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Variable Clinical Manifestations of COVID-19: Viral and Human Genomes Talk

Zeinab Imani-Saber[1], Hajar Vaseghi[1], Mojdeh Mahdian[1], Fatemeh Safari[1], and Mohsen Ghadami[1,2]

[1] Department of Medical Genetics, School of Medicine, Tehran University of Medical Sciences, Tehran, Iran
[2] Cardiac Primary Prevention Research Center, Tehran Heart Center, Tehran University of Medical Sciences, Tehran, Iran

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ABSTRACT

The new coronavirus, known as "SARS-CoV-2"; is the cause of one of the most prevalent infectious viral diseases that was recently announced pandemic by the world health organization. Ongoing research in the fields of prevention, management, and therapy establishes a functional scaffold for clinics during the time of crisis. To obtain this goal, it is necessary that all pathophysiologic aspects of COVID-19 from infection to predisposing backgrounds of infection be identified, so that all the ambiguities of researchers regarding transmission mechanisms, variable clinical manifestation, and therapeutic response can be solved. Here, we firstly discuss about the homology screening between nCoV-2019 and beta-coronavirus family using phylogenetic analyses. Secondly, we analyzed the viral motifs to show that viral entry into the host cells requires a primary activation step performed by FURIN and FURIN-like-mediated enzymatic cleavage on the structural glycoprotein. The cleavage increases viral performance by 1000 folds. We then present a comprehensive view on host cells and the significance of gene variants affecting activation enzymes, supportive entry, and spread mechanisms in humans including renin-angiotensin-aldosterone system (RAAS) a pathway results in certain phenotypes or exacerbate infection-related phenotypes in different organs, hence causes variable clinical manifestations. This is followed by discussing about the importance of personalized medicine in nCoV-2019 exposure. Moreover, chemical drugs prescribed for individuals affected with COVID-19, as well as genes involved in drug transport and metabolisms are reviewed as a prelude to drug response. Finally, we suggest some therapeutic approaches developed based on new methods and technology such as anti-sense therapy and antibodies.

Keywords: Angiotensin-converting enzyme 2; Coronavirus; FURIN protein; Personalized medicine; Severe acute respiratory syndrome coronavirus 2

Corresponding Author: Mohsen Ghadami, MD, PhD; Department of Medical Genetics, School of Medicine, Tehran University of Medical Sciences, Tehran, Iran. Tel: (+98 912) 2931 362, E-mail: mghadami@tums.ac.ir
* First and second authors contributed equally to this paper

INTRODUCTION

Coronaviruses (CoVs) comprise a large family of viruses that contribute to the pathogenesis of a various

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range of conditions from the common cold to more severe diseases including severe acute respiratory syndrome (SARS-CoV) and the Middle East respiratory syndrome (MERS-CoV), which began to spread in 2002 and 2013, respectively. It affected a great number of individuals with serious symptoms such as pneumonia, bronchiolitis in susceptible populations.[1] A new type of CoV, known as nCoV-2019 or SARS-CoV-2, lead to the break-out of a viral infection termed coronavirus disease (COVID-2019 or COVID-19), which causes acute respiratory problems and leads to death in 2.1% of cases.[2] COVID-19 is characterized by symptoms including fever, cough, and breathing difficulties. In severe cases, the infection can cause pneumonia, acute respiratory syndrome, kidney failure, and can even become fatal. Current approaches have focused on isolation and quarantine to inhibit COVID-19 spread. This review firstly discusses findings of phylogenetic analyses and subsequent analysis of viral motifs that show viral entry into host cells requires primary activation mediated by FURIN. This helps researchers to identify the origin of coronavirus. For instance, some studies declare coronavirus originates from bats. Therefore, understanding how bats control virus-mediated pathogenesis may help researchers identify novel therapeutic targets and molecules to treat infections caused by these viruses in other mammals, including humans and agricultural animals.[3]

Herein, we present a comprehensive review of host cells and the significance of gene variants affecting activation enzymes, supportive entry, and transmission mechanisms in humans. We then discuss the importance of personalized medicine in nCoV-2019 as well as chemical drugs prescribed for individuals affected with COVID-19. Finally, new therapeutic approaches in this field are suggested.

METHODS

In this paper, the phylogenetic tree was designed by aligning the spike glycoprotein using MEGA X software. For this purpose, firstly, the Refseqs of beta-CoV family glycoproteins were extracted from National Center for Biotechnology Information (NCBI) and UniPropt in FASTA format, and then, they were aligned by the ClustalW multiple sequence criterion. Next, amino acid sequences were separately analyzed for cleavage site screening.

Furthermore, a literature search was performed using Pubmed, Elsevier, Scopus, and Google scholar search engine using a combination of medical subject headings (MESH) terms including coronavirus, angiotensin-converting enzyme 2, severe acute respiratory syndrome coronavirus 2, FURIN protein, and personalized medicine. Finally, 75 related articles with full-text access that were published between 1990 and 2020 were considered in this study.

Coronavirus Genome, Functional Domains, and Viral Entry

CoV contains an RNA genome and starts to proliferate upon entering host cells and produces new viral particles for further excretion and infection of other cells. Studies on the genome show that both CoV and SARS belong to the family of beta-coronaviruses.[4] However, these types of viruses are remarkably different in, rate of transmission and infection as well as pathogenesis.[5]

Beta-coronaviruses are classified into four lineages of A: human coronavirus OC43، bovine coronavirus, porcine hemagglutinating encephalomyelitis virus ،murine hepatitis virus and human coronavirus HKU; B: severe acute respiratory syndrome coronavirus 2 (ncov2019) bat SARS coronavirus HKU3-3; C: bat coronavirus HK, tylonycteris bat coronavirus HKU4, Middle East respiratory syndrome-related coronavirus; D: bat coronavirus HKU9.[4]

As shown in Figure 1, the phylogenetic tree depicts the close relationship between SARS-COV2 and HKU3. The tree was generated on a homology basis in the S glycoprotein sequence since the variance in this region of the genome has granted the virus with new features which in turn, is suspected to explain the reason behind the high transmission rate as well as pathogenicity.[5,6] The glycoprotein domains are further discussed to better clarify the importance of this protein in the new CoV pathogenesis and infectivity.

Analyses of the new CoV and SARS virus have demonstrated target sites in S1/S2 and S2 for cleavage by the aforementioned host protease. According to a group of studies, these sites are targeted by FURIN protease of the seine protease family while a couple of other studies attribute them to FURIN-like protease enzymes.[7] The S glycoprotein on the surface is crucial in viral attachment to the host cell and subsequent fusion (Figure 2).

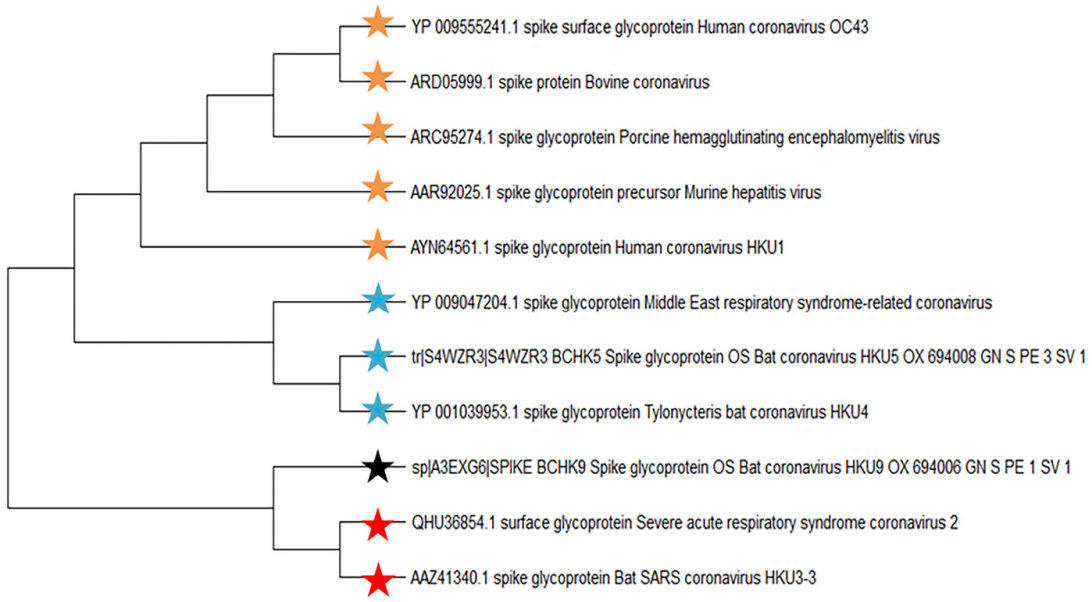

Figure 1. Phylogenetic tree of beta-CoV family generated by MEGA X software based on sequence alignment of S glycoprotein of beta-CoVs using the Neighbor-Joining algorithm. The orange labels indicate lineage A viruses, red labels indicate lineage B, blue labels indicate lineage C and black labels indicate lineage D. As it is shown, the new coronavirus 2019 is co-categorized with HKU3 in lineage B. COVID-19 glycoprotein is co-categorized with bat coronavirus HKU3-3 surface glycoprotein due to their extreme sequence similarity. These sequences are labeled with red stars. Both these viruses have the same ancestor with bat coronavirus HKU9.

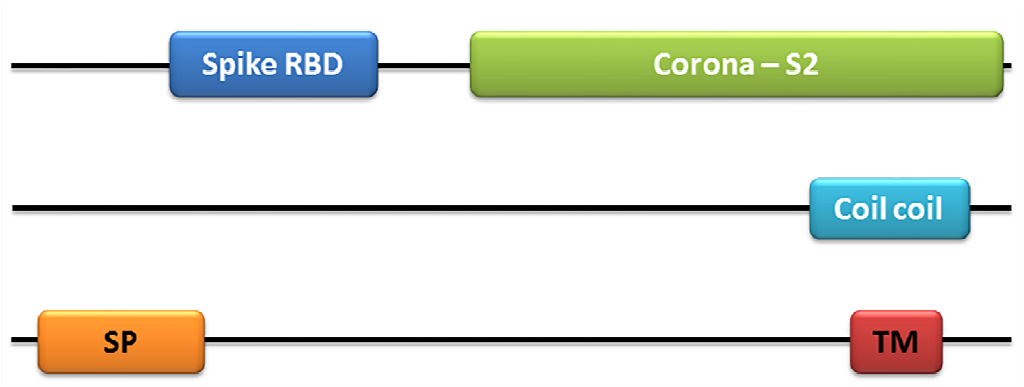

Figure 2. A schematic view of different proteins produced by the new coronavirus with a focus on structures involved in viral maturation. Domains in each row are characterized specifically; domains in the first row are surface glycoproteins involved in binding and cell fusion; domains in the second row are not functionally defined; domains in the third row are often structurally important. SP domain: signal peptide; Spike RBD: Spike receptor-binding domain (binds host receptor); S2 domain (comprises IFP and FP that promote surface fusion and intracellular fusion, respectively); TM transmembrane domain): primarily found in severe acute respiratory syndrome (SARS) and the Middle East respiratory syndrome (MERS). (https://www.ebi.ac.uk/Tools/hmmer/results/392B55CE-7098-11EA-B5F2-E7F5E876C163.1/score. Accession: PF09408.10 - PF01601.16)

Multialignment of the S sequence within the beta-coronavirus family; using MEGA-X revealed several cleavage motifs of the FURIN Serine protease and FURIN-like families (Figure 3). Target sites of these enzymes comprise a sequence as (R/K)-(2X)n-(R/K)↓ located in S1 and RRR/S/R or RRR/A/R S2 domains.[6,8] The linking region between S1/S2 in other family members such as SARS CoV is not affected by FURIN and its cleavage mechanism is not revealed yet. Interestingly, the following findings of different studies can enhance our understanding of how the virus transmits and how it can be prevented or even treated. First, for the virus to act in its active infectious form within a cell, both Arginine-rich target sites must be cleaved by FURIN and FURIN-like enzymes. Second, various studies have shown that the new CoV is dependent on binding to a protein_ ACE2_ through its spike Receptor Binding Domain RBD, to be able to enter the cells. This domain is activated by a protease before binding and cellular infection. Therefore,

Figure 3. Conserved regions containing cleavage sites for FURIN and FURIN-like enzymes. To obtain this figure, the Refseqs of beta-CoV family glycoproteins were extracted from NCBI and UniPropt in FASTA, and then MEGA X software was employed to perform ClustalW multiple sequence alignment. Next, amino acid sequences were separately analyzed for cleavage site screening.

A: S1 sequence contains the receptor-binding domain, defined by amino acids 350-550. Amino acid sequence analysis of S1 shows arginine and lysine-rich cleavage sites with/without a spacer. This motif is indicated by a blue box.

B: S2 sequence comprises amino acids 600-1273. The arginine-rich cleavage sites shown in the blue box contain tandem repeats of RRR/S/R. This sequence exists only in lineage A and the new CoV, but not in SARS. Spike surface glycoprotein of the new coronavirus owns arginine-rich cleavage sites indicated by a blue box. These sites are cleaved by FURIN and FURIN-like enzymes.

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inhibitors of the activating protease could be of therapeutic value and are discussed in continue. Third, the new CoV owns a considerably higher infection rate compared to the SARS virus, which is due to a new mutation leading to the formation of a FURIN target site. This may be a proposed hypothesis as to why the new virus is highly contagious.

As the final point, it is worth mentioning that comparative studies have found that the rate of pathogenicity in affected individuals with a FURIN target site was higher compared to those without the aforementioned site. Moreover, the first group of individuals was more severely affected. As a result, variable expressivity of the phenotype and organ involvement in patients might be attributed to this matter.[6]

Viral Entry into the Cells

S1 protein is involved in receptor attachment while S2 takes part in virus-host cell membrane fusion.[6] The rate of influenza virus activation in infected cells depends on FURIN expression level; higher levels of FURIN mRNA are observed in more contagious and severe disease cases.[9] Nonetheless, further investigation is required to find out if this holds for COVID-19 as well. In some investigations, it was shown that FURIN plays a role in different pathways.[10-20] Somehow, FURIN participates in a multitude of pathways among which, aging-related processes such as collagen destruction and plaque formation are outstanding. FURIN serves as an activator in these processes and is unsurprisingly highly expressed in the elderly. This could be a hypothetical explanation for the higher rate of involvement and fatality of COVID-19 in the elderly.[4] A history of lung diseases could also contribute as a risk factor of COVID-19 as the encoding FURIN gene shows the highest level of expression in the lungs. Also, the level of FURIN is higher in individuals with lung inflammation and therefore, the virus is more pathogenic.[21]

A history of cardiovascular and coronary heart diseases was reported to make individuals highly susceptible to COVID-19 and the mortality rate of COVID-19 in these individuals is approximately 30%.[22] In these individuals, vascular remodeling is likely to occur and as FURIN is highly expressed in the blood and acts as a positive regulator of vascular remodeling that has been shown in mice models.[17] It could be suggested that high levels of FURIN in the vessels are associated with higher infection susceptibility. Intriguingly, hypoxia and iron deficiency can affect regulatory elements in the FURIN gene promoter and increase its expression level by 3 to 400-fold. Therefore, the co-occurrence of viral infection and hypoxia causes lung cell destruction on one hand, and overexpression of the activating protein, FURIN, on the other hand, which leads to further progression of the disease. Provision of individuals with oxygen and higher iron intake is considered as management strategies to reduce the level of activating viral protein and viral spread.[9]

In some individuals affected with COVID-19, the blood dimer index is above 1 microgram. The index is correlated with thrombophilic conditions and is increased in most of the COVID-19 cases, especially in those who are severely affected. Regarding the activating role of FURIN in the production of F8 and the positive interaction with Von Willebrand factor, a boost in the level of FURIN is linked to increased thrombophilic phenomena as well as di-dimers, which altogether re-propose the hypothetical role of FURIN in viral activation and the role of the virus in FURIN production.[22,23] In a genome-wide association study (GWAS), it was shown that some polymorphisms (rs17514846 A→C) are associated with susceptible risk factors that contribute to infection severity.[24] There might be a correlation between an increase of FURIN with COVID-19 risk factors, disease severity, and infection probability.

Based on the findings of a recent study in China, the 2019-nCoV enters cells using the receptor angiotensin-converting enzyme II (ACE2),[25,26] the same receptor used by SARS-CoV.[27] During the entrance of both viruses, the S1 subunit of Spike protein on the virion binds ACE2 while S2 subunit is responsible for virus-host cell membrane fusion.[28] Upon cleavage of ACE2, a possible mechanism for viral entry to take place is through the L-Cathepsin-dependent (pH-dependent endo-/lysosomal protease of the host) pathway and endosomal fusion (Figure 4).[29]

Human Genes Involved in Viral Entry into Cells with ACE2 Receptor

ACE2 is one of the many proteins involved in the Renin Angiotensin Aldosterone System (RAAS) and regulates the blood pressure as well as fluid and

electrolyte balance. ACE2 is located on the inner layers of blood vessels in the heart, kidney, lung, and is also highly expressed on the mucosa of the oral cavity.[30] As a result, these organs are more prone to damage upon exposure to nCoV-2019.

Among all organs, the lungs seem to be the most vulnerable to the virus. A possible explanation could be that the lungs provide a vast surface for respiratory viruses. However, there is a biological factor involved. Zhao et al. reported that 83% of ACE2-expressing cells are alveolar epithelial type II cells (AECII), showing that these cells are of storage capacity for viral infections. Moreover, gene ontology enrichment analysis revealed that ACE2-expressing AECII contains a great load of viral genes that participate in a variety of viral processes such as monitoring, life cycle, assembly, and replication, indicating facilitated viral proliferation.[31] ACE2 has two isoforms: one that owns a full-length transmembrane domain that helps the extracellular domain to anchor the cell membrane. In the case of SARS-CoV2, the extracellular domain of ACE2 binds Spike protein.[29]

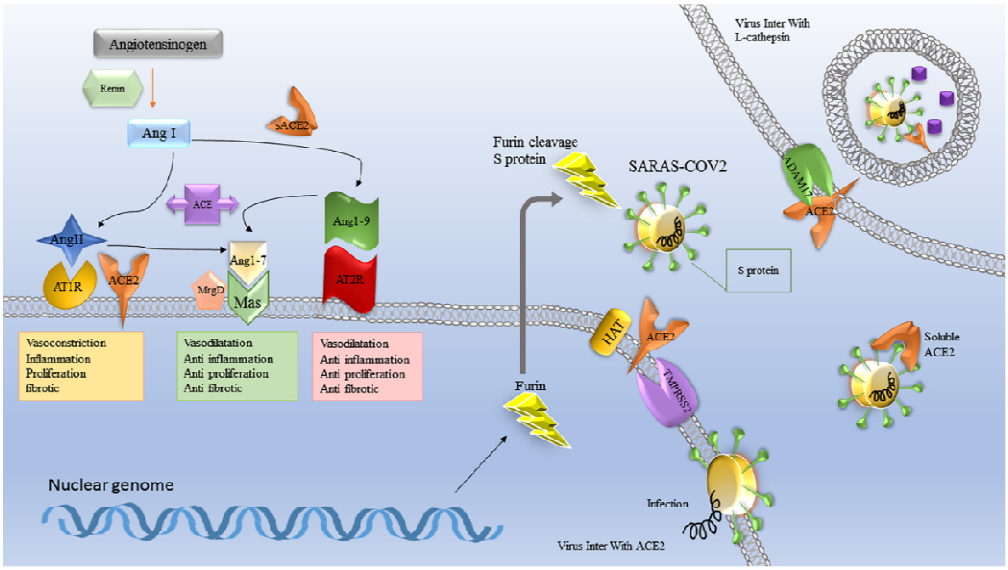

Figure 4. A schematic view of COVID-19 viral entry into host cells. The top left depicts the L-cathepsin pathway of viral entry in which cathepsins (purple cylinders) are in charge of intracellular cleavage within lysosomes. The transmembrane domain of ACE2 is cleaved by ADAM17 to produce soluble ACE2 (it can prevent the binding of viral particles to ACE2 in the cell membranes). At the bottom, viral entry through binding of S protein to ACE2 accompanied by HAT and TMPRSS2 (protease) is shown. S protein is cleaved and activated by FURIN before cellular entry. On the left, the RAAS pathway along with involved enzymes and their interactions are exhibited. AngII: Angiotensin II; ACE2: angiotensin-converting enzyme 2; ACE: angiotensin-converting enzyme; AT1R: angiotensin type-1 receptor; AT2R: angiotensin type-2 receptor; Mas: Mas receptor; MrgD: Mas-related G protein-coupled receptor.

The other isoform of ACE2 has a soluble form that lacks the transmembrane domain and could be found in blood circulation at low levels. [29]

The trans-membrane domain is cleaved by ADAM17, a type of tumor necrosis factor-alpha (TNFα) [29] converting enzyme. It has been recently proposed that the soluble form can prevent the binding of viral particles to ACE2, and therefore, act as a competing receptor for SARS-CoV and other CoVs.[32] In this regard, production of recombinant ACE2 protein might be a new potential biologic treatment to fight against the virus or restrict disease progression caused by CoVs that rely on ACE2 as a means of viral entry. [32]

ACE2 is necessary for viral entry, but not sufficient. To be more specific, viral entry requires the simultaneous presence of transmembrane serine protease 2 (TMPRSS2) and human airway trypsin-like protease (HAT). These enzymes are both expressed in human ACE2+ lung cells and aim to activate S protein by cleaving the ACE molecule bound to the virus,

facilitating cellular entry.[33] It is demonstrated that SARS-CoV-2 uses the receptor ACE2 for entry and serine protease TMPRSS2 for S protein priming.[34] Increased levels of TMPRSS2 in cell cultures could perform a key role in the spread of SARS-CoV within the human respiratory system.[35]

Due to lack of comprehensive clinical reports on SARS-CoV in different populations on one side, and the heavy load of the unknown in the genetic basis of ACE2 transcription and function on the other side, it is required to perform genetic analysis of quantitative expression trait loci (eQTLs)[36] and potentially functional variants of ACE2 in populations to perform epidemiologic studies regarding SARS-CoV spread in all populations.

One of the studies in this field using mass spectrophotometry pinpointed arginine residue 621(R621) as the cleavage site for ACE2. However, mutations in this residue do not interfere with further processing of TMPRSS2 and HAT unless it disrupts protein folding or accessing the cleavage sites. They find five clusters of arginine and lysine residues located between R619 and the transmembrane domain of ACE2 (Residues 741–761) as a cleavage target. Findings indicate that arginine and lysine residues of amino acids in the positions 697 to 716 in cluster 4 of ACE2 are fundamental for the cleavage reaction. In case a mutation takes place within target sites of this cluster, enzymatic cleavage does not occur.[29] If mutations occur at key residues in C4 along with C0, cleavage will decrease, which might increase immunity against viral pathogenicity in individuals with specific genotypes of these clusters. This is another possible explanation for the variable expression of clinical manifestations of COVID-19 in patients. Mutagenesis studies demonstrated that arginine and lysine residues of amino acids 652 to 659 of ACE2 affect the function of ADAM17, but not the processing of ACE2 via TMPRSS2 / HAT.[29] In conclusion, mutations in these residues despite normal ADAM17 variants could nullify the therapeutic utility of ADAM17 overexpression for COVID-19. Also, they showed the necessity of these residues for ADAM17 to cleave the receptor and to attend the competitive processing of ACE2 by TMPRSS2. Different variants in these residues could impact viral entry. TMPRSS2 inhibitors might also be efficient for therapeutic candidates.[25,34] Expression levels of ACE2 gene and gene variants in the binding region between ACE2 and S protein of the new coronavirus might impact the level of interaction, hence, viral entry, especially into lung cells. The interaction level can surely impact the variable expressivity of COVID-19. Based on the systematic analysis of 1700 candidate coding variants and allele frequency of ACE2 obtained from databases in China, there was no straightforward evidence of protein binding-resistance in ACE2 toward S protein in various populations. Further investigation using variant distribution and allele frequency data might pave the way toward defining the role of variants and allele frequency in lung function and acute lung damage. In East Asia, the frequency of eQLT variants and ACE2 expression is considerably high, which might explain the reason behind diverse susceptibility or reactions of different populations toward SARS-CoV2 under similar conditions.[37] Moving on, another important enzyme in RAAS is ACE with I/D polymorphisms, comprising insertion (I) or deletion (D) of 287bp in intron 16 of the ACE gene.[38] Compared to carriers of allele I, carriers of allele D have higher levels of angiotensin II and experience a faster reduction of blood pressure during exercise, which in turn reduces the maximal heart rate and oxygen absorption (VO_2 max). Therefore, these carriers are more prone to increased risk of cardiovascular diseases by 10%. On the other hand, carriers of allele I have low levels of ACE and as a result, higher VO2max and maximal heart rates, and in turn, show higher endurance.[38,39] Hypothesizing that increased blood pressure and reduced oxygen absorption are among risk factors and diagnostic criteria of COVID-19, respectively, it could be concluded that allele D is associated with severe manifestations in patients. Theses variants have proven to be responsible for conditions such as diabetes, high blood pressure, and chronic renal disease.[40,41] This is while each of these conditions is a risk factor for COVID-19 and can influence its clinical manifestations.

RAAS is negatively regulated by ACE2 through the conversion of angiotensin II to Angiotensin-(1-7) vasodilator through binding Mas receptor_ and Angiotensin-(1-9) vasodilator through binding AT2R (Figure 4) and reduces the effect of Ang II.[42] Interaction of ACE2, ACE, angiotensin II and other elements of RAAS are complex and partly contradictory. Furthermore, ACE2 expression in heart, kidney, and lung varies among healthy individuals, patients affected with cardiovascular and CoV. The role

of ACE2 in COVID-19 infectivity of individuals with cardiovascular disease is unknown.

Another member of RAAS is Angiotensin II receptor type 2 (AT2R) with a key role in the regulation of blood pressure and atherogenesis. The genetic polymorphism "A1675G" on AT2R is associated with systemic inflammation and high blood pressure, with the latter especially found in men. It also affects cardiovascular risk and atherosclerosis severity.[43] After binding its ligand, Angiotensin II receptor type 1 (AT1R) provokes vasoconstriction, inflammation, and cell proliferation. This further highlights the importance of AT1R activity, as well as variants in AT1R in clinical manifestation of COVID-19, which is due to their disruptive effect on ligand-receptor interactions or aberrant activation of AT1R. Missense variants might affect ligand binding and AT1R signaling directly (A163T, C289 and, T282M variants) and indirectly (L48V, L222V, and, A244S).[44] Also, these variants might impact the effect of antagonist drugs used to lessen the activity of AT2R and its agonists and cause variable drug response among individuals.

In conclusion, due to the importance of differential effects related to variants in genes encoding enzymes and receptors in RAAS, further studies on these variants and polymorphisms might result in the identification of genotype-phenotype correlations in individuals with COVID-19.

Risk Factors for Coronavirus Infection

Predisposing risk factors for COVID-19 and more severe clinical symptoms include environmental factors such as age, smoking, and underlying diseases. It seems that accurate prediction of COVID-19 phenotypic manifestation in each individual is facilitated through the analysis of well-known associated genes and polymorphisms.

Reports indicate that approximately 70% of people over the age of 70 died as a result of viral infection whereas the mortality risk elevated among older patients hospitalized in health care organizations.[22] FURIN acts as an activator and impeller of many aging-related pathways, some of which are listed in the Reactome database including degradation of extracellular matrix and lipoprotein metabolism. Also, investigations showed that FURIN plays an important role in several pathways related to vascular remodeling, as seen in coronary artery disease and high blood pressure.[17]

Back to the hypothesis raised in the "viral genome" section, it can be concluded that it is more likely for the elderly to get infected by the virus and show a higher mortality rate.[20]

People with allele D in ACE2, who show a remarkable increase in blood pressure during heavy physical activity seem to be at higher risk of severe COVID-19. Since ACE genotyping data is not available in general populations, it can be suggested that heavy exercise activities should be avoided to eliminate the pandemic feature of coronavirus.[39]

Underlying pulmonary diseases is one of the most important risk factors that put patients affected with COVID-19 at risk as FURIN and ACE2 genes have the highest expression level in lung tissue. Furthermore, individuals with inflammatory lung disease have higher levels of FURIN expression, and therefore, a more severe condition.[19]

Hypertension and diabetes are next in the list of COVID-19 risk factors. About 32% of the disease affected by underlying chronic medical conditions such as diabetes (20%), hypertension (15%), and cardiovascular disease (15%).[45]

Studies conducted among patients with type 1 or type 2 diabetes, hypertension and cardiovascular diseases who were treated with ACE inhibitors and type I Angiotensin II receptor blockers have shown that these therapies lead to up-regulation of ACE2.[46-48] On the other hand, experimental studies have shown that consumption of ARB and ACE inhibitors reduce severe lung damage in some cases of viral pneumonia.[49] It is still debating whether these therapies should be prescribed for patients with COVID-19 and hypertension/diabetes or change the therapeutic approach toward the use of other antihypertensive drugs.

To make the best possible prediction about clinical manifestation and drug response of individuals affected with COVID-19, it is suggested that we apply a type of personalized medicine to different populations and races through the examination of ACE2 gene polymorphisms associated with these underlying diseases and the risk of SARS-CoV-2 infection.

The weak immune system is another significant available risk factor for COVID-19. A recent study consisted of 30 hospitalized patients with COVID-19 in Huizhou municipal central hospital suggested that platelet counts and their dynamical changes during

treatment may have implications for the severity and prognosis of the disease.[50] A remarkable increase in the platelet level and longer hospitalization periods may be correlated with cytokine storm. Cytokine storm may exacerbate disease severity, and along with the weakened immune system, the patient may experience the final and the second inflammatory phase, which eventually leads to tissue damage. Regarding this issue, a history of underlying diseases of the immune system, such as congenital or acquired immune defects and the use of immunosuppressive drugs and genome-wide studies of these individuals may be associated with the severity of clinical manifestations of COVID-19.

Among cases of SARS-CoV-2 infection confirmed by the National Health Commission of China (NHC), some patients were first referred to a physician for cardiovascular symptoms. These patients suffered from chest tightness and heart palpitations rather than respiratory symptoms, fever, and cough, and they were later diagnosed with COVID-19. According to NHC, 10% of patients who died from COVID-19 affected with underlying cardiovascular diseases had significant heart damage with increased Cardiac troponin I (cTnI) levels or cardiac arrest during hospitalization. Consequently, due to systemic inflammatory response and immune system defects during disease progression in patients with COVID-19, the incidence of cardiovascular manifestations was increased.[51]

Other suggested mechanisms of cardiac damage in COVID-19 patients include cytokine storm can due to imbalanced response of T helper 1 and 2 and respiratory dysfunction and hypoxemia induced by COVID-19.[52]

It has been demonstrated that there is a significant association between rs17514846 polymorphism, located in the Upstream Region of the FURIN gene, with cardiovascular disease and increased expression of this gene.[24] Based on previous studies, it can be concluded that the presence of this variant in the FURIN gene may be considered as a factor in increasing the probability of infection by the COVID-19 virus mentioned earlier. Physical or mental impairments can potentially increase the risk of COVID-19 due to non-compliance with health recommendations. Physical or mental impairments can potentially increase the risk of COVID-19 due to non-compliance with health recommendations.

Making decisions about hospitalization is one of the most difficult stages of therapy due to the lack a of direct relationship between radiological and clinical findings and prognostic evidence. Based on epidemiological studies for all these risk factors and by considering the specific score, a final score is obtained to predict the COVID-19 infection and the severity of clinical manifestations.

Proposed Drugs for COVID-19 Treatment

According to the Center for Disease Control and Prevention Guidelines, patients with COVID-19 are divided into 3 different groups of mild to moderate (mild symptoms up to mild pneumonia), severe (dyspnea, hypoxia, or >50% lung involvement on imaging), and Critical (respiratory failure, shock, or multiorgan system dysfunction) patients.[53]

Hydroxychloroquine and Azithromycin are common drugs used for all COVID-19 patients.

Hydroxychloroquine: Mechanism of Action, Metabolism and its Transporters

Investigations focus on hydroxychloroquine and chloroquine for the treatment of SARS-CoV-2.[54] Furthermore, hydroxychloroquine may lead to a severe hypoglycemia.[55] Therefore, COVID-19 patients who have diabetes are advised to monitor their blood glucose levels.

Accumulation of hydroxychloroquine in human organelles increases the intracellular pH of vacuoles and reduces antigen processing and inflammatory response by acidic hydrolases in the lysosome.[56] Increased pH in endosomes inhibits the activity of viruses like SARS-CoV and SARS-CoV-2 for fusion and entrance into the cell. In addition to its antiviral activity, chloroquine has an immunomodulatory activity that may synergistically increase its antiviral effect in vivo.[57]

The terminal glycosylation of ACE2, which is the receptor that targets SARS-CoV and SARS-CoV-2 during cell entry, is inhibited by hydroxychloroquine.[56] Non-glycosylated ACE2 may be less interactive with SARS-CoV-2 Spike protein and inhibit viral entry.[54,56]

Hydroxychloroquine is metabolized to N-desethyl hydroxychloroquine by cytochrome P450 enzymes (CYP2D6, 2C8, 3A4, and 3A5).[58,59]

Molecules that act as carriers of this drug include P-glycoprotein 1 with Xenobiotic-transporting ATPase activity and Solute carrier organic anion transporter family member 1A2, encoded by ABCB1 and SLCO1A2 genes, respectively.[60,61]

The variants found in each of these hydroxychloroquine metabolizing or transporter genes may alter the intensity and speed of drug metabolism.[62]

Azithromycin: Mechanism of Action, Metabolism and its Transporters

Azithromycin is being investigated as a potential treatment for patients with COVID-19.

Azithromycin attaches to the 23S rRNA of the bacterial 50S ribosomal subunit. This process controls bacterial infections.[63] Also, azithromycin inhibits the Arginine Deiminase Protein 4 (PADI4) activity.[64]

Azithromycin is minimally metabolized by CYP3A4 enzyme.[65]

Molecules that act as transporters of this drug include P-glycoprotein 1 with Xenobiotic-transporting ATPase activity and Canalicular multispecific organic anion transporter 1, which are transcribed from ABCB1 and ABCC2 genes, respectively.[65]

Investigations on the effect of ABCB1 gene polymorphisms on the pharmacokinetics of azithromycin among Chinese people (Han ethnicity) were carried out by prescribing the same oral dose of azithromycin to each individual and measuring the concentration of this drug in the blood and finally comparing it with the genotype of individuals. For the first time, obtained results indicated that pharmacokinetics of azithromycin might be affected by specific polymorphisms of ABCB1 gene.[66]

ACE Inhibitors and Angiotensin Receptor Inhibitor as Drugs for Patients with COVID-19; Personalized Medicine

Personalized medicine defines why different people respond to the same medication differently. Perhaps personalized medicine is a confusing factor in answering the question of whether taking ACE inhibitors and angiotensin receptor blockers are helpful or harmful in patients with COVID-19 who suffer from underlying diseases such as high blood pressure and cardiovascular conditions. In this section, two contradictory approaches related to the effects of taking medications for hypertension and cardiovascular disease will be discussed:

The Use of AT1R Antagonist (Losartan) and ACE Inhibitor Decrease the Entry Rate of COVID-19

Deshotels et al. found an interaction between ACE2 and AT1R. Angiotensin-II-based therapy led to ACE2 internalization into lysosomes and its degradation. These outcomes were inhibited by the AT1R blocker losartan, the lysosomal inhibitor leupeptin, and ACE2 ubiquitination, assuming that angiotensin-II reduces physical interactions between ACE2 and AT1R, and also induces ubiquitination and internalization of ACE2 and its degeneration in lysosomes (Figure 4). Losartan inhibits entrance/ degradation and ubiquitination of ACE2.

Therefore, using angiotensin receptor blockers such as losartan, valsartan, telmisartan could be suggested as a novel therapeutic approach to block ACE2, thus inhibit SARS-CoV-2 infection.[42]

In the absence of angiotensin-II, AT1R and ACE2 interact physically to form a complex on the cell membrane, which could potentially reduce the interaction of ACE2 and virus.[67]

The clinical impact of the study by Deshotels et al. is unclear. However, based on their hypothesis, ACE and angiotensin receptor inhibitors may have positive and diminishing effects on the entrance rate of nCoV-2019 into host cells by decreasing ACE2 levels. If the interaction of the viral protein with ACE2 is reduced in the ACE2-AT1R complex, Angiotensin II receptor blockers (ARBs) might be useful. Prescription of an ARB may stabilize ACE2-AT1R interaction and prevent the interaction and entry of the viral protein-ACE2 complex. To our knowledge, Angiotensin-(1-7) generation, which may also play a role in preventing severe lung infection, has not been evaluated in studies. Angiotensin-(1-7) is produced by ACE2 cleavage and exerts its effects on vasodilation and prevention of tissue damage through its Mas and MrgD receptors.[42] It is not clear whether the prevention of ACE2-dependent entry through this mechanism can prevent viral infection with SARS or COVID-19 and this should be further investigated.

The Use of ACE2 Stimuli and Angiotensin II Type 2 Receptor Agonists Increase Viral Entry

People with diabetes and hypertension, who naturally have high rates of angiotensin 2, are treated with ACE2-stimulating drugs. This can increase the likelihood of getting infected by COVID-19 in terms of severity and fatality. If this hypothesis is confirmed, it will pose many challenges for the treatment of COVID-19 since ACE2 is one of the agents that reduces inflammation and has also been suggested as a potential new treatment for inflammatory lung disease, diabetes,

and hypertension.[68,69] However, it may act as a gateway for viral entry in COVID-19. Also, if different impacts of variants and polymorphisms of each of these receptor molecules and drug-metabolizing genes are taken into account, this might be much more challenging in individuals with COVID-19 suffering from these underlying diseases.

Currently, no laboratory or clinical information is indicating the advantages and disadvantages of ACE inhibitors, ARBs, or other Renin–Angiotensin–Aldosterone System (RAAS) antagonists in COVID-19 patients with a history of cardiovascular disease. Based on the statement issued on March 17, 2020, by the American Heart Association and the Heart Failure Society of America, it has been recommended to continuously use RAAS antagonists for patients with heart failure, hypertension, or ischemic heart disease. If patients suffering from cardiovascular diseases are diagnosed as COVID-19 infected cases, new decisions should be made for treatment according to hemodynamic status and clinical manifestations of each patient. It is necessary to implement urgent and accurate investigations on the theoretical concerns, contradictions, and findings regarding COVID-19 with cardiovascular disease. Therefore, it is maybe possible to correlate the genotype with manifestation and severity of COVID-19 for individuals by studying variants of genes encoding enzymes and receptors involved in drug metabolism.

New Innovative Therapies for COVID-19

Nucleic Acids-based Strategies

New therapies against respiratory viral infections include suppression and destruction of the viral genome.[70] RNA-based strategies include short interfering RNAs (siRNAs) and ribozyme therapy, and DNA-based strategies include deoxyribozyme-based approaches and antisense oligonucleotides.

The major obstacle of using these tools is drug delivery into target cells as these molecules possibly trigger immunogenicity, and therefore, are phagocytized by immune cells and digested by enzymes in body fluids. However, recent developments helped overcome such problems through optimizations such as using non-viral vectors including cationic liposomes, especially in the field of respiratory viral infections. Because of the positive charge, cationic liposomes remarkably interact with both negatively-charged nucleic acids and cell membranes.

Regarding the availability of lung tissue through the intranasal pathway in comparison to other organs, the spraying of nucleic acid molecules seems to be an effective way to treat respiratory viral infections. To enhance the stability of these nucleic acids, especially RNAs, they can be aggregated with nanoparticles like Chitosan.[71]

A study demonstrated the ability of ribozymes to inhibit viral replication up to 60% by using a chimeric DNA-RNA hammerhead ribozyme targeting SARS-CoV.[72] In this research, the authors also showed that the chimeric structure that targets SARS-CoV significantly reduced the expression of SARS-CoV RNA in transfected 3T3 cells with recombinant plasmids. Therefore, these structures could be suggested as possible therapeutic approaches for SARS.

Peptide-conjugated Phosphorodiamidate Morpholino Oligomers (PPMOs) may rapidly enter cells and interfere with viral protein expression through inhibition of the complementary RNA (Figure 5). Neuman et al. designed specific PPMOs against specific sequences of SARS-CoV genome.[73]

Positive results from studies on SARS-CoV can be inspirational for the treatment of COVID-19. CoV-2019 target sequences should be selected according to phylogenetic analysis and targeted by genome editing tools. These sequences are associated with early activation of the virus and include motifs that are considered as the cleavage site of different proteases. Also, appropriate designing of the tools mentioned above and the selection of a suitable vector may be a reliable solution for COVID-19 treatment. The proposed therapeutic technique based on the use of PPMO for the treatment of patients with COVID-19 is illustrated in Figure 5.

Antibody-based Therapy Strategies

Due to the structural similarity of envelope proteins in the Beta-coronaviruses family, especially among MERS-CoV, SARS-CoV, and 2019-nCoV, employment of monoclonal antibodies generated against these viruses may also be helpful for the treatment of COVID-19.[74]

One of the possible solutions to overcome SARS-CoV-2 infection is convalescent plasma therapy. In this approach, the blood of formerly infected patients is collected after recovery, and then the serum, which is rich in antibodies, is separated and injected into a

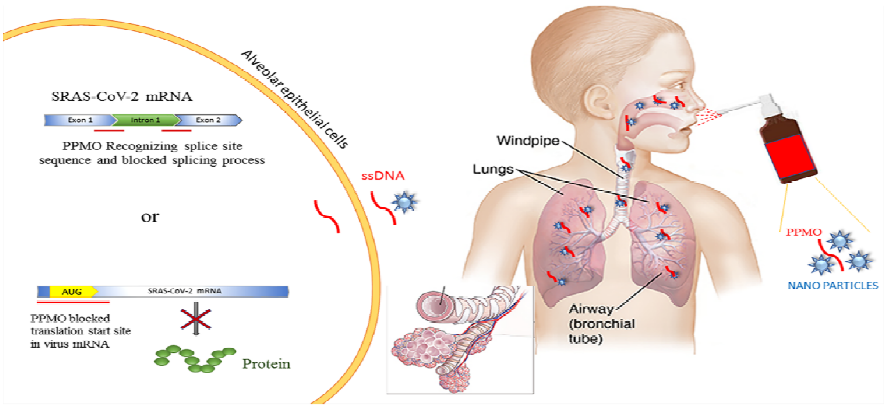

Figure 5. Schematic illustration of Peptide phosphorodiamidate morpholino oligomers (PPMOs) inhibiting severe acute respiratory syndrome (SARS)-CoV-2 mRNA translation. Since nanoparticles can bind nucleic acid molecules, conjugation of PPMOs as single-stranded DNAs with nanoparticles improves molecular stability and provides easier absorption of these molecules into the body and cells. Due to the accessibility of lung tissue, these molecules could be sprayed by a nebulizer to treat respiratory viral infection. Furthermore, due to the complementarity of PPMO to the viral RNA genome, it is possible to inhibit the growth and proliferation of SARS-COV-2 in two ways: first, PPMOs can be designed as a complementary sequence to splice sites and prevent splicing upon binding them. Second, PPMOs can be used to recognize the translation start site in viral RNA and inhibit translation. Therefore, it can be concluded that these approaches could have therapeutic effects on patients with COVID-19.

newly infected case. Antibodies are proteins produced by B cells of the immune system with the ability to bind a specific molecule on pathogens that invade the human immune system and directly neutralize or activate an immune response.[75]

Another option for disease prevention is the production of an appropriate monoclonal antibody against RBD to play as a domain that is involved in both viral activation and entry into the host cell.

Furthermore, the use of cocktail antibodies against both of these viruses may be one of the potential strategies for vaccine preparation. Plasma donation from recovered COVID-19 patients with antibodies in their plasma could be another treatment strategy against the virus.

CONCLUSION

In the present study, potential reasons for COVID-19 variable manifestation, such as gene variants whose products are involved in versatile pathways or cellular functions, host cells, and availability of viral equipment for proliferation, as well as age, smoking, underlying diseases, and weak immune system were discussed. Delineation of the main cause(s) of clinical heterogeneity might be possible by comprehensive genomic profiling of affected individuals with variable clinical manifestation using high-throughput methods such as whole genome/exome sequencing. Findings of genomic studies could provide prognostic prediction, proper drug dosage prescription, and development of well-fit therapeutics regarding COVID-19-associated variants in individuals.

CONFLICT OF INTEREST

All the authors declare no conflict of interest.

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