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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

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33 **Abstract**

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

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62 **Introduction**

63 Over the past 20 years, seven coronaviruses responsible for more or less severe respiratory
64 diseases have emerged in humans. Several of them, including SARS-CoV-2 (a
65 *Betacoronavirus* lineage b/*Sarbecovirus*), can cause patients lung injury and sometimes multi-
66 organ failure with adverse myocardial remodeling, myocardial stress, and cardiomyopathy^{1,2}.
67 Recently, SARS-CoV-2 was reported to be a human angiotensin I converting enzyme 2
68 (ACE2)-tropic virus^{3,4} able to bind the alveolar pneumocytes which express ACE2 at their
69 surface^{5,6}. Yet, in humans the ACE2 mRNAs were found expressed in virtually all organs
70 including the heart, blood vessels, kidney and testis, opening the possibility for this virus to
71 infect other tissues beside lung^{7,8}. ACE2 is a known peptidase that regulates the renin-
72 angioten-aldosterone system (RAAS), thus controlling blood pressure. Therefore, it is not
73 surprising that initials reports suggested that hypertension, diabetes and cardiovascular
74 diseases were the most frequent comorbidity in COVID-19 disease⁹.

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77 **The human coronaviruses**

78 Coronaviruses (CoV) circulate in bats and generally pass over an intermediate animal host
79 before crossing species barrier to infect humans¹⁰. Different species of bats in China carry
80 genetically diverse coronaviruses, some of which are direct ancestors of SARS-CoV¹¹⁻¹³.
81 Indeed, the first SARS-CoV that caused a human outbreak derived from SARS-like CoV
82 circulating in Chinese horseshoe *Rhinolophus* bats which apparently adapted to wild
83 Himalayan palm-civet before spreading in humans¹⁴. The MERS-CoV originated from a
84 *Pipistrellus* bat CoV and was probably transmitted to humans through contact with infected
85 camels¹⁵⁻¹⁷. Soon after the first outbreak of SARS-CoV-2 in humans, it was reported that this
86 new virus was related to a bat-borne coronavirus (BatCoV RaTG13) present in the
87 *Rhinolophus affinis* bat species¹⁸. The identification of an intermediate animal hosts has been
88 the subject of intense research and it was claimed that a pangolin (*Manis javanica*) was the
89 intermediate host for SARS-CoV-2¹⁹. The SARS-CoV-2 receptor ACE2 from bat and
90 pangolin and several other species, were found to resemble that of human²⁰.

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

95 HCoV-HKU1 (*Betacoronavirus* lineage a). Even if the health authorities pay little attention to
96 these viruses, sometimes they can cause deaths in people with fragile health. A study in
97 Switzerland reported that among 279 subjects who had bronchoalveolar lavage for
98 investigation of respiratory symptoms, 29 were tested positive for HCoV (detection rate:
99 10.4%)²¹. A large-scale polymerase chain reaction (PCR) screening of 11,661 nasal samples
100 from European patients with respiratory disease, found 35 HCoV-229E (0.30%), 61 HCoV-
101 HKU1 (0.52%), 75 HCoV-NL63 (0.64%), and 111 HCoV-OC43 (0.85%)²². A similar study
102 in Africa on 5,573 nasal samples from child hospitalized for pneumonia found 114 HCoV-
103 229E (2.05%), 163 HCoV-NL63 (2.93%), and 111 HCoV-OC43 (1.99%)²³. Two Chinese
104 studies involving almost 25,000 throat and nasal swab samples from patients with acute
105 respiratory tract infections revealed 114 HCoV-229E (0.37%-0.57%), 61 HCoV-HKU1
106 (0.18%-0.33%), 104 HCoV-NL63 (0.33%-0.52%), and 523 HCoV-OC43 (1.36%-3.04%),
107 respectively^{24,25}. The fatality rate of the coronaviruses causing the common winter cold was
108 estimated 0.5% to 1.5%²⁶.

109 Coronaviruses strongly gained in notoriety when SARS-CoV (*Betacoronavirus* lineage b)
110 emerged in China in March 2003 and was proven responsible for the severe acute respiratory
111 syndrome (SARS) outbreak in humans²⁷. The SARS-CoV adapted to humans and became
112 able to spread from person-to-person leading to a fatality rate of 9.6% in infected patients,
113 causing global concern. The Middle East Respiratory Syndrome (MERS) caused by the
114 MERS-CoV (*Betacoronavirus* lineage 2c), was reported in Saudi Arabia in 2012. This
115 epidemic which has been one of the least deadly in absolute number of deaths, was the one
116 which has created the most fears in health authorities and the most important panic in the
117 populations due to its high fatality rate (case fatality rate of 34.7%)²⁸. The SARS-CoV-2 that
118 emerged in China at the end of 2019, is responsible for respiratory infections including
119 pneumonia with a mortality rate estimated about 1%-2.5%², increasing with age and the
120 existence of underlying diseases. Under chest computerized tomography (CT) scans, the
121 majority of patients show bilateral ground glass-like opacities and subsegmental areas of
122 consolidation indicative of SARS-CoV-2 induced pneumonia.

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

126 Already for SARS-CoV, it was demonstrated that this virus used the angiotensin I converting
127 enzyme 2 (ACE2) to enter human cells²⁹. The novel *Betacoronavirus* SARS-CoV-2
128 (formerly 2019-nCoV), that cause COVID-19 disease, has 79.5% nucleotide identity with

129 SARS-CoV¹. It is worth noting that HCoV-NL63, SARS-CoV and SARS-CoV-2 spike
130 proteins bind ACE2³⁰ expressed at high levels in type I and II alveolar cells in the lung,
131 whereas MERS-CoV bind the dipeptidyl peptidase 4 (DPP4)/CD26), a multifunctional serine
132 peptidase known involved in T cell activation³¹. The analysis of SARS-CoV-2 spike (S)
133 protein and ACE2 three-dimensional (3-D) structures allowed identification of regions in the
134 peptidase domain of ACE2 required for viral spike binding³. Three very elegant papers
135 published in the recent weeks characterized SARS-CoV-2 entry in target cells through
136 interactions with ACE2 and serine protease TMPRSS2 priming as well as the 3-D structures
137 involved in these interactions^{3,32,33}.

138 The human monocarboxypeptidase ACE-2 was originally cloned from human heart failure
139 and lymphoma cDNA libraries⁷. Although the *ACE2* gene is usually considered silent in
140 immune cells, the expression of ACE2 mRNAs was reported in a subset of CD14+ CD16-
141 human monocytes³⁴. ACE2 is also expressed by enterocytes of the small intestine and
142 expected to regulate the expression of the gut antimicrobial peptides³⁵. Moreover, this
143 peptidase is also present on the arterial and venous endothelial cells, and arterial smooth
144 muscle³⁶. In normal human lung, the ACE2 protein is found on type I and II alveolar
145 epithelial lung cells³⁷. High expression of ACE2 was also reported on the epithelial cells of
146 oral mucosa³⁸. Single-cell RNA-seq analysis indicated that Asian men have a higher ACE2
147 mRNA expression in lung than women and that Asian people express higher amount of ACE2
148 than Caucasian and African American populations³⁹, but this observation remains
149 controversial⁴⁰. Until recently, the genetic basis of ACE2 expression in different populations
150 remained largely unknown⁴¹.

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153 **ACE2 structure and function**

154 The *ACE2* gene span 39.98 kb of genomic DNA and contains 18 exons. It maps to
155 chromosome X at position Xp22⁸. It encodes a type I cell-surface glycoprotein of about
156 100kDa, composed by 805 amino acids and characterized by a N-terminal signal peptide of 17
157 amino acid residues, a peptidase domain (PD) (residues 19-615) with its HEXXH zinc
158 binding metalloprotease motif, a C-terminal Collectrin (a regulator of renal amino acid
159 transport and insulin)-like domain (CLD) (residues 616-768) that includes a ferredoxin-like
160 fold "Neck" domain (615-726), that end with an hydrophobic transmembrane hydrophobic
161 helix region of 22 amino acid residues followed by an intracellular segment of 43 amino acid

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

167 ACE2 belongs to the family of ACE members which have a wider tissues distribution. The
168 juxtamembrane, transmembrane and cytoplasmic tail of ACE2 do not resemble ACE but these
169 two proteins share the CLD region, a 220 amino-acid domain. Angiotensin converting
170 enzymes (ACE) are zinc metallopeptidases. ACE, is a widely distributed protein of 170 kDa
171 encoded by a 21 kb gene located on chromosome 17 (17q23) ^{45,46}, that converts the inactive
172 decapeptide, angiotensin (Ang) I to an active vasoconstrictor octapeptide Ang II [Asp-Arg-
173 Val-Tyr-Ile-His-Pro-Phe] that controls the blood pressure ⁴⁷⁻⁵⁰, and through inactivation of
174 bradykinin vasodilator ⁵¹. AngII also triggers the release of aldosterone that regulates the
175 capacity of kidney to absorb sodium and water ⁵². Moreover, Ang II stimulates DPP4 activity
176 likely via the seven-transmembrane receptor (7TM) angiotensin II type A receptor (AT₁R)-
177 mediated transactivation of epidermal growth factor receptor ⁵³ and DPP4 inhibitors are
178 described as a new class of anti-diabetic treatments the cardiovascular safety of which has
179 been confirmed whereas their impact on hypertension is under evaluation ⁵⁴. Ang II also
180 mediates cell proliferation by stimulating various cytokines ⁵⁵. ACE2, known for its diverse
181 biological functions, including regulation of blood pressure through the renin-angiotensin-
182 aldosterone system (RAAS), converts the octapeptide AngII to the heptapeptide Ang(1-7) by
183 hydrolysis of the C-terminal residue. Ang(1-7) is expect to exert its action through the MAS-
184 related (MAS1) G protein-coupled receptor (GPGR) ^{56,57}. In the pancreas ACE2 play an
185 important glycemia-protective role ⁵⁸. Low ACE2 expression in the kidney is also associated
186 with progressive renal diseases including diabetic nephropathy ⁵⁹. A soluble form of the
187 catalytic ACE2 ectodomain can be released in the circulation following cleavage between
188 amino acids 716 and 741 by sheddase ADAM10 and ADAM17 ⁶⁰⁻⁶². The transcriptional
189 regulation of ACE2 is under the control of DNA-binding protein such as Sirtuin 1 (SIRT1) ⁶³.
190 **(Figure 1A).**

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195 **ACE2 polymorphism and diseases**

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

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

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

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220 **Viral ACE2 receptor polymorphism and coronaviruses infection**

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

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

227 triggers cell signaling. These molecules play a crucial role in the permanent molecular
228 crosstalk between the cell and its environment. In this viral model, the study of the CCR5 co-
229 receptor polymorphism clearly showed that a natural $\Delta 32$ deletion prevented the infection by
230 HIV of homozygous people carrying this genotype^{85, 86}. For the MERS-CoV, attachment of
231 the spike (S) glycoprotein to human cells require the host cell typeII transmembrane protein
232 dipeptidyl peptidase 4 (DPP4/CD26)^{87,88}. Following interaction with DPP4, the S protein of
233 MERS-CoV undergoes proteolytic activation through the cellular serine protease TMPRSS2
234 and cysteine protease cathepsin L once inside endosomes⁸⁹. Soluble forms of DPP4 can be
235 released in the blood circulation after cleavage by the kallikrein-related peptidase 5 (KLK5)
236⁹⁰. It was recently reported that among fourteen characterized mutants forms of DPP4, four
237 polymorphisms (K267E, K267N, A291P and $\Delta 346-348$) strongly reduce the binding and
238 penetration of MERS-CoV into target cells and the viral replication⁹¹.

239 Regarding SARS-CoV, the S1 domain of the spike protein mediates ACE2 receptor binding
240 whereas the S2 domain is a membrane-associated portion that likely undergoes post-binding
241 transconformational modifications allowing membrane fusion. The viral receptor binding
242 domain (RBD) located in S1 has been narrowed down to amino acid residues 318 to 510⁹². A
243 co-crystal structure of ACE2 to the RBD revealed that residues 424 to 494 are involved in
244 direct contact with the first α -helix and Lys353 and proximal residues at the N-terminus of β -
245 sheet 5 of ACE2⁹³. By altering the His353 amino acid in rat ACE2 and modifying a
246 glycosylation site (Asp 90) that may alter the conformation of the α -helix 1 of ACE2, Li and
247 colleagues⁹³ converted the rat ACE2 into an efficient receptor for SARS-CoV. A point
248 mutation Leu584Ala in ACE2, markedly attenuated the shedding of the enzyme and
249 facilitated SARS-CoV entry into target cells⁶¹. A soluble form of ACE2 lacking the
250 cytoplasmic and transmembrane domain of the molecule was reported capable of blocking
251 binding of SARS-CoV spike protein to ACE2⁹⁴. Expression of ACE2 was found down
252 regulated in cells infected by SARS-CoV⁹⁵. A recombinant SARS-CoV spike protein was
253 found to down regulated ACE2 expression through release of sACE2 and thereby promotes
254 lung injury⁹⁶. Among other antiviral effect of Chloroquine on SARS-CoV *in vitro* one could
255 be attributable to a deficit in the glycosylation of the ACE2 virus cell surface receptor^{97, 98}.
256 Regarding the HCoV-NL63 that also employ ACE2 for cell entry a recombinant SARS-
257 CoV/HCoV-NL63 spike protein trigger shedding of sACE2⁹⁹.
258 Very recently, investigation of SARS-CoV-2 cell entry through ACE2 binding showed
259 important commonalities between SARS-CoV and SARS-CoV-2 infection, including similar

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

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278 **Discussion**

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

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

296 It is known that ACE2 can shift the RAAS balance by conversion of Ang II to Ang (1-7).
297 Consequently, HT and COVID-19 recently become a question of concern for international
298 professional societies of cardiology regarding: i) the susceptibility of patients with HT to get
299 COVID-19; ii) the severity of the disease; and, iii) the use of ACE inhibitors (ACEi) and
300 AngII receptor blockers (ARBs, that targets the AT₁R). It is known that HT inhibitors
301 increase the cell-surface expression of ACE2. It was demonstrated that ACEi can increase
302 intestinal ACE2 mRNA expression ¹⁰². Although data are lacking regarding the effects of
303 such drugs on ACE2 mRNA expression in lung epithelial cells, there is a concern that patients
304 taking those treatments can favor virus capture. In patients with HT who received long-term
305 olmesartan (ARB) treatment, urinary ACE2 levels were higher than among untreated control
306 patients ¹⁰³. In contrast to HT, in patients suffering from idiopathic pulmonary fibrosis, the
307 expression levels of ACE2 are markedly decreased ³⁰. ACE2 is a major actor toward
308 resolution of inflammation and fibrosis ¹⁰⁴. In an animal model of bleomycin-induced
309 pulmonary fibrosis, treatment with intraperitoneal injection of recombinant human ACE2
310 improved the lung function and decreased lung inflammation and fibrosis ¹⁰⁵. Moreover,
311 impaired phosphorylation of ACE2 Ser680 by AMP-activated protein kinase in pulmonary
312 endothelium leads to a labile ACE2 and hence pulmonary HT ¹⁰⁶. We must also paid attention
313 to molecules such as xanthenone (XNT) and dimiazene aceturate (DIZE, an anti-
314 trypanosomal drug) described as ACE2 activators ¹⁰⁷. In a rat model of ischemic heart disease,
315 the subcutaneous infusion of DIZE significantly increased cardiac ACE2 mRNA expression
316 and ACE2 protein catalytic activity, reduced ACE mRNA expression, and improved cardiac
317 remodeling ¹⁰⁸. The possible beneficial properties of other molecules such as exenatide (a
318 glucagon-like peptide-1 agonist) which induces an increase in vasodilatory and a decrease
319 in vasoconstrictive mediators must also be investigated ¹⁰⁹. In addition, it was recently
320 reported that heparin (anticoagulant) treatment is associated with decreased mortality in
321 severe COVID-19 patients with coagulopathy ¹¹⁰.

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

325 patients with COVID-19, 58 (30%) were patients with hypertension and 48% of them died,
326 which is surprisingly high percentage (14.6%)¹¹². These results suggest that the prevalence of
327 patients with HT was higher in patients who developed severe COVID-19 disease than those
328 who do not. By mid march 2020, the international professional societies of cardiology
329 recommended continuing patients' treatment¹¹³. Indeed, when the SARS-CoV-2 spike binds
330 its ACE2 receptor in the α helix 1 (Lys31, Tyr 41) and β 5 region (Lys353) it likely reduces
331 the catalytic properties of ACE2 that is usually associated with reduced inflammation. The
332 lack of Ang(1-7) generation may increase lung injury^{114, 115} and cardiovascular risks, Ang II
333 acting like an inflammatory cytokine¹¹⁶. In a murine model it was observed that lung
334 inflammation aggravates AngII-induced induced abdominal aortic aneurysms¹¹⁷.

335 Mutations might modify the expression level of ACE2 protein as shown in a murine model
336¹¹⁸. The deletion of ACE2 in mice model was associated with increased circulation and tissue
337 AngII levels and led to cardiovascular damage^{119, 120}. It remain possible that i) mutations
338 affecting the human *ACE2* gene; ii) transcriptional variation in ACE2 mRNA expression; iii)
339 post-transcriptional modifications that act on the ACE2 viral receptor (such as N-
340 glycosylation), and; iv) putative ACE2 protein mutations, may influence the outcome of
341 COVID-19 by acting on blood pressure through the RAAS and possible increasing of lung
342 and heart damages through the oxidative stress triggered by Ang II. Recently an high rate
343 fatality of SARS-CoV-2 was reported in Iran¹²¹, without satisfactory explanation. If
344 underreporting of the number of infected people can be excluded, it could be hypothesized: i)
345 a more aggressive variant clade of SARS-CoV-2¹²²; ii) a variation in *ACE2*; or, iii) a
346 variation in genes like those encoding Toll-like receptors. Since it is known that Ang(1-7)
347 prevents inflammation by inhibiting the resistin/ Toll-like receptor 4 (TLR4)/MAPK/NF- κ B
348 pathway¹²³ and that there is a high variability of the TLR4 gene in different ethnic groups in
349 Iran¹²⁴, it remains possible that SARS-CoV-2 triggers increased inflammation in Iranian
350 patients by suppressing the ACE2-mediated metabolism of AngII to Ang(1-7). This could be
351 related to the observation that mice deficient in the TLR3/TLR4 adaptor TRIF are highly
352 susceptible to SARS-CoV infection including severe inflammatory induction¹²⁵.

353 The mechanism of acute myocardial injury caused by SARS-CoV-2 during severe COVID-19
354 disease might be related to the inhibition of ACE2 catalytic activity¹²⁶. (**Figure 2**).
355 Interestingly, a recent study posted as a pre-print paper¹²⁷ pointed out a list of 97 approved
356 drugs that may have a therapeutic potential against COVID-19 including anti-diabetics
357 (metformin), statins (simvastatin) and ARBs (sartans). Medical records of patients currently

358 treated with these compounds may help to identify whether those drugs have a beneficial or
359 adverse effect on COVID-19 patients. Metformin was also identified as a potential drug-
360 repurposing against SARS-CoV-2 in another study ¹²⁸. It should be remembered that a large
361 number of data suggest that there is a mild or severe cytokine storm in severe COVID-19
362 patients which is an important cause of death. To reduce the pro-inflammatory effect of AngII
363 and the cytokine storm observed in severe cases of COVID-19, it might make sense to
364 continue treating patients with ACE inhibitors and ARBs, a conclusion shared by recent
365 recommendations of the international societies of cardiology¹²⁹. However, Fang and
366 colleagues¹¹⁵ recently reported that the most distinctive comorbidities in patients who died
367 from COVID-19 are HT, coronary heart diseases, cerebrovascular diseases and diabetes, and
368 among them several were treated by ACE inhibitors. How should clinicians navigate this
369 uncertainty for patients who are taking ACE inhibitors and ARBs and become infected with
370 SARS-CoV-2? Do these molecules have a harmful effect in the outcome of the disease or is
371 the link that is made highlights only a confounding factor which confirms that HT is a major
372 factor of comorbidity? In agreement with others ¹³⁰⁻¹³² , we consider that it is of special
373 importance to rapidly evaluate whether these drugs are more beneficial than harmful in severe
374 COVID-19 patients.

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396

397 **Competing Interests**

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

400

401 **Ethical Approval**

402 Not required

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421 **Figure legends**

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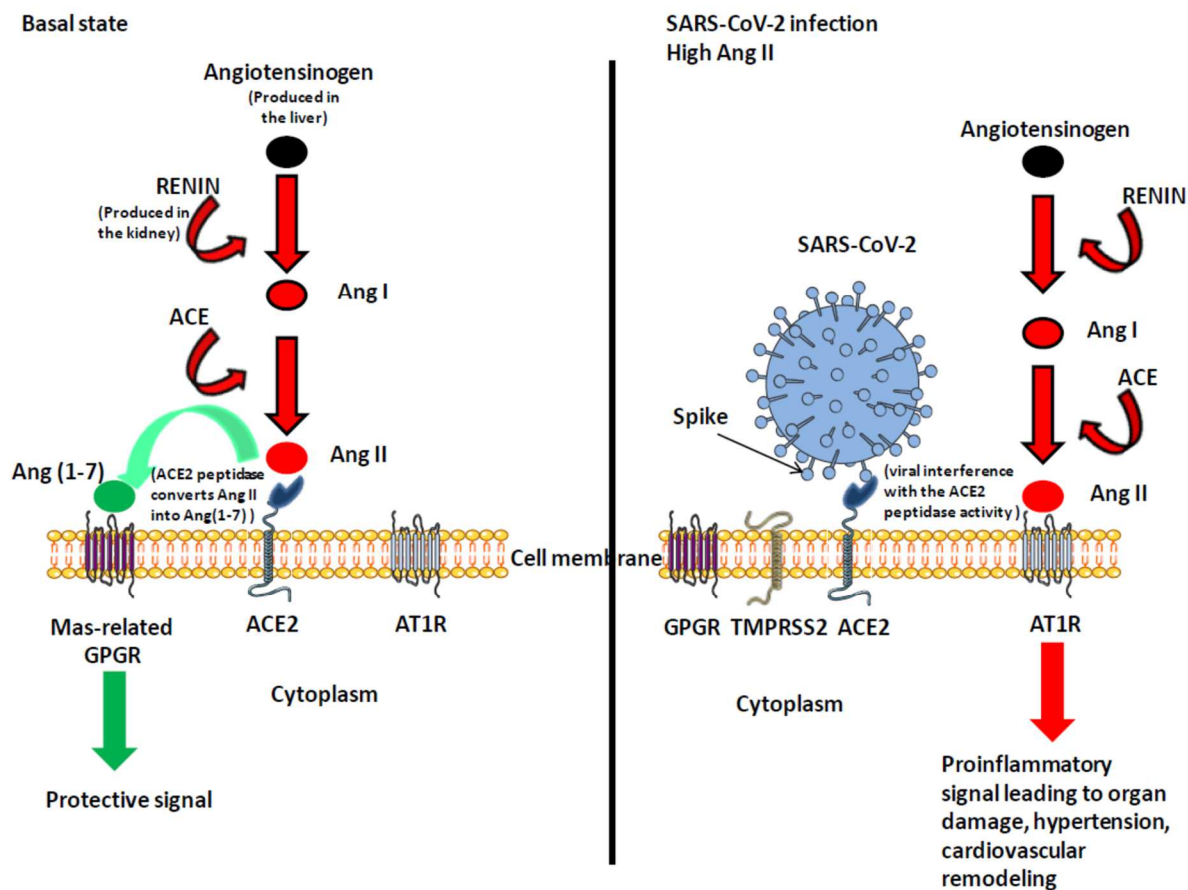
423 **Figure 1**

424 1A. Schematic representation of the regulation of ACE2. The transcription of the *Ace2* gene is
425 under control of the SIRT1 DNA-binding protein that binds the *Ace2* gene promotor. Post-
426 transcriptional regulation by miRNA (miRNA143, miRNA421) could occur (not shown).
427 Following translation the newly synthesized ACE2 proteins are likely target of post-
428 transcriptional modifications such as phosphorylation of Ser680 by AMPK that enhances the
429 stability of ACE2, and N-glycosylations. Once expressed at the cell membrane the ACE2
430 protein can be regulated by sheddases (ADAM10, ADAM17) that cleave the ACE2
431 extracellular domain and release a circulating soluble form sACE2 capable to interact with
432 integrins (ITGB1).

433 1B. Schematic representation (left) of the ACE2 molecule and its major domains. The amino
434 acids position is in black. Some of the amino acids important for viral tropism are in red
435 (previous studies showed that residues 31, 41, and regions 82-84 and 353-357 are important
436 for viral spike binding). Clustal Omega multiple sequence alignment (EMBL-EBI
437 bioinformatic tool; Copyright © EMBL 2020) of human ACE2 and its different isoforms
438 (right). The comparison of the reference *Homo sapiens* ACE2 protein sequence
439 (S1=Genbank: BAB40370.1) with 9 others ACE2 sequences from the NCBI reference
440 sequence database (S2=UniProtKB Q9BYF1.2; S3=NCBI NP_001358344.1; S4=NCBI
441 NP_068576.1; S5= GenBank EAW98892.1; S6= GenBank AAH48094.2; S7= GenBank
442 AAH39902.1; S8= GenBank AAO25651.1; S9= GenBank BAD99267.1; GenBank
443 AAF99721.1), showed 100% amino acids identity (not shown). The Clustal MSA was also
444 used for the comparison of the human ACE2 S1 sequence and available sequences of ACE2
445 isoforms: the isoform X1 (I1)= NCBI XP_011543851.1; isoform X2 (I2)= NCBI
446 XP_011543853.1; isoform X3(I3)=NCBI XP_011543854.1; isoform CRA (I4)=GenBank
447 EAW98891.1. The figure illustrates that these isoforms correspond to deletions in the CLD
448 domain, or truncations in the transmembrane domain. A very elegant work by Cao and
449 colleagues⁴² has recently analyzed 1700 ACE2 variants in search of ACE2 protein
450 polymorphism. The mutations and truncations found by this team are shown in light blue.

454 **Figure 2**

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



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