ACE2 receptor polymorphism: Susceptibility to SARS-CoV-2, hypertension, multi-organ failure, and COVID-19 disease outcome

Christian Devaux, Jean-Marc Rolain, Didier Raoult

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Minireview

Full title: ACE2 receptor polymorphism : susceptibility to SARS-CoV-2, hypertension, multi-organ failure, and COVID-19 disease outcome

Short title: ACE2 polymorphism and COVID-19 disease

Christian A. Devaux a,b,c,*, Jean-Marc Rolain a,c, Didier Raoult a,c

a *Aix-Marseille Université, IRD, APHM, MEPHI, IHU–Méditerranée Infection, Marseille, France*

b *CNRS, Marseille, France*

c *IHU–Méditerranée Infection, 19–21 boulevard Jean Moulin, 13005 Marseille, France*

* Corresponding author. Present address: IHU–Méditerranée Infection, 19–21 Boulevard Jean Moulin, 13385 Marseille, France. Tel.: +33 4 13 73 20 51; fax: +33 4.13 73 20 52.

E-mail address: christian.devaux@mediterranee-infection.com (C.A. Devaux).

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Abstract

The severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), has emerged in Chinese people in December 2019 and has currently spread worldwide causing the COVID-19 pandemic with more than 150,000 deaths. In order for a SARS-CoV like virus circulating in wild life for a very long time to infect the index case-patient, a number of conditions must be met, foremost among which is the encounter with humans and the presence in *homo sapiens* of a cellular receptor allowing the virus to bind. Recently it was shown that the SARS-CoV-2 spike protein, binds to the human angiotensin I converting enzyme 2 (ACE2). This molecule is a peptidase expressed at the surface of lung epithelial cells and other tissues, that regulates the renin-angiotensin-aldosterone system. Humans are not equal with respect to the expression levels of the cellular ACE2. Moreover, ACE2 polymorphisms were recently described in human populations. Here we review the most recent evidence that ACE2 expression and/or polymorphism could influence both the susceptibility of people to SARS-CoV-2 infection and the outcome of the COVID-19 disease. Further exploration of the relationship between the virus, the peptidase function of ACE2 and the levels of angiotensin II in SARS-CoV-2 infected patients should help to better understand the pathophysiology of the disease and the multi-organ failures observed in severe COVID-19 cases, particularly heart failure.

Introduction

Over the past 20 years, seven coronaviruses responsible for more or less severe respiratory diseases have emerged in humans. Several of them, including SARS-CoV-2 (a *Betacoronavirus* lineage b/*Sarbecovirus*), can cause patients lung injury and sometimes multi-organ failure with adverse myocardial remodeling, myocardial stress, and cardiomyopathy [1,2] . Recently, SARS-CoV-2 was reported to be a human angiotensin I converting enzyme 2 (ACE2)-tropic virus [3,4] able to bind the alveolar pneumocytes which express ACE2 at their surface [5,6]. Yet, in humans the ACE2 mRNAs were found expressed in virtually all organs including the heart, blood vessels, kidney and testis, opening the possibility for this virus to infect other tissues beside lung [7,8]. ACE2 is a known peptidase that regulates the renin-angioten-aldosterone system (RAAS), thus controlling blood pressure. Therefore, it is not surprising that initials reports suggested that hypertension, diabetes and cardiovascular diseases were the most frequent comorbidity in COVID-19 disease [9].

The human coronaviruses

Coronaviruses (CoV) circulate in bats and generally pass over an intermediate animal host before crossing species barrier to infect humans[10]. Different species of bats in China carry genetically diverse coronaviruses, some of which are direct ancestors of SARS-CoV [11-13]. Indeed, the first SARS-CoV that caused a human outbreak derived from SARS-like CoV circulating in Chinese horseshoe *Rhinolophus* bats which apparently adapted to wild Himalayan palm-civet before spreading in humans [14]. The MERS-CoV originated from a *Pipistrellus* bat CoV and was probably transmitted to humans through contact with infected camels [15-17]. Soon after the first outbreak of SARS-CoV-2 in humans, it was reported that this new virus was related to a bat-borne coronavirus (BatCoV RaTG13) present in the *Rhinolophus affinis* bat species[18]. The identification of an intermediate animal hosts has been the subject of intense research and it was claimed that a pangolin (*Manis javanica*) was the intermediate host for SARS-CoV-2 [19]. The SARS-CoV-2 receptor ACE2 from bat and pangolin and several other species, were found to resemble that of human [20].

Before 2003, although human coronavirus 229E (HCoV-229E) (*Alphacoronavirus*) and HCoV-OC43 (*Betacoronavirus* lineage a) described in the 1960s were known to be agents of respiratory infections, they lent little attention. In the early 2000s, two other coronaviruses responsible for similar diseases were identified, the HCoV-NL63 (*Alphacoronavirus*) and

HCoV-HKU1 (*Betacoronavirus* lineage a). Even if the health authorities pay little attention to these viruses, sometimes they can cause deaths in people with fragile health. A study in Switzerland reported that among 279 subjects who had bronchoalveolar lavage for investigation of respiratory symptoms, 29 were tested positive for HCoV (detection rate: 10.4%) [21]. A large-scale polymerase chain reaction (PCR) screening of 11,661 nasal samples from European patients with respiratory disease, found 35 HCoV-229E (0.30%), 61 HCoV-HKU1 (0.52%), 75 HCoV-NL63 (0.64%), and 111 HCoV-OC43 (0.85%) [22]. A similar study in Africa on 5,573 nasal samples from child hospitalized for pneumonia found 114 HCoV-229E (2.05%), 163 HCoV-NL63 (2.93%), and 111 HCoV-OC43 (1.99%) [23]. Two Chinese studies involving almost 25,000 throat and nasal swab samples from patients with acute respiratory tract infections revealed 114 HCoV-229E (0.37%-0.57%), 61 HCoV-HKU1 (0.18%-0.33%), 104 HCoV-NL63 (0.33%-0.52%), and 523 HCoV-OC43 (1.36%-3.04%), respectively [24,25]. The fatality rate of the coronaviruses causing the common winter cold was estimated 0.5% to 1.5% [26].

Coronaviruses strongly gained in notoriety when SARS-CoV (*Betacoronavirus* lineage b) emerged in China in March 2003 and was proven responsible for the severe acute respiratory syndrome (SARS) outbreak in humans [27]. The SARS-CoV adapted to humans and became able to spread from person-to-person leading to a fatality rate of 9.6% in infected patients, causing global concern. The Middle East Respiratory Syndrome (MERS) caused by the MERS-CoV (*Betacoronavirus* lineage 2c), was reported in Saudi Arabia in 2012. This epidemic which has been one of the least deadly in absolute number of deaths, was the one which has created the most fears in health authorities and the most important panic in the populations due to its high fatality rate (case fatality rate of 34.7%) [28]. The SARS-CoV-2 that emerged in China at the end of 2019, is responsible for respiratory infections including pneumonia with a mortality rate estimated about 1%-2.5% [2], increasing with age and the existence of underlying diseases. Under chest computerized tomography (CT) scans, the majority of patients show bilateral ground glass-like opacities and subsegmental areas of consolidation indicative of SARS-CoV-2 induced pneumonia.

The MERS-CoV, SARS-CoV, SARS-CoV-2 and their cellular receptors

Already for SARS-CoV, it was demonstrated that this virus used the angiotensin I converting enzyme 2 (ACE2) to enter human cells [29]. The novel *Betacoronavirus* SARS-CoV-2 (formerly 2019-nCoV), that cause COVID-19 disease, has 79.5% nucleotide identity with

SARS-CoV [1]. It is worth noting that HCoV-NL63, SARS-CoV and SARS-CoV-2 spike proteins bind ACE2 [30] expressed at high levels in type I and II alveolar cells in the lung, whereas MERS-CoV bind the dipeptidyl peptidase 4 (DPP4)/CD26), a multifunctional serine peptidase known involved in T cell activation [31]. The analysis of SARS-CoV-2 spike (S) protein and ACE2 three-dimensional (3-D) structures allowed identification of regions in the peptidase domain of ACE2 required for viral spike binding [3]. Three very elegant papers published in the recent weeks characterized SARS-CoV-2 entry in target cells through interactions with ACE2 and serine protease TMPRSS2 priming as well as the 3-D structures involved in these interactions [3,32,33].

The human monocarboxypeptidase ACE-2 was originally cloned from human heart failure and lymphoma cDNA libraries [7]. Although the *ACE2* gene is usually considered silent in immune cells, the expression of ACE2 mRNAs was reported in a subset of CD14+ CD16- human monocytes [34]. ACE2 is also expressed by enterocytes of the small intestine and expected to regulate the expression of the gut antimicrobial peptides [35] . Moreover, this peptidase is also present on the arterial and venous endothelial cells, and arterial smooth muscle [36]. In normal human lung, the ACE2 protein is found on type I and II alveolar epithelial lung cells [37]. High expression of ACE2 was also reported on the epithelial cells of oral mucosa [38]. Single-cell RNA-seq analysis indicated that Asian men have a higher ACE2 mRNA expression in lung than women and that Asian people express higher amount of ACE2 than Caucasian and African American populations [39], but this observation remains controversial [40]. Until recently, the genetic basis of ACE2 expression in different populations remained largely unknown [41].

ACE2 structure and function

The *ACE2* gene span 39.98 kb of genomic DNA and contains 18 exons. It maps to chromosome X at position Xp22 [8]. It encodes a type I cell-surface glycoprotein of about 100kDa, composed by 805 amino acids and characterized by a N-terminal signal peptide of 17 amino acid residues, a peptidase domain (PD) (residues 19-615) with its HEXXH zinc binding metalloprotease motif, a C-terminal Collectrin (a regulator of renal amino acid transport and insulin)-like domain (CLD) (residues 616-768) that includes a ferredoxin-like fold "Neck" domain (615-726), that end with an hydrophobic transmembrane hydrophobic helix region of 22 amino acid residues followed by an intracellular segment of 43 amino acid

residues [7, 42]. The histidine motif HEXXH identified as an important component in a wide variety of zinc-dependent metalloproteases consists of five residues, the first histidine followed by glutamic acid being conserved, then the two variable amino acids and a final histidine [43]. Crystal structure analysis have suggested the presence of several hinge regions and N-glycosylations [44].

ACE2 belongs to the family of ACE members which have a wider tissues distribution. The juxtamembrane, transmembrane and cytoplasmic tail of ACE2 do not resemble ACE but these two proteins share the CLD region, a 220 amino-acid domain. Angiotensin converting enzymes (ACE) are zinc metallopeptidases. ACE, is a widely distributed protein of 170 kDa encoded by a 21 kb gene located on chromosome 17 (17q23) [45,46], that converts the inactive decapeptide, angiotensin (Ang) I to an active vasoconstrictor octapeptide Ang II [Asp-Arg-Val-Tyr-Ile-His-Pro-Phe] that controls the blood pressure [47-50], and through inactivation of bradykinin vasodilatator [51]. AngII also triggers the release of aldosterone that regulates the capacity of kidney to absorb sodium and water [52]. Moreover, Ang II stimulates DPP4 activity likely via the seven-transmembrane receptor (7TM) angiotensin II type A receptor (AT_1R)-mediated transactivation of epidermal growth factor receptor [53] and DPP4 inhibitors are described as a new class of anti-diabetic treatments the cardiovascular safety of which has been confirmed whereas their impact on hypertension is under evaluation [54]. Ang II also mediates cell proliferation by stimulating various cytokines [55]. ACE2, known for its diverse biological functions, including regulation of blood pressure through the renin-angiotensin-aldosterone system (RAAS), converts the octapeptide AngII to the heptapeptide Ang(1-7) by hydrolysis of the C-terminal residue. Ang(1-7) is expect to exert its action through the MAS-related (MAS1) G protein-coupled receptor (GPGR) [56,57]. In the pancreas ACE2 play an important glycemia-protective role [58]. Low ACE2 expression in the kidney is also associated with progressive renal diseases including diabetic nephropathy [59]. A soluble form of the catalytic ACE2 ectodomain can be released in the circulation following cleavage between amino acids 716 and 741 by sheddase ADAM10 and ADAM17 [60-62] . The transcriptional regulation of ACE2 is under the control of DNA-binding protein such as Sirtuin 1 (SIRT1) [63]. (**Figure 1A**).

ACE2 polymorphism and diseases

ACE2 limits the adverse vasoconstrictor and profibrotic effects of AngII. The hydrolysis of AngII into Ang (1-7) reduces the oxidative stress of AngII on endothelial cerebral arteries [64]. Ang(1-7) was reported to have vasodilatory and antifibrotic actions [65]. Disruption of ACE2 results in increased AngII levels and impaired cardiac function [66]. Reduced levels of cardiac ACE2 have been reported in hypertension (HT) and diabetic heart disease [67,68]. Low expression of ACE2 mRNA was associated to HT, dyslipidemia and/or heart failure [69].

A polymorphism of ACE2 gene was first documented in the Chinese population with three ACE2 variants (rs4240157, rs4646155, and rs4830542) associated with HT [70-74], in a Nicotine Dependence in Teens Canadian cohort rs2074192, rs233575, and rs2158083 mutations were significantly associated with pathological variations of blood pressure [75]. ACE2 rs21068809 mutation (C>T) has been reported associated with clinical manifestations of HT [76]. In Indian the study of 246 HT patients and 274 normotensive people indicated an association of HT with ACE2 rs21068809 mutation [77]. In Brazilian patients, the combination of ACE I/D and ACE2 G8790A polymorphisms revels susceptibility to HT [78]. The RAAS pathway can also be regulated by a polymorphism in ACE. In African-American with hypertension an ACE polymorphism was reported [79].

Very recently, Cao and colleagues reported the results of a large investigation (1700 variants) of coding sequences variants in *ACE2* and the allele frequency differences between populations in ACE2 gene from the China Metabolic Analytics Project and 1000 Genome Project database and other large scale genome databases [41]. They found one variant with a truncation Gln300 in China. In addition, they reported 32 variants among which seven hotspot variants in different populations.

Viral ACE2 receptor polymorphism and coronaviruses infection

It remains possible that *ACE2* gene polymorphism, human ACE2 mRNA expression and human ACE2 protein polymorphism influence SARS-CoV-2 susceptibility and COVID-19 disease outcome.

For more than two decades, in the field of the human immunodeficiency virus (HIV), a retrovirus transmitted by sexual intercourse, it was demonstrated that the binding of the gp120 viral envelope glycoprotein to the CD4 receptor [80,81], to CXCR4 [82,83] or CCR5 coreceptor [84],

triggers cell signaling. These molecules play a crucial role in the permanent molecular crosstalk between the cell and its environment. In this viral model, the study of the CCR5 co-receptor polymorphism clearly showed that a natural Δ32 deletion prevented the infection by HIV of homozygous people carrying this genotype [85, 86]. For the MERS-CoV, attachment of the spike (S) glycoprotein to human cells require the host cell typeII transmembrane protein dipeptidyl peptidase 4 (DPP4/CD26) [87,88]. Following interaction with DPP4, the S protein of MERS-CoV undergoes proteolytic activation through the cellular serine protease TMPRSS2 and cysteine protease cathepsin L once inside endosomes [89]. Soluble forms of DPP4 can be released in the blood circulation after cleavage by the kallikrein-related peptidase 5 (KLK5) [90]. It was recently reported that among fourteen characterized mutants forms of DPP4, four polymorphisms (K267E, K267N, A291P and Δ346-348) strongly reduce the binding and penetration of MERS-CoV into target cells and the viral replication [91].

Regarding SARS-CoV, the S1 domain of the spike protein mediates ACE2 receptor binding whereas the S2 domain is a membrane-associated portion that likely undergoes post-binding transconformational modifications allowing membrane fusion. The viral receptor binding domain (RBD) located in S1 has been narrowed down to amino acid residues 318 to 510 [92]. A co-crystal structure of ACE2 to the RBD revealed that residues 424 to 494 are involved in direct contact with the first α-helix and Lys353 and proximal residues at the N-terminus of β-sheet 5 of ACE2 [93]. By altering the His353 amino acid in rat ACE2 and modifying a glycosylation site (Asp 90) that may alter the conformation of the α-helix 1 of ACE2, Li and colleagues [93] converted the rat ACE2 into an efficient receptor for SARS-CoV. A point mutation Leu584Ala in ACE2, markedly attenuated the shedding of the enzyme and facilitated SARS-CoV entry into target cells [61]. A soluble form of ACE2 lacking the cytoplasmic and transmembrane domain of the molecule was reported capable of blocking binding of SARS-CoV spike protein to ACE2 [94]. Expression of ACE2 was found down regulated in cells infected by SARS-CoV [95]. A recombinant SARS-CoV spike protein was found to down regulated ACE2 expression through release of sACE2 and thereby promotes lung injury [96]. Among other antiviral effect of Chloroquine on SARS-CoV *in vitro* one could be attributable to a deficit in the glycosylation of the ACE2 virus cell surface receptor [97, 98]. Regarding the HCoV-NL63 that also employ ACE2 for cell entry a recombinant SARS-CoV/HCoV-NL63 spike protein trigger shedding of sACE2 [99].

Very recently, investigation of SARS-CoV-2 cell entry through ACE2 binding showed important commonalities between SARS-CoV and SARS-CoV-2 infection, including similar

choice of entry receptors [32]. SARS-CoV and SARS-CoV-2 share about 76% amino acids identity and most amino acid residues essential for ACE2 binding were conserved in the SARS-CoV-2 spike S1 domain. Another recent paper published reported the structural basis of SARS-CoV2 interaction with ACE2 [3]. The trimeric SARS-CoV-2 S1 spike binds the PD domain of ACE2 and the cleavage of ACE2 C-terminal segment (residues 697 to 716) by the transmembrane protease serine 2 (TMPRSS2) enhances the S-protein-driven viral entry. By comparing the 805 amino acid residues of the 10 human ACE2 proteins and the 4 different ACE2 isoforms available through GeneBank using Clustal Omega multiple sequence alignment, a 100% identity among the complete ACE2 sequences was observed and the isoforms corresponded to a deletion in the CLD domain, or truncation in the transmembrane domain. The role of these isoforms in SARS-CoV-2 infection and COVID-19 outcome, remains speculative. According to the recent work by Cao and colleagues [41], 32 variants of ACE2 where characterized among which seven hotspot variants (Lys26Arg, Ile486Val, Ala627Val, Asn638Ser, Ser692Pro, Asn720Asp, and Leu731Ile/Phe) in different populations (**Figure 1B**). This open the possibility that some people could be less susceptible to SARS-CoV-2 infection than others.

Discussion

ACE2 protein at the surface of lung alveolar epithelial cells allows infection of the respiratory tract with SARS-CoV-2. It can be hypothesized that the ACE2 levels correlate with susceptibility to SARS-CoV-2 infection. Apparently, men have a higher ACE2 expression in lung than women and Asian people express ACE2 higher than Caucasian and African American populations [37]. This is in agreement with the finding that conversion of Ang II to Ang (1-7) by ACE2 was higher in males than female [100], suggesting an over-expression of ACE2 in men. Because ACE2 is encoded by a gene located on the X chromosome and men express more ACE2 than women it could be speculated that depending the allele expressed by women, they could be considered of lower sensitivity against the most severe adverse effects of the infection [99, 101]. All clinical reports published to date indicate that men represent between 66% and 75% of the most severe cases of COVID-19. During early SARS-CoV-2 infection and viral spread within body tissues, the ACE2 function is likely impaired either by steric hindrance of the peptidase domain of ACE2 following virus binding or by down

regulation of ACE2 mRNA expression and ACE2 protein. In severe COVID-19 disease, the presence of the viral receptor on other tissues than lung may explain the multi-organ failure sometimes observed in clinic. We therefore suggest that quantification of ACE2 and AngII be added to the COVID-19 patients biological monitoring.

It is known that ACE2 can shift the RAAS balance by conversion of Ang II to Ang (1-7). Consequently, HT and COVID-19 recently become a question of concern for international professional societies of cardiology regarding: i) the susceptibility of patients with HT to get COVID-19; ii) the severity of the disease; and, iii) the use of ACE inhibitors (ACEi) and AngII receptor blockers (ARBs, that targets the AT_1R). It is known that HT inhibitors increase the cell-surface expression of ACE2. It was demonstrated that ACEi can increase intestinal ACE2 mRNA expression [102]. Although data are lacking regarding the effects of such drugs on ACE2 mRNA expression in lung epithelial cells, there is a concern that patients taking those treatments can favor virus capture. In patients with HT who received long-term olmesartan (ARB) treatment, urinary ACE2 levels were higher than among untreated control patients [103]. In contrast to HT, in patients suffering from idiopathic pulmonary fibrosis, the expression levels of ACE2 are markedly decreased [30]. ACE2 is a major actor toward resolution of inflammation and fibrosis [104]. In an animal model of bleomycin-induced pulmonary fibrosis, treatment with intraperitoneal injection of recombinant human ACE2 improved the lung function and decreased lung inflammation and fibrosis [105]. Moreover, impaired phosphorylation of ACE2 Ser680 by AMP-activated protein kinase in pulmonary endothelium leads to a labile ACE2 and hence pulmonary HT [106]. We must also paid attention to molecules such as xanthenone (XNT) and dimiazene aceturate (DIZE, an anti-trypanosomal drug) described as ACE2 activators [107]. In a rat model of ischemic heart disease, the subcutaneous infusion of DIZE significantly increased cardiac ACE2 mRNA expression and ACE2 protein catalytic activity, reduced ACE mRNA expression, and improved cardiac remodeling [108]. The possible beneficial properties of other molecules such as exenatide (a glucagon-like peptide-1 agonist) which induces an increase in vasodilatory and a decrease in vasoconstrictive mediators must also be investigated [109] . In addition, it was recently reported that heparin (anticoagulant) treatment is associated with decreased mortality in severe COVID-19 patients with coagulopathy [110].

In a Chinese cohort of 1,099 patients with COVID-19, 165 (13%) individuals were patients with HT, among which 24% suffered from severe COVID-19, a percentage of 3.7%, slightly higher to that of the general population of COVID-19 patients [111]. In a smaller cohort of 191

patients with COVID-19, 58 (30%) were patients with hypertension and 48% of them died, which is surprisingly high percentage (14.6%) [112]. These results suggest that the prevalence of patients with HT was higher in patients who developed severe COVID-19 disease than those who do not. By mid march 2020, the international professional societies of cardiology recommended continuing patients' treatment [113]. Indeed, when the SARS-CoV-2 spike binds its ACE2 receptor in the α helix 1 (Lys31, Tyr 41) and β5 region (Lys353) it likely reduces the catalytic properties of ACE2 that is usually associated with reduced inflammation. The lack of Ang(1-7) generation may increase lung injury [114, 115] and cardiovascular risks, Ang II acting like an inflammatory cytokine [116]. In a murine model it was observed that lung inflammation aggravates AngII-induced induced abdominal aortic aneurysms [117].

Mutations might modify the expression level of ACE2 protein as shown in a murine model [118]. The deletion of ACE2 in mice model was associated with increased circulation and tissue AngII levels and led to cardiovascular damage [119, 120]. It remain possible that i) mutations affecting the human *ACE2* gene; ii) transcriptional variation in ACE2 mRNA expression; iii) post-transcriptional modifications that act on the ACE2 viral receptor (such as N-glycosylation), and; iv) putative ACE2 protein mutations, may influence the outcome of COVID-19 by acting on blood pressure through the RAAS and possible increasing of lung and heart damages through the oxidative stress triggered by Ang II. Recently an high rate fatality of SARS-CoV-2 was reported in Iran [121] , without satisfactory explanation. If underreporting of the number of infected people can be excluded, it could be hypothesized: i) a more aggressive variant clade of SARS-CoV-2 [122] ; ii) a variation in *ACE2*; or, iii) a variation in genes like those encoding Toll-like receptors. Since it is known that Ang(1-7) prevents inflammation by inhibiting the resistin/ Toll-like receptor 4 (TLR4)/MAPK/NF-kB pathway [123] and that there is a high variability of the TLR4 gene in different ethnic groups in Iran [124], it remains possible that SARS-CoV-2 triggers increased inflammation in Iranian patients by suppressing the ACE2-mediated metabolism of AngII to Ang(1-7). This could be related to the observation that mice deficient in the TLR3/TLR4 adaptor TRIF are highly susceptible to SARS-CoV infection including severe inflammatory induction [125].

The mechanism of acute myocardial injury caused by SARS-CoV-2 during severe COVD-19 disease might be related to the inhibition of ACE2 catalytic activity [126]. (**Figure 2**). Interestingly, a recent study posted as a pre-print paper[127] pointed out a list of 97 approved drugs that may have a therapeutic potential against COVID-19 including anti-diabetics (metaformin), statins (simvastatin) and ARBs (sartans). Medical records of patients currently

treated with these compounds may help to identify whether those drugs have a beneficial or adverse effect on COVID-19 patients. Metaformin was also identified as a potential drug-repurposing against SARS-CoV-2 in another study [128]. It should be remembered that a large number of data suggest that there is a mild or severe cytokine storm in severe COVID-19 patients which is an important cause of death. To reduce the pro-inflammatory effect of AngII and the cytokine storm observed in severe cases of COVID-19, it might make sense to continue treating patients with ACE inhibitors and ARBs, a conclusion shared by recent recommendations of the international societies of cardiology[129]. However, Fang and colleagues[115] recently reported that the most distinctive comorbidities in patients who died from COVID-19 are HT, coronary heart diseases, cerebrovascular diseases and diabetes, and among them several were treated by ACE inhibitors. How should clinicians navigate this uncertainty for patients who are taking ACE inhibitors and ARBs and become infected with SARS-CoV-2? Do these molecules have a harmful effect in the outcome of the disease or is the link that is made highlights only a confounding factor which confirms that HT is a major factor of comorbidity? In agreement with others [130-132] , we consider that it is of special importance to rapidly evaluate whether these drugs are more beneficial than harmful in severe COVID-19 patients.

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Competing Interests

CD declare a link of interest with the Sanofi and Merck pharmaceutical companies. JMR and DR declare that they have no competing interests.

Ethical Approval

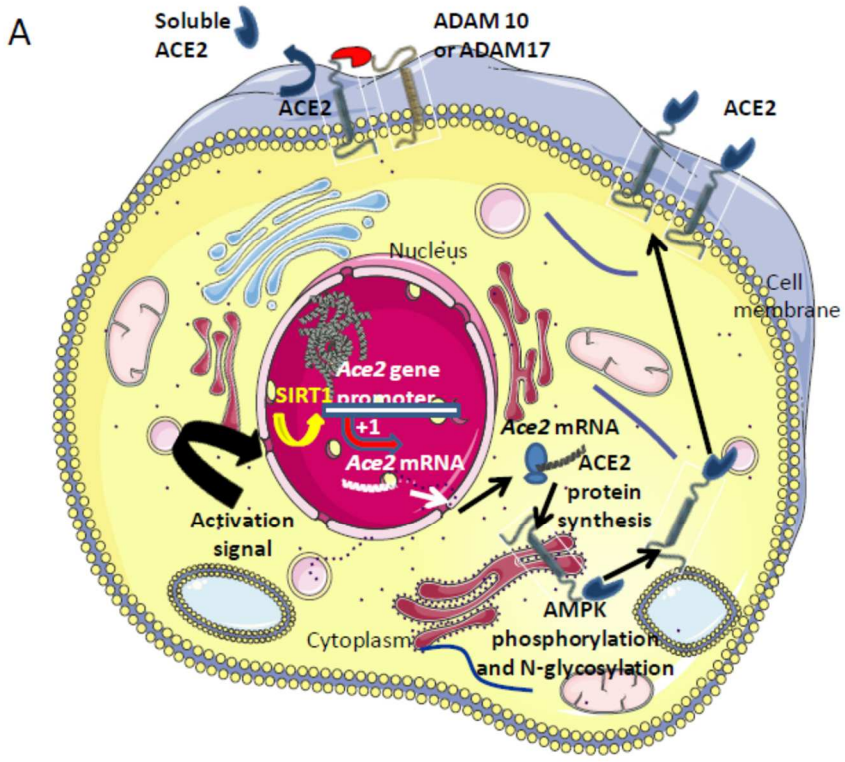
Not required

Figure legends

Figure 1

1A. Schematic representation of the regulation of ACE2. The transcription of the *Ace2* gene is under control of the SIRT1 DNA-binding protein that binds the *Ace2* gene promotor. Post-transcriptional regulation by miRNA (miRNA143, miRNA421) could occur (not shown). Following translation the newly synthesized ACE2 proteins are likely target of post-transcriptional modifications such as phosporylation of Ser680 by AMPK that enhances the stability of ACE2, and N-glycosylations. Once expressed at the cell membrane the ACE2 protein can be regulated by sheddases (ADAM10, ADAM17) that cleave the ACE2 extracellular domain and release a circulating soluble form sACE2 capable to interact with integrins (ITGB1).

1B. Schematic representation (left) of the ACE2 molecule and its major domains. The amino acids position is in black. Some of the amino acids important for viral tropism are in red (previous studies showed that residues 31, 41, and regions 82-84 and 353-357 are important for viral spike binding). Clustal Omega multiple sequence alignment (EMBL-EBI bioinformatic tool; Copyright © EMBL 2020) of human ACE2 and its different isoforms (right). The comparison of the reference *Homo sapiens* ACE2 protein sequence (S1=Genbank: BAB40370.1) with 9 others ACE2 sequences from the NCBI reference sequence database (S2=UniProtKB Q9BYF1.2; S3=NCBI NP_001358344.1; S4=NCBI NP_068576.1; S5= GenBank EAW98892.1; S6= GenBank AAH48094.2; S7= GenBank AAH39902.1; S8= GenBank AAO25651.1; S9= GenBank BAD99267.1; GenBank AAF99721.1), showed 100% amino acids identity (not shown). The Clustal MSA was also used for the comparison of the human ACE2 S1 sequence and available sequences of ACE2 isoforms: the isoform X1 (I1)= NCBI XP_011543851.1; isoform X2 (I2)= NCBI XP_011543853.1; isoform X3(I3)=NCBI XP_011543854.1; isoform CRA (I4)=GenBank EAW98891.1. The figure illustrates that these isoforms correspond to deletions in the CLD domain, or truncations in the transmembrane domain. A very elegant work by Cao and colleagues[42] has recently analyzed 1700 *ACE2* variants in search of ACE2 protein polymorphism. The mutations and truncations found by this team are shown in light blue.


A
Soluble ACE2
ADAM 10 or ADAM17
ACE2
ACE2
Nucleus
Cell membrane
Ace2 gene promoter
SIRT1
+1
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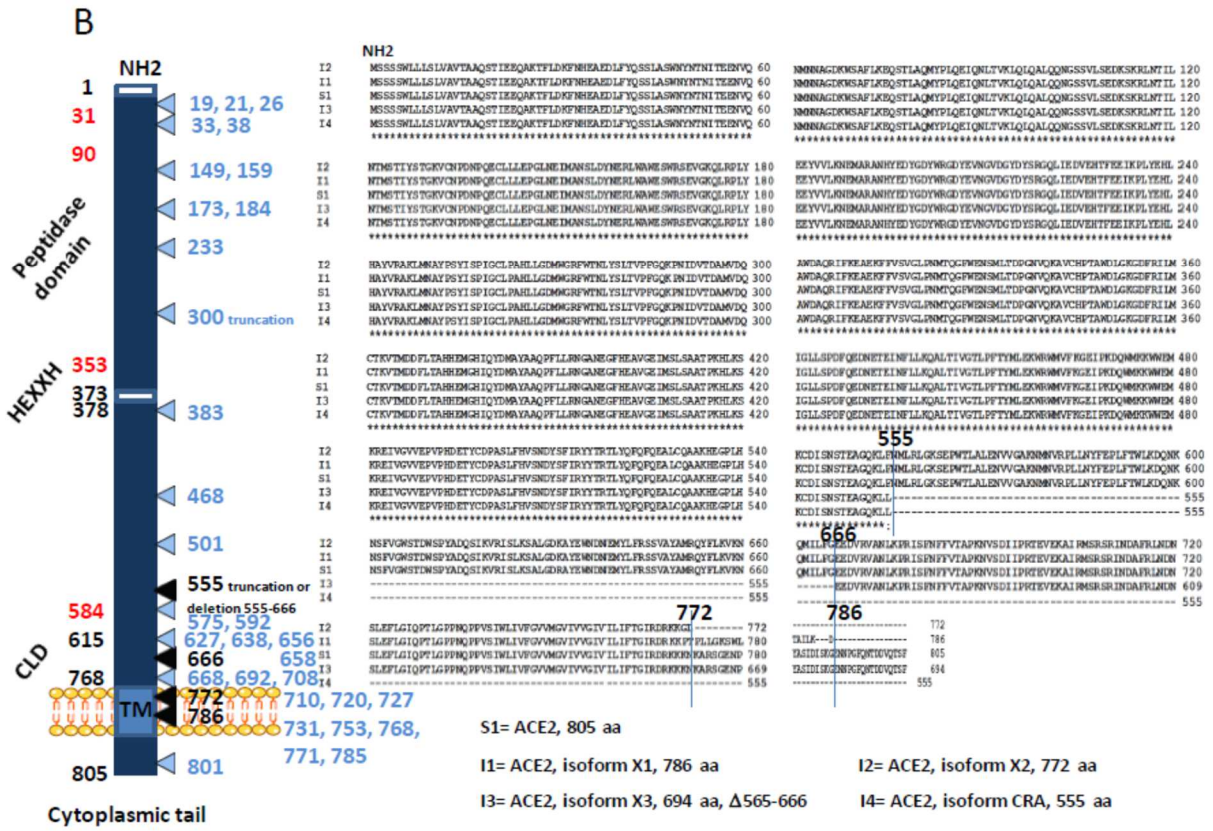
Ace2 mRNA
ACE2 protein synthesis
Activation signal
AMPK phosphorylation and N-glycosylation
Cytoplasm

451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31
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353
373
378
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615
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Peptidase domain
HEXXH
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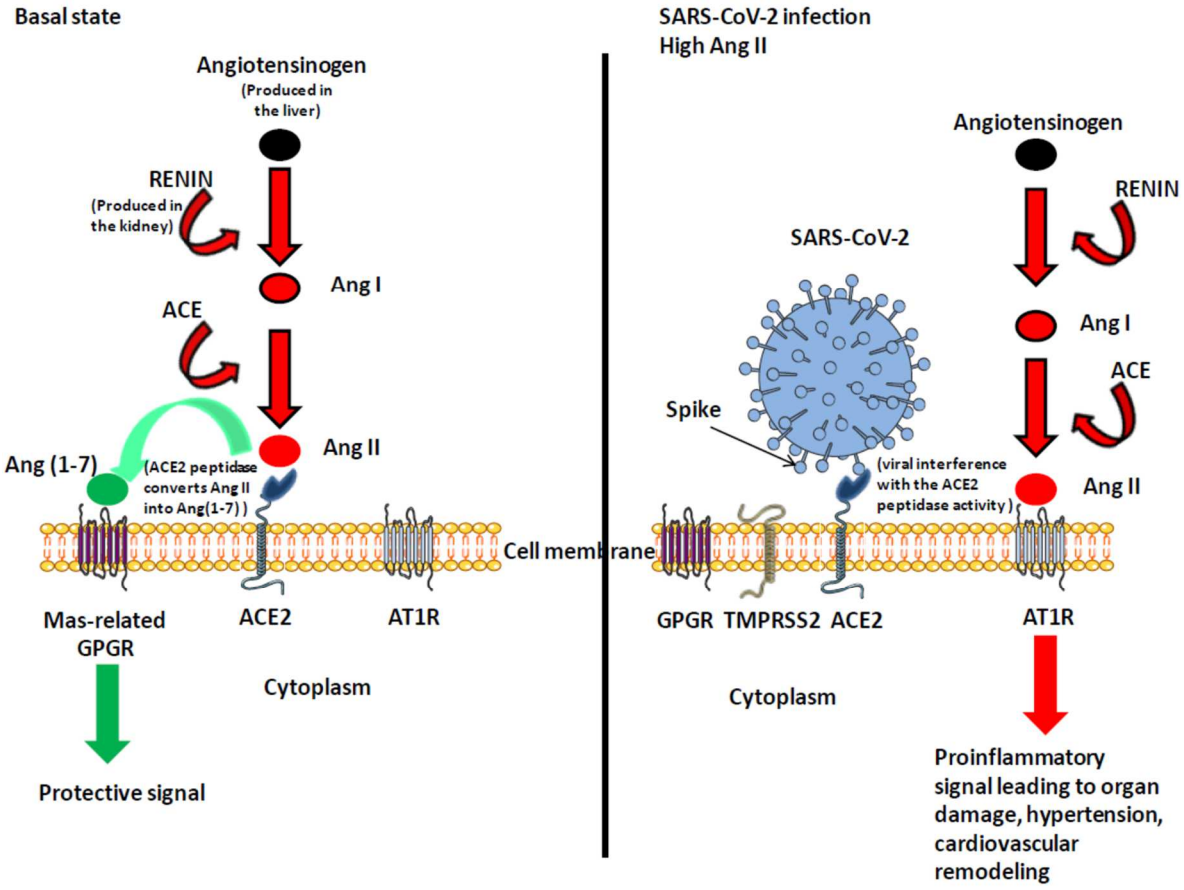
Cytoplasmic tail
19, 21, 26
33, 38
149, 159
173, 184
233
300 truncation
383
468
501
555 truncation or deletion 555-666
575, 592
627, 638, 656
666
658
668, 692, 708
772
786
710, 720, 727
731, 753, 768, 771, 785
801
S1= ACE2, 805 aa
I1= ACE2, isoform X1, 786 aa
I2= ACE2, isoform X2, 772 aa
I3= ACE2, isoform X3, 694 aa, Δ565-666
I4= ACE2, isoform CRA, 555 aa

452

453

Figure 2

Simplified diagram of the renin-angiotensin system in normal and pathologic conditions. The left panel indicates that ACE2 converts Ang II to Ang(1-7) leading to protective signal. The right panel illustrates the possible dysfunction of signals when SARS-CoV-2 is attached to its ACE2 receptor. Under this condition Ang(1-7) is no longer synthetized, Ang II accumulates and binds AT1R, leading to proinflammatory signals that trigger both tissues damage (in particular lung and heart) and hypertension.



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