Opinion Paper

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Do genetic polymorphisms in angiotensin converting enzyme 2 (ACE2) gene play a role in coronavirus disease 2019 (COVID-19)?

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Abstract: Although some demographic, clinical and environmental factors have been associated with a higher risk of developing coronavirus disease 2019 (COVID-19) and progressing towards severe disease, altogether these variables do not completely account for the different clinical presentations observed in patients with comparable baseline risk, whereby some subjects may remain totally asymptomatic, whilst others develop a very aggressive illness. Some predisposing genetic backgrounds can hence potentially explain the broad inter-individual variation of disease susceptibility and/or severity. It has been now clearly established that severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), the virus causing COVID-19, infects the host cell through binding and being internalized with angiotensin converting enzyme 2 (ACE2), a surface protein expressed in a noticeable number of human cells, especially in those of upper and lower respiratory tracts, heart, kidney, testis, adipose tissue, gastrointestinal system and in lymphocytes. Accumulating evidence now suggests that genetic polymorphisms in the ACE2 gene may modulate intermolecular interactions with the spike protein of SARS-CoV-2 and/or contribute to pulmonary and

systemic injury by fostering vasoconstriction, inflammation, oxidation and fibrosis. We hence argue that the development of genetic tests aimed at specifically identifying specific COVID-19-susceptible or -protective ACE2 variants in the general population may be a reasonable strategy for stratifying the risk of infection and/or unfavorable disease progression.

Keywords: angiotensin; COVID-19; enzyme; polymorphism; receptor.

Introduction

Nearly 20 years after the outbreak of the severe acute respiratory syndrome (SARS), caused by a beta coronavirus, now renamed SARS-CoV-1, a new and more infectious virus emerged in China at the end of the 2019. This novel virus has spread across the planet, finally reaching the pinnacle of a pandemic disease [1]. As of mid-June 2020, over 8 million people have been infected by this new coronavirus (called SARS-CoV-2), resulting in at least 450,000 deaths.

Many biological and clinical features of this new pathology, which is called coronavirus disease 2019 (COVID-19), are similar to those observed in SARS-CoV-1. This is not surprising given that SARS-CoV-1 and SARS-CoV-2 share remarkable genetic identity [2]. Although the number of people with asymptomatic or mildly symptomatic COVID-19 remains mostly undefined, it is estimated to be at a rate somewhere between 40 and 80% [3]. On the other hand, in a substantial number of symptomatic patients, COVID-19 progresses to severe disease which encompasses a sequence of progressively worsening conditions, beginning with a severe form of interstitial pneumonia, variably associated with a systemic pro-inflammatory state, which may subsequently evolve into acute respiratory distress syndrome (ARDS), intravascular thrombosis, multiple-organ failure, ultimately leading to death in 5.4% of patients (case fatality rate updated as for the mid-June, 2020) [4].

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The heterogeneous risk for progression to the severe form of SARS-CoV-2 infection is one of the most controversial aspects in the pathogenesis and clinical course of COVID-19. Some important clinical predictors have been identified in different populations worldwide, mostly encompassing male sex, advanced age (i.e., >80 years), high body mass index or obesity, and the presence of severe co-morbidities, such as hypertension (HTN), diabetes mellitus, cardiovascular disease (CVD), chronic pulmonary disease, and impaired renal and liver function [5–8]. Although the severity of COVID-19 has been positively associated with air pollution [9] and gross domestic product [10], as well as negatively linked to current smoking status [11], it now seems rather clear that clinical and environmental factors do not completely account for the different clinical presentations observed in patients with a comparable baseline risk. In effect, some patients remain totally asymptomatic until definitive viral shedding, while others develop a very aggressive form of illness [12–14]. Such extremes in COVID-19 clinical picture strongly suggest that additional factors may play an important role in modulating the risk of disease onset and progression. In this article, we discuss a hypothesis regarding individual differences in binding-affinity between SARS-CoV-2 and the host cell receptors due to the existence of genetic polymorphisms.

Pathways of cellular infection by SARS-CoV-2

Both SARS-CoV-1 and SARS-CoV-2 bind to an identical receptor at the surface of human cells, which has been identified as the angiotensin converting enzyme 2 (ACE2) [15]. This binding especially involves a receptor binding domain (RBD) located within the spike protein of the virus (Figure 1). The pivotal residues in the RBD of SARS-CoV-2 for interacting with human ACE2 seem to be L455, F486, Q493, S494, N501 and Y505, whilst those on ACE2 seem to be K31, E35, D38, M82 and K353. Notably, although five of the six SARS-CoV-2 RBD key residues are different from those of SARS-CoV-1, the general structure of the interface between RBD and ACE2 is considerably similar between the two viruses [16]. However, *in vitro* studies have recently shown that unlike SARS-CoV-1, the RBD of SARS-CoV-2 tends to generate a larger binding interface (i.e., 1204 vs. 998 Å) through a higher number of cumulatively interacting residues (i.e., 30 vs. 24), along with an extended contact with the N-terminal helix of ACE2, which would explain the higher affinity of SARS-CoV-2 for binding ACE2 as compared to SARS-CoV-1 [15, 17].

Figure 1: Putative intermolecular interactions between the spike protein of SARS coronavirus 2 (SARS-CoV-2) and its host cellular receptor angiotensin converting enzyme 2 (ACE2). Effective binding is dependent upon spike protein activation by transmembrane serine protease 2 (TMPRSS2) or furin.

It is now clear is that the pathway used by SARS-CoV-2 to enter and infect the cell is via the RBD within the spike protein binding to ACE2. However, the spike protein needs to be cleaved by some human proteases, so that the S1 and S2 subunits dissociate from each other, with the latter domain undergoing important structural changes necessary for fusion with the host cell membrane [18]. Unlike SARS-CoV-1, the RBD within the spike protein of SARS-CoV-2 is characterized by ineffective receptor binding at rest [19]. This would, hence, translate into the RBD of SARS-CoV-2 having major affinity for binding to ACE2 at the surface of human cells, though it is cumulatively less accessible when it is not primed through a process of host protease-mediated activation. One of the most important enzymes in this process, along with lysosomal cathepsins, is the transmembrane serine protease 2 (TMPRSS2) [20].

Furin, a type 1 membrane-bound protease belonging to the subtilisin-like proprotein convertase family, also cleaves the site between both S1 and S2 subunits of the SARS-CoV-2 spike protein. A furin cleavage site is absent in SARS-CoV-1, which is another unique aspect in the pathogenesis of COVID-19 (Figure 1). Importantly, furin is expressed in many organs, including the lungs. After SARS-CoV-2 binds to ACE2 with its RBD, furin catalyzes the cleavage of the spike protein (S1/S2), which is otherwise necessary to for viral entry into the cell (Figure 1)[19]. This alternative pathway, encompassing furin-mediate activation, would hence permit SARS-CoV-2 to have a lower dependency on TMPRSS2 co-expressions at the cell surface for infecting cells. Thus, SARS-CoV-2 may be capable of entering a vast array of cells with lower TMPRSS2 expression. It is important to mention here that this necessary pre-

activation step that the spike protein must undergo for enabling efficient virus penetration into the host cell may represent an important immune evasion strategy, whereby the antibodies generated against the virus may be unable to efficiently recognize and bind to the “hidden” and/or “inactivated” RBD, and thus may be unable to neutralize the virus.

Besides the ACE2-mediated host cell entry, it has also been reported that special intercellular adhesion molecule-3-grabbing non-integrin (SIGN) may be an alternative receptor for SARS-CoV-2, but displaying lower affinity as compared to ACE2. However, its role remains largely speculative so far [21].

Finally, the disintegrin and metalloproteinase domain 17 (ADAM17) not only functions by cleaving the precursor of tumor necrosis factor- α (pro-TNF- α) [22], but also catalyzes the release of ectodomains of a number of transmembrane proteins, including ACE2. Reduced expression of ADAM17 concomitantly decreases ACE2 shedding, whilst ADAM17 overexpression enhances its cellular release [23]. It is, therefore, conceivable that an increase ADAM17 activity would be associated with increased ACE2 shedding. This, in theory, reduces the likelihood that SARS-CoV-2 would be able to find an available host receptor for cellular entry [24].

Angiotensin converting enzyme 2

Genetic and biochemical structure of angiotensin converting enzyme 2 (ACE2)

The human gene encoding for ACE2 (*ACE2*), mapped on chromosome Xp22.2, is 41,116 base pair long, contains 21 exons and belongs to the ACE family of dipeptidyl carboxydipeptidases [25]. The *ACE2* gene encodes a 788 amino acids mature protein with a molecular mass of ~92.5 kDa, sharing considerable homology (i.e., ~40% identity and ~60% similarity) with the human gene encoding for angiotensin converting enzyme (*ACE1*) [26]. ACE2 is now universally recognized as a type I integral membrane protein, thus containing an HEXXH + E zinc-binding consensus sequence and consisting of a 17 amino acid N-terminal signal peptide (residues 1–17), a 724 amino acid extracellular domain (residues 18–740), a 22 amino acid transmembrane helical domain (residues 741–761), and a 43 amino acid intracellular (cytoplasmatic) domain (residues 762–805) (Figure 2) [27].

ACE2 exerts a pivotal function in humans, whereby this enzyme catalyzes the conversion of angiotensin I (Ang I) into angiotensin 1–9 (Ang 1,9), which can be further

Figure 2: Crystal structure and main functional domains of angiotensin converting enzyme 2 (ACE2).

converted by ACE into angiotensin 1–7 (Ang 1,7). Angiotensin II (Ang II) is also directly converted into Ang 1,7 by ACE2 [28]. In doing so, not only does ACE2 reduce the unfavorable activities of Ang II, which is a vasoconstrictive, pro-inflammatory, pro-oxidative and pro-fibrotic peptide, but also promotes vasodilatation and anti-fibrotic activity by generating Ang 1,7.

Tissue distribution of angiotensin converting enzyme 2 (ACE2)

The expression of *ACE2* in various human tissues has been a matter of debate until this protein was recently recognized as the main receptor through which SARS-CoV-2 penetrates human cells. Subsequently, several new studies evaluating the types of cells where *ACE2* is expressed, and which can hence represent possible targets (or even reservoir) for SARS-CoV-2, have been rapidly performed. Li et al. carried out a comprehensive study to address *ACE2* expression in many normal human tissues, and analyzed their findings with respect to sex and age to investigate why a worse prognosis in COVID-19 is often seen in older males [29]. High *ACE2* expression was detected, in decreasing order, in the small intestine, testis, kidney, heart, thyroid, adipose tissue and salivary glands. Intermediate expression was found in pancreas, esophagus, lungs, colon, liver, bladder and adrenal gland, whilst lower expression was detected in nerves, stomach, uterus, muscle, blood vessels, brain, bone marrow and spleen. However, *ACE2* was not found to be differentially expressed between sexes or between younger and older patients.

In another important study, Sungnak et al. investigated *ACE2* gene expression, and thereby SARS-CoV-2

potential tropism, in multiple scRNA-seq datasets obtained from different human tissues of healthy donors, with a specific focus on the respiratory tree [30]. In line with previous findings, *ACE2* was found to be highly expressed in the airway epithelium, cornea, esophagus, ileum, colon, liver, gallbladder, heart, kidney and testis. *ACE2* was also present in specific airway epithelial cell types, such as alveolar epithelial type II cells and nasal epithelial (i.e., goblet and ciliated cells). In nasal epithelia, *ACE2* was found to be highly co-expressed with the *TMPRSS2* gene, thus explaining the special predisposition (and vulnerability) of these cells to be infected by SARS-CoV-2.

Interestingly, Xu et al. [31] demonstrated that *ACE2* is also expressed on the mucosa of oral cavity (i.e., in the epithelial cells of tongue), as well as in lymphocytes, thus providing a reasonable explanation for the lymphocyte injury commonly observed in COVID-19, leading to lymphopenia, especially in patients with the most severe form of illness [32].

Importantly, *ACE2* is expressed on the surface of endothelial cells [33], thus supporting direct SARS-CoV-2 infection of these cells. This may explain the diffuse endothelial inflammation and pyroptosis observed in COVID-19, which may in part explain the considerable enhanced risk of developing thrombotic events [34].

It should be noted that *ACE2* tissue expression alone does not necessarily constitute that SARS-CoV-2 actively targets and infects such organs. However, a recent autopsy found that in addition to the respiratory tract, SARS-CoV-2 was molecularly detected in the kidneys, liver, and heart, brain and blood, demonstrating the *in vivo* multiorgan tropism of this novel virus [35]. The authors suggest that comorbidities may contribute to organotropism. However, whether these detected viral loads in tissues outside the lung contribute to pathology and clinical outcomes remains to be elucidated.

Angiotensin converting enzyme 2 (*ACE2*) gene polymorphisms

Twenty-five different *ACE2* gene variants are included in the Leiden Open Variation Database (LOVD) [36], whilst as many as 1700 *ACE2* variants have been identified in the ChinaMAP and 1KGP databases [37].

Major emphasis has been given to some of these polymorphisms over the past decades, due to its implications in the onset and progression of cardiovascular disease and, more specifically, their link with HTN. In a large meta-analysis published by Yang and colleagues in 2015 which included 17 studies totaling 14,122 patients [38], the

authors concluded that two *ACE2* polymorphisms (i.e., G8790A and rs2106809) were significantly associated with a higher risk of developing essential HTN. Several studies were then published in support of these findings. For example, Zhang et al. [39] explored the presence of different *ACE2* single-nucleotide polymorphisms (SNPs) in 956 normotensive subjects and 1024 HTN patients, showing that five of them were significantly associated with HTN in women. In a separate study, Luo et al. [40] also investigated *ACE2* polymorphisms in 233 normotensive subjects and 402 HTN patients, reporting that one SNP was associated with essential HTN whilst, even more importantly, three other SNPs were associated with enhanced risk of HTN-related atrial fibrillation and left atrial remodeling.

Such considerable genetic heterogeneity in the *ACE2* gene inevitably leads to the intriguing question as to whether structural protein variation may influence binding with SARS-CoV-2 and thereby modulate the virulence and pathogenicity of the virus in patients. During the outbreak of SARS in 2002–2003, it was demonstrated that some *ACE2* mutations were associated with cell surface *ACE2* expression and with diverse SARS-CoV-1 entry efficiency [41, 42].

In a preliminary study, Cao et al. [37] explored the allele frequency distribution of 1700 variants in the *ACE2* gene among different worldwide populations. Notably, 11 common variants and one rare variant were found to be associated with enhanced *ACE2* expression, and their expression was found to be unevenly distributed among different populations. The authors found that a specific *ACE2* gene polymorphism (variant rs4646127) was strongly associated with higher expression levels in the East-Asian population, thus paving the way to further studies aimed to more specifically address this important issue. Analogous results have been made available by Chen et al. [43], who also showed that the allele frequency of variants associated with *ACE2* over-expression was higher in East Asian populations.

In a subsequent study, Hussain and colleagues investigated the binding efficiency of different proteins encoded by a number of *ACE2* gene variants to SARS-CoV-2 spike protein, each differing in the presence of polymorphisms in the RBD binding sequence [44]. Notably, although most genetic variants displayed high structural homologies, considerable differences were found in spatial orientation of the pivotal binding residues. Two specific *ACE2* alleles (i.e., rs73635825 and rs143936283) exhibited a relatively low binding affinity for the spike protein of SARS-CoV-2, which might imply a lower likelihood of viral attachment and potential resistance to infection.

A much broader study has become available in pre-print, in which Stawiski and colleagues explored the possibility that some natural *ACE2* gene variants, especially those predicated to interact with the spike protein of SARS-CoV-2, may be associated with variable virus-host interaction, thus potentially modulating virulence and pathogenicity [45]. Briefly, the authors carried out a huge genomic dataset analysis, including more than 290,000 samples from over 400 populations, which were used to construct synthetic mutant maps of *ACE2*. Notably, at least nine human *ACE2* variants (i.e., S19P, I21V, E23K, K26R, T27A, N64K, T92I, Q102P and H378R) were found to have predictable enhanced susceptibility to viral binding, whilst 17 other *ACE2* variants (i.e., K31R, N33I, H34R, E35K, E37K, D38V, Y50F, N51S, M62V, K68E, F72V, Y83H, G326E, G352V, D355 N, Q388L and D509Y) were considered to be protective against viral entry, since they exhibited a lower binding propensity to SARS-CoV-2 spike protein. This evidence has been confirmed by another recent study that compared the efficiency of many variants in *ACE2* library to bind the RBD of SARS-CoV-2 [46], reporting that some of these variants fail to bind to the spike protein, whilst others displayed higher affinity.

In an ensuing investigation, Renieri et al. [47] integrated genomic data obtained from five different Italian centers, with the purpose of identifying *ACE2* gene variations which may explain differential SARS-CoV-2 spike protein affinity, binding, processing and/or internalization. Notably, three variants (i.e., p.Lys26Arg, p.Gly211Arg and p.Asn720Asp) could be identified. These three variants were more frequently expressed in the Italian rather than Eastern Asian populations. All these variants are located closely to the sequence that is essential for SARS-CoV-2 spike protein binding and are hence predicted to modulate the cleavage-dependent viral intake (e. g., Asn720Asp is located just four amino acids from the cleavage site of TMPRSS2). This may in part explain the higher case fatality rate recorded in Italy as compared to China [48]. Importantly, several other rare variants were identified in this study, some of which are likely capable to determine conformational variations in the RBD binding sequence, which would modify the affinity for the SARS-CoV-2 spike protein.

Although in SARS the limited published evidence failed to find significant associations between *ACE2* gene polymorphisms and disease outcomes [49], preliminary data suggests that genetic variants in *ACE2* gene may not only play an important role in modulating the host susceptibility to SARS-CoV-2 infection, but are also key in determining the severity of local and systemic tissue injury. Some *ACE2* gene polymorphisms were found to

exhibit differential efficiency in activating neutrophils, monocyte/macrophages, natural killer (NK) cells, T helper (Th) 1, 2 and 17 cells, thus potentially either promoting or attenuating the so-called inflammatory or “cytokine storm” [50], as well as catalyzing the conversion of Ang II to Ang 1,7, thus ameliorating or worsening vasoconstriction and contributing to improve or aggravate local or systemic tissue injury [38, 51]. Further studies should be urgently performed to identify putative *ACE2* gene variants that may be associated with abnormal immune response and exaggerated pyroptosis [52], as shown in *ACE2* null mice [53]. This would also pave the way to targeted mutagenesis and development of recombinant *ACE2* containing sequence variations that may confer enhanced vasodilating and anti-inflammatory properties, counteracting the risk of developing ARDS and/or systematic inflammatory response [54].

Conclusions

Several months after its initial spill-over in China, and the following diffusion across the world, many uncertainties remain regarding the mechanisms underlying the interplay between SARS-CoV-2 and its human host. Though it is now unquestionable that some demographical, clinical and environmental factors may explain a substantial part of virulence and pathogenicity of this coronavirus, the specific biological reasons for a better or worse prognosis are still elusive, raising the strong probability of various genetic factors being involved. Indeed, virus recombination and possible emergence of different SARS-CoV-2 haplotypes in certain geographical regions, or even within a single patient, are possibilities that cannot be ruled out, as recently emphasized by Becerra-Flores et al. [55] and by Shen et al. [56]. Overall, genomic diversity in specific domains of SARS-CoV-2, especially in the RBD of its spike protein, may favor enhanced binding to *ACE2*, thus fostering host virulence and pathogenicity [57].

Besides SARS-CoV-2 recombination, it is plausible that polymorphisms in the *ACE2* gene, most of which has been detected so far in Chinese populations, may modulate intermolecular interactions with the SARS-CoV-2 spike protein and/or worsen pulmonary and systemic injury in patients with COVID-19 by fostering increased vasoconstriction, inflammation, oxidation and fibrosis [58]. The fact that *ACE2* has an X-chromosome linked phenotype would then be mirrored by higher risk of developing variants in the male sex, which may contribute to explain - at least partially - the higher severity and enhanced death rate of COVID-19 in men compared to women [59].

At this point in time, it becomes clear that the development of genetic tests aimed at identifying specific COVID-19-susceptible or -protective *ACE2* variants in the general population may be a strategy for obtaining clinical and societal advantages. The identification of subjects with more vulnerable *ACE2* variants would imply that these individuals need reinforced precautions (i.e., major social distancing) to avoid contact with the virus, and that infected patients bearing these more vulnerable *ACE2* variants may benefit from more aggressive treatment from initial presentation to prevent progression towards unfavorable outcomes. A genetic analysis of *ACE2* may not only be beneficial for detecting enhanced spike protein binding and infectivity, but also for predicting the risk for developing ARDS and cytokine storm resulting from the synergistic effect of *ACE2* polymorphisms and comorbidities such as HTN, which is a well-known predictive factor for developing severe COVID-19 [60]. On the other hand, the identification of subjects with protective *ACE2* variants, once definitely established that they are resistant to SARS-CoV-2 binding or that they may be protected from dysregulated immune response and inflammation, would still nonetheless need to be protected by maintaining standard precaution measures. However, such patients may take advantage of a different, less aggressive clinical management plan. Finally, regarding this genetic perspective, the COVID-19 Host Genetics Initiative (<https://www.covid19hg.org/>) deserves to be mentioned here. Briefly, this project is aimed to engage the scientific community in generating, sharing and analyzing data to dissect potential genetic determinants of COVID-19 susceptibility, severity and outcomes. Importantly, further research on the relationship between *ACE2* gene variants and SARS-CoV-2 interaction at the host cell surface may also pave the way to developing innovative therapeutic strategies based on splice-switching antisense oligonucleotides (SSOs), which could be designed to specifically target critical domains underlying SARS-CoV-2 virulence, thus minimizing the risk of infection and unfavorable clinical progression [61].

It is also worthwhile to mention here that not only *ACE2* polymorphisms, but also transmembrane serine protease 2 (*TMPRSS2*) gene variance may influence the interplay between SARS-CoV-2 and *ACE2*, thus potentially altering virulence and pathogenicity of the virus. A recent study showed that, from the over 11184 SNPs that could be identified throughout the *TMPRSS2* gene, 92 of these have different frequency distribution between Asian and other populations, whilst 15 alter the splicing processing through several mechanisms [62]. It is hence reasonable that some splice variations, which impair the expression of *TMPRSS2* at the cell surface, may have an impact on individual

susceptibility to SARS-CoV-2 infection and COVID-19 severity.

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