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SARS-CoV-2 receptor and renin-angiotensin system regulation: impact of genetic variants in *ACE2* gene

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Abstract:- The ACE2 has a physiological role in the regulation of the Renin-Angiotensin System. It is also described your function as a receptor for SARS-CoV-2 and other coronaviruses. Genetic variants in *ACE2* are associated with cardiovascular diseases in different human populations and drug response. There is no direct evidence that mutations in *ACE2* confer resistance to coronavirus spike protein binding. The evolutionary relationship between spike protein binding and ACE2 is complex. Significant genetic variants are present in *ACE2*, meanwhile, the evolutionary time of contact of the human ACE2 to the virus is short and, therefore, it did not suffer sufficient selective pressures to offer resistance to viral spike protein binding at the population level. More efforts are needed to identify genetic variants in human *ACE2* and other genes, and, consequently, conducting case-control studies to validate these variants and their possible association with infection rates by SARS-CoV-2 and/or clinical outcome.

Keywords:- *coronaviruses, COVID-19, Angiotensin I Converting Enzyme 2*

I. INTRODUCTION

At the end of 2019, in China, were described a cluster of human pneumonia cases [1], the illness was named as coronavirus disease 2019 (COVID-19) [2], [3]. The common symptoms included fever, dry cough, and fatigue. Rare symptoms included headache, loss of smell, nasal congestion, sore, throat, coughing up sputum, shortness of breath, chills, nausea and/or vomiting, diarrhea, and pain in muscles or joints. In severe cases, has confusion, high fever, bluish face or lips, coughing up blood, persistent chest pain, decreased white blood cells, kidney failure, and difficult walking [4]. The complications can include organ failure, heart problems, blood clots, acute kidney injury, and additional viral and bacterial infections [5], [6].

The causal agent was a novel coronavirus, initially termed 2019-nCoV by the World Health Organization (WHO) [2], [7], [8], and recently, the International Committee on Taxonomy of Viruses called the virus SARS-CoV-2 [9]. As of March 11, 2020, the WHO declares a pandemic of the new coronavirus. Until July 17, 2020, have 13. 575. 158 confirmed cases, 584. 940 deaths and cases were reported in 216 countries, areas, or territories (https://www.who.int/emergencies/diseases/novel-coronavirus-2019).

Two genres of coronaviruses cause human disease: alphacoronaviruses (alphaCoVs): hCoV-229E and hCoV-NL63, and the betacoronaviruses (betaCoVs): HKU1, HCoV-OC43, Middle East respiratory syndrome CoV (MERS-CoV), severe acute respiratory syndrome CoV (SARS-CoV), and SARS-CoV-2 [2], [7], [8], [10], [11]. These genres have a most recent common ancestor, estimated from 10.000 to millions of years [12]. A mutation rate of 1.19 to 1.31 × 10−3 per site per year was calculated, based on several SARS-CoV-2 strains, it is proposing a very recent origin, probably from 22 to 24 November 2019 [13].

In the last decade the betaCoVs cased two coronaviral epidemics. Between 2002-2003, in China, SARS-CoV emerged and spread to other parts of the world, causing approximately 8.096 infections and 774 deaths worldwide (https://www.who.int/csr/sars/country/table2004_04_21/en/) . Was identified in 2012 MERS-CoV in the Middle East, and until the end of November 2019 affected 27 counties with 2.494 infections and 858 associated deaths (https://www.who.int/emergencies/mers-cov/en/).

Both previous infections were caused for zoonotic pathogens originating from animals [14]–[16]. Is still being investigated the origin of SARS-CoV-2, and then it is related to an animal market [2], [8], [17]. In this review, we first summarize the nature of coronaviruses, as special attention to SARS-CoV-2. The ACE2 receptor-binding characteristics and genetics variants, and which this could implicate in infection mechanism and future treatments.

II. CORONAVIRUSES: INTERSPECIES TRANSMISSION

Coronaviruses are enveloped viruses with positive-sense and single-stranded RNA genomes that can infect animals and humans [2], [10], [18]. The recent metagenomic study corroborates that pangolins (Manis javanica) could be an intermediate-host of SARS-CoV-2, and that assimilated these viruses autonomously from bats or other animal hosts

[19]. Supposed recombinant signals between the pangolins, bat, and human SARS-CoV-2 coronaviruses were described [19]. The origin of the new human coronaviruses is complex and it is unclear which the intermediate host is. However, mutations and/or recombination of coronaviruses could permit cross-species infection [20], [21].

The zoonotic transmission of an agent from an animal to a human comprises viral, social, environmental, and climatic factors [22]. The stage of identifying the host binding receptor is critical, and establishes the virus binding to a species, tissue, or cell type [23]. The specificity of coronaviruses is determined by surface-located trimeric spike (S) glycoprotein. This glycoprotein is composed of an N-terminal S1 subunit, which is specialized in identifying host-cell receptors, and a membrane-embedded C-terminal S2 subunit, that realize the membrane fusion [10], [24], [25]. Two domains folding independently are presented in the S1 N- and C-terminal portion, both may function as a receptor-binding entity. The S1 N-terminal domain (NTD) is involved in receptor recognition. Nevertheless, in most coronaviruses, the receptor-binding domain (RBD) is present in the S1 C-terminal domain (CTD) [26]–[30], in this case, the NTD can afford the attachment of the virus to the cell by identifying exact sugar molecules. Therefore, the S1 interaction is the basis for tissue tropism and host interaction of coronaviruses. [26], [31].

The ability of the virus binding to the receptor in other organisms is a criterion for interspecies transmission [32]. HCoV-OC43 and HKU1 use sugars for cell attachment [27]. Other human coronaviruses identify proteinaceous peptidases as a receptor [32]. Despite being classified into different genres, SARS-CoV and hCoV-NL63 use the human angiotensin-converting enzyme 2 (hACE2) for virus entry [33]–[35]. Recently, was found the SARS-CoV-2 also use the hACE2 [8] (Fig. 1).

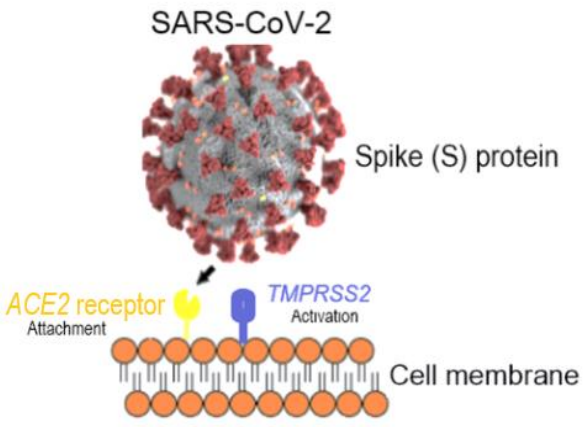

Fig 1:- SARS-CoV-2 uses hACE2 for cell entry. TMPRSS2 cleaves the spike protein of SARS-CoV-2 for activation.

The difference between SARS-CoV-2 and SAR-CoV is 380 amino acid replacements. This translates into five different amino acids of the six vital amino acids in the receptor-binding domain of the viral spike (S) protein [36]. The SARS-CoV-2-CDT is an important region of interaction with the hACE2 receptor. This is similar to observed in SARS-CoV. However, SARS-CoV-2-CDT has a higher affinity for the receptor [37]. Also, SARS-CoV-2 utilizes host proteases including cathepsin L, cathepsin B, trypsin, factor X, elastase, furin, and TMPRSS2 (transmembrane protease serine 2) for S-protein cleavage and facilitates cell entry [38]. The TMPRSS2 is decisive for SARS-CoV-2 entrance into host cells, it cleaves protein spike at the S1 and S2 sites, leading to the fusion of viral and cellular membranes [38], [39] (Fig.1). This leads to an efficient tropism with hACE2 [19], [23].

III. GENETIC VARIANTS IN *ACE2* AND BINDING TO SARS-COV-2

The Angiotensin I Converting Enzyme 2 (*ACE2*) gene is located on the short arm of the X chromosome and encodes the ACE2 protein, that is a receptor for coronaviruses, and may have an important role in the viral replication [8], [33], [34], [37]. In mice infected with a hepatitis virus, a group 2 coronavirus, the allelic variants of viral receptor were associated with altered virus binding activity, which mediated host predisposition [40], [41]. Were shown a spike protein and receptor adaptation in rat ACE2 with introducing residues of *hACE2*, allowed an effective infection of human cells [42].

A case-control study composed of 168 SARS-CoV patients and 328 healthy individuals compared the frequencies of five single nucleotide polymorphisms (SNPs) in *ACE2*. No significant difference was found in the allele and genotype frequencies for these loci in any of the comparison groups. These results showed no association between these genetic variants in *ACE2* and SARS-CoV predisposition or outcome. Regardless of its X-chromosome location, male SARS-CoV patients with poor outcomes not are correlated with these SNPs of *ACE2* [43].

Despite a large number of infected people and areas of the globe with case records of SARS-CoV-2, epidemiological, association and the genetic of populations studies are still at an early stage. Genomic comparison of 70 *ACE2* placental mammal orthologues identifies that 4% of the *ACE2* variants located in the catalytic domain are under positive selection. It is proposed a taxon-specific adaptation associated with the *ACE2*. The variants located at the critical ACE2 binding sites showed a high diversity between placental mammal species and no found SNPs in the human population. These results suggest a high and effective tropism for all human populations. Also, the contagion and dispersion of coronaviruses can by facilitated by demographic and cultural conditions of each human population [44]. Regarding ACE2 expression, no difference is found between Asian and other populations, as well as any SNPs in the *ACE2* locus [45].

An extensive analysis of the *ACE2* variant in two date banks results in 62 variants located in the coding region, and this, 32 variants can be potentially affecting the amino acid sequence. These variants have low allele frequency in different populations. Remarkably, the rs2285666 has a

higher allele frequency in the Asian populations compared to Ad Mixed American, African, and European. Moreover, were found 15 expression quantitative trait loci (eQTL) variants in 20 tissues from the Genotype-Tissue Expression (GTEx) database. Most of these eQTLs had higher allele frequency in the Asian population compared to the European population. These eQTLs are related to the high expression of ACE2. These results showed an inexistence of evidence of *ACE2* mutations in populations that confer resistance. The higher allele frequency in the eQTLs related to higher ACE2 expression can suggest different predispositions or responses to SARS-CoV-2 infection in different populations under similar conditions [46].

Seventeen coding variants were identified in *ACE2* in the position of binding with viral protein. The rs73635825, rs1299103394, and rs766996587 can interfere in the stability of the encoded protein compared to the wild-type. Nevertheless, no was reported detrimental features for these variants in humans. Moreover, all *ACE2* variants binding to a viral protein have a similar topology to the resolved ACE2-SARS-CoV-2 conformation. Though, the strongest and fewer variances were found between SARS-CoV-2 spike protein and rs961360700 and rs143936283, respectively. These findings suggest that rs143936283 can offer some level of resistance for the binding of SARS-CoV-2 to the ACE2 [47]. Though, all coding variants identified in this study have low AF in al databases, hindering population studies. Therefore, the rapid dispersion of SARS-CoV-2 in continents and different susceptibility between individuals in distinct human populations can be for another reason [48].

It showed a reduction in incidence and effect of COVID-19 disease in populations that dwell at a height of over 3,000 mean sea levels. Environmental and biological factors may be the reason for the reduction of severity in these conditions [49]. Reinforcing the hypothesis that several factors are related to different rates of SARS-CoV-2 infection, including the accumulation of evolutionary adaptations and variable host immune responses to viral infection [46], [48].

In 200.000 people were found several variations in a binding region of the ACE2 receptor. There is no description of pathogenicity for these variants. However, they can alter the interaction of the receptor with the viral protein. [50]. In the Italian population, don't have evidence of *ACE2* genetic variants related to features of the disease. However, *TMPRSS2* genetic variants and protein levels can be a possible candidate for disease modulators [51]. It suggests that together with *ACE2*, *TMPRSS2* genetic variants modulate predisposition to SARS-CoV-2 infection, justifying the epidemiological data observed in patients of this population [51]. Other similar data suggest the existence of genetic predisposition acting on the variability of responses to SARS-CoV-2 infection [52]–[54]. Highlighting, all variants identified have low or rare allele frequency in different populations, given the absence of selective pressure, as the contact of the viral protein and the human receptor is recent. All these genetic variants identified in these studies need experimental validation on case-control patients with different clinical manifestations (Fig. 2).

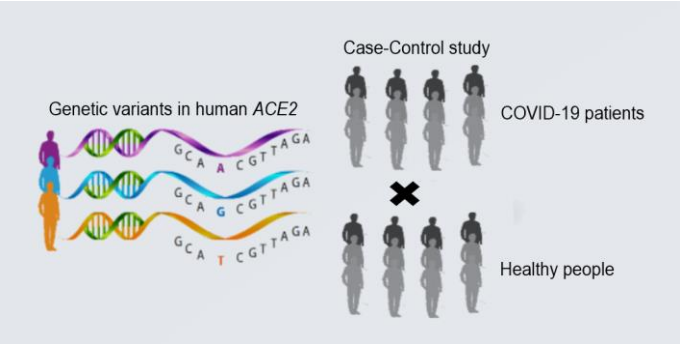

Fig 2:- Use of genetic variants in the human *ACE2* gene in association study type case-control.

IV. BIOLOGY AND PHYSIOLOGY ROLES OF *ACE2* IN CARDIOVASCULAR DISEASES

It has been accumulated knowledge of biology and physiology of *ACE2* over the last 20 years since the discovery [55]–[58]. The ACE2 is a monocarboxypeptidase that regulates the Renin-Angiotensin System (RAS), participates in the transport of amino acids, and is a coronaviruses receptor [34], [37], [59]–[61]. In the RAS, ACE2 converts Angiotensin (Ang) I in a nonapeptide (Ang 1-9) and Ang II in a heptapeptide (Ang 1-7) [55], [58]. The negative regulation of RAS by the enzymatic pathway of degradation of Ang I and II reduces the detrimental actions conducted by Ang II. The Ang 1-7 is an active peptide with vast effects opposites of initiated by Ang II [62]–[65].

Cardiovascular disease is a global public health problem. The negative regulation of RAS is cardioprotective, limiting the action of ACE in Ang I and II. The reduction of Ang II detrimental effects through degradation of the peptide, and formation of Ang 1–7 exercises cardioprotective effects. The reduction of ACE2 activity leads to the activation of Ang II, which contributed to the increased progression of cardiovascular diseases. The increased ACE2 level and activity result in the development of Ang 1–9 and Ang 1–7, protecting from cardiovascular diseases [66]. Experimental and clinical studies corroborate the biological role of ACE2 in cardiovascular and lung disease [55], [67]–[69]. It is clinically proven that an increase and activation of ACE2 could offer protective effects against cardiovascular disease [55], [67]–[69]. The ACE2 is located in cardiomyocytes, cardiac fibroblasts, epicardial adipose tissue, and coronary vascular endothelium. The heptapeptide Ang 1-7 is also present in the same tissue, additionally in the vascular smooth muscle cells [70]–[73].

The ACE2 expression levels are higher in the kidneys, heart, intestine, testis, thyroid, and adipose tissues, and are lowermost in the blood, spleen, bone marrow, brain, blood vessels, and muscle. The middle expression is present in the lungs, colon, liver, bladder, and adrenal gland [74]. Therefore, the SARS-CoV-2 can interact with the receptor

in all of the tissue and people with different sexes and ages [74]. It is known that respiratory involvement and multiorgan dysfunction occur in SARS-CoV-2 infections [1], [5], [6].

After the entrance of SARS-CoV and SARS-CoV-2 in the cells has a downregulation of surface ACE2 expression [34], [75]. Was found increased circulating levels of Ang II in 12 SARS-CoV-2 infected people compared to controls. This exhibits a correlation between tissue ACE2 downregulation with systemic RAS imbalance, and facilitates the development of multiorgan damage from SARS-CoV-2 infection [76]. Was detected viral genome in seven of 20 postmortem heart tissue infected by SARS-CoV, and increased myocardial fibrosis, inflammation, and reduced myocardial ACE2 level [77]. The pre-existence of diabetes mellitus, hypertension, and lung disease carries an increased risk of SARS-CoV-2 infection [1], [78] probably because the dysregulation in RAS, which occurs in these conditions. Therefore, an imbalance in the RAS and a decrease of ACE2 levels in patients with COVID-19 contribute to tissue and systemic inflammation [8], [58], [79]. Moreover, in SARS-CoV-2 infected people, prior cardiovascular complications led to poor outcomes, and most deaths secondary to fulminant inflammation, lactic acidosis, and thrombotic states [80].

Susceptibility to infection can be mediated by sex and age [5]. Oher findings do not describe the difference between sex or age in any tissue [74]. Were described age-dependent ACE2 levels in nasal epithelium adjusted for sex in 305 individuals aged 4 to 60 years. Compared to adults, children have lower ACE2 levels. This can explain why COVID-19 is less frequent in children [81]. Finally, Asian males have low ACE2 expression compared to females and other ethnic groups and decreased expression of ACE2 with the age [45].

V. *ACE2* AND THERAPY TARGET

Presently, no antiviral drugs available are effective in treat COVID-19. Nevertheless, there are numerous clinical trials with drugs that were originally designed for other pathogens. The therapies can be function directly in SARS-CoV-2, impeding the viral enzyme replication or viral entry to human cells. Alternative drugs can modulate the immune system, increasing the innate response, or impeding the inflammatory process that causes lung damage [82]. Additionally, there are efforts for the advance in vaccine production and antibodies specifically targeting SARS-CoV-2, focusing on the viral protein that is the target for therapeutic and vaccine development [66].

It has been suggested the potential action the ACE2 in therapies for COVID-19 disease [64], [83], [84]. The soluble recombinant human Angiotensin-converting Enzyme 2 (rhACE2) probable blocks the entry of SARS-CoV-2 into the cell, interfering in the interaction of the viral protein with the human receptor. Also, the rhACE2 could inhibit viral reproduction in cellular models [85]. It is proposed that the administration of the rhACE2 can decrease the serum level of Ang II. This may prevent receptor activation and maintain pulmonary vascular integrity and inhibits acute respiratory distress syndrome (ARDS) [86]. This therapy is in step II of test for ARDS and an initial protocol in China evaluates the role of rhACE2 in COVID-19 disease [82].

The important role of RAS in cardiovascular disease is well known [87]. It is proposed that use of inhibitors of ACE (ACEI) and blockers of the receptor of Ang II (ARBs) may lead to an increased risk of SARS-CoV-2 infection by upregulation of ACE2 [88], [89], also is proposed that ACEI/ARBs are beneficial to treat this infection, directing the host response to the virus [90]. In this system ARBs intensifies the ACE2 levels and activity, increasing the levels of Ang 1-7, which has protective action. Additionally, ARBs inhibit inflammation and acute lung damage caused by Ang II [90], [91].

Cardiology associations recommend following treatment with ACEI/ARBs in patients who already use these drugs (https://newsroom.heart.org/news/patients-taking-ace-i-and-arbs-who-contract-covid-19-should-continue-treatment-unless-otherwise-advised-by-their-physician). Additionally, a review showed that there is no scientific and clinical evidence to recommend stopping the use of these drugs [92]. The *ACE2* polymorphisms are associated with cardiovascular diseases in different human populations [93]–[102] and drug response [97], [103]. The COVID-19 disease rapidly evolving and differentially affects risk groups that have dysregulation in the RAS; the genetic polymorphisms in *ACE2* should be considered in the process of development of therapies where ACE2 is the target since the response can be variable.

VI. FINAL CONSIDERATIONS AND FUTURE PERSPECTIVES

It is important to adopt massive testing in populations. Some countries confront concomitant epidemics with similar symptoms. In Brazil, a constant epidemic of arboviruses transmitted by Aedes Aegypti leaves the health system always on alert. Until June were reported 823.738 dengue fever cases and 374 deaths [104], [105]. It has been reported, cases of patients diagnosed with dengue fever and then progress to COVID-19 [106], [107]. Dengue virus and SARS-CoV-2 infections share clinical and laboratory characteristics, making it difficult to distinguish them [108]. Demonstrating the importance of laboratory tests associated with clinical criteria for diagnosis.

Studies of epigenetic dysregulation of *ACE2* and other genes of immunological systems might better clarify different outcomes of COVID-19 disease. Other coronaviruses that circulate in Asian countries used ACE2 as a receptor. It is not known whether these populations have any protective factor not present in European and American populations, which were more susceptible to infection and transmission.

Given the initials findings available in the literature so far, there is no direct evidence that mutations in *ACE2* confer resistance to coronavirus spike protein binding. Moreover, mutations in SARS-CoV-2 increased contagion, however not the virulence. The evolutionary relationship between spike protein binding and ACE2 is complex. Significant genetic variants are present in *ACE2*, meanwhile, the evolutionary time of contact of the virus with the human receptor ACE2 is short and, therefore, it did not suffer sufficient selective pressures to offer resistance to viral spike protein binding at the population level. More efforts are needed to identify genetic variants with the minor allele frequency >5% in hACE2 and other genes, and, consequently, conducting case-control studies to validate these variants and their possible association with infection rates by SARS-CoV-2 and/or clinical condition outcome.

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