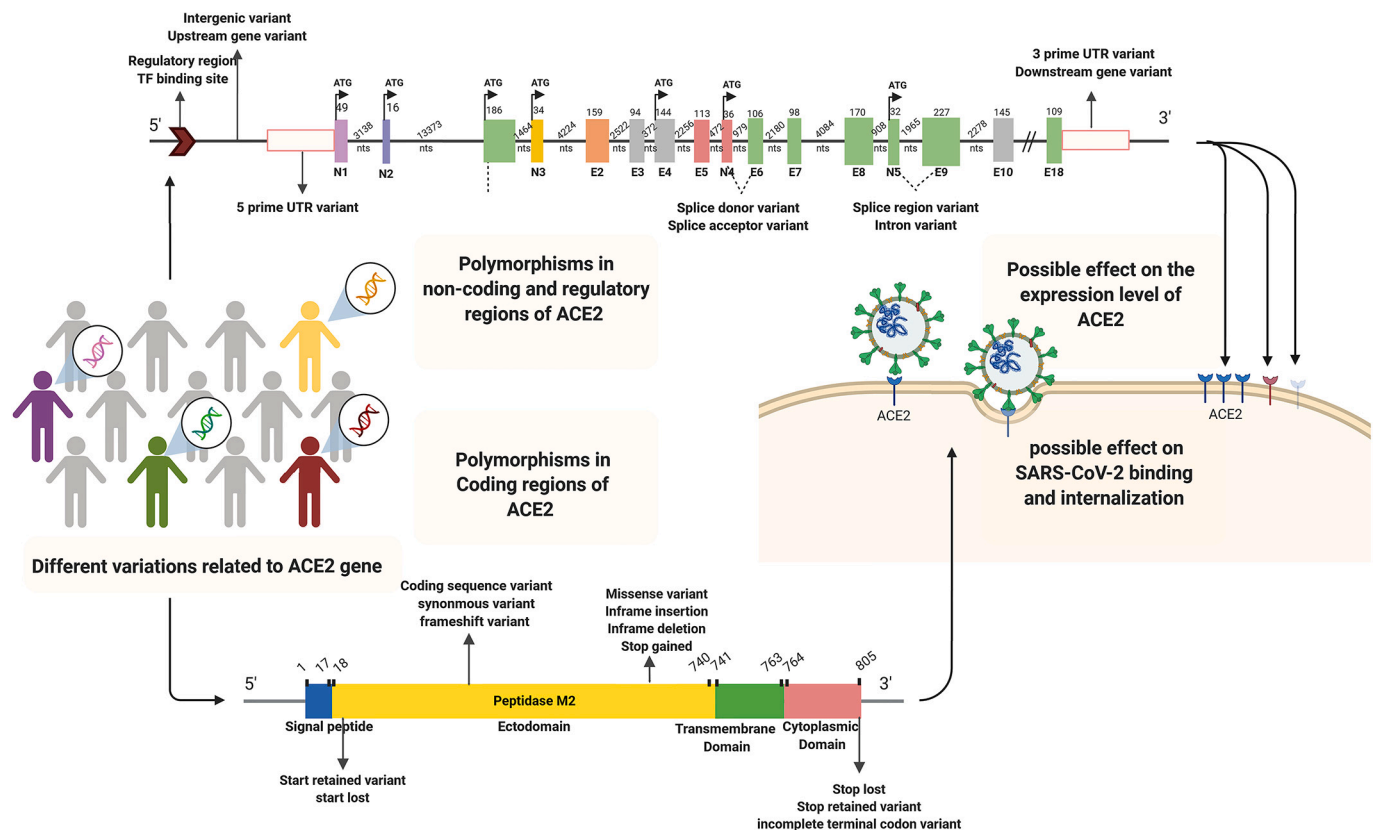
Fig. 6. Schematic representation of the distribution of gnomAD (v2.1.1, v3) missense, synonymous, and stop gained variants in the coding sequence of ACE2. Altogether, 338 ACE2 coding variants in gnomAD are reported, of which 241 numbers of them are missense and 88 synonymous variants. In the illustration, 44 deleterious coding variants of the ACE2 gene identified in the gnomAD databases and 33 coding variants (red) mentioned in Table 4, possibly affecting the host-virus interactions (Hou et al., 2020a). Yellow, green and red dots indicate the missense, synonymous, and stop gained variants. Synonymous variants do not change the protein sequence and subsequently are less likely to have a functional effect. Generally, the influence of missense variants depends on the functional and structural context of the residues that have been mutated and the mutant's physicochemical features. Indeed, missense variants are determined in all the main functional domains in ACE2, involving the peptidase M2, collectrin domains. Synonymous variants, including frameshifts and stop, are also reported in gnomAD for ACE2. These mutation types can influence the expression levels and the protein structure, but their influence is less associated with the residue context than missense ones (Cao et al., 2020).

biobank in which some subjects were infected by SARS-CoV-2, showed that none of the variants of ACE2 and TMPRSS2 determine COVID-19 severity (Curtis, 2020). However, MacGowan and Barton from UK analyzed the variants in gnomAD for ACE2 and predicted some variants of ACE2 modify the structure and function of ACE2 and affect its binding affinity to spike protein (MacGowan and Barton, 2020). The impact of the missense variant depends on the functional and structural context of mutating residue, besides the physicochemical characteristics of the mutant residue. In Fig. 6, according to gnomAD database, possible interactions between the ACE2 affinity variants and ACE2 expression polymorphism are highlighted.

Altogether the variant could potentially affect ACE2 expression and function, which can contribute to SARS-CoV-2 spread among populations around the worldwide. Indeed, this study will be useful to identify patients at high risk of complications of the underlying disease, which may need monitoring and treatment strategies.

7. Conclusion

The increasing data available from SARS-CoV-2 infections highlighting the potential correlation to be made between particular gene loci and variable susceptibility to COVID-19. The immune system components react to SARS-CoV-2 mainly related to the inter-individual variation in COVID-19 severity, whereas genes linked with the binding affinity to the ACE2 cell receptor and the viral entry at the early stages of infection, considerably determine the variable COVID-19 severity and

different susceptibility to SARS-CoV-2 infection. At this time, it is not clear whether the structure and function modulation of ACE2 in different variants could have a role in the probability of the infection or not. There are contradictory results for the impact of ACE2 variants in COVID-19 severity. Obviously, variants and mutations of spike protein and mainly its RBD have influenced the interaction between ACE2 and SARS-CoV-2.

There can be regarded as two possible scenarios for the effect of ACE2 variants in the results of SARS-CoV-2 infection. In the first scenario, multiple variants of ACE2 may have a synergistic effect on disease progression, and in the second, one/multiple rare variants may have a major impact on infection outcome. In order to confirmation of the findings, many studies need to be performed. Such studies could benefit the biopharmaceutical industry to design effective approaches for therapy and vaccination of COVID-19 disease.

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Declaration of Competing Interest

The authors declare no conflicts of interest.

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