ANGIOTENSIN- CONVERTING ENZYME 2 GENE POLYMORPHISM AND SUSCEPTIBILITY TO COVID-19 INFECTION

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Abstract:
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 can cause patients lung injury and sometimes multi-organ failure with adverse myocardial remodeling, myocardial stress, and cardiomyopathy. Recently, SARS-CoV-2 was reported to be a human angiotensin I converting enzyme 2 (ACE2)-tropic virus.

Keywords: ACE2, SARS-CoV-2, COVID-19.

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Introduction:

Angiotensin-converting enzyme (ACE) is a critical component of the renin- angiotensin system (RAS) that is involved in blood pressure homeostasis **(1).** Cleavage of angiotensin I (Ang I) by ACE leads to the production of angiotensin II (Ang II) that binds to either angiotensin II type 1 receptor (AT1R) or angiotensin II type 2 receptor (AT2R). The binding of Ang II to AT1R is mainly involved in blood pressure regulation. On the other side, ACE2 encoded by human X-chromosome can either act on Ang I to give rise to angiotensin 1-9 or on Ang II to generate angiotensin **(2).**

Genetic structure

The ACE gene is located on the long arm of chromosome 17 (17q23) and has 21 kilobases long containing 25 introns and 26 exons; exons 1-12 encode for the amino domain, and exons 13-26 encode for the carboxyl domain. More than 160 ACE gene polymorphisms are known and most of them are single nucleotide polymorphisms **(3)**.

Unlike the ACE gene, which is located on human chromosome 17, the 40kb ACE2 gene is located on chromosome Xp22 and contains 18 exons, most of which resemble exons in the ACE gene. Whereas ACE contains 2 active sites, ACE2 possesses only a single catalytic domain. Both ACE and ACE2 act as zinc metallopeptidases but of differing substrate specificities defining their distinct and counterbalancing roles in the RAS **(4)**.

Whereas ACE cleaves C-terminal dipeptide residues from susceptible substrates (a peptidyl dipeptidase), ACE2 acts as a simple carboxypeptidase able to hydrolyze Ang I, forming Ang 1–9 and Ang II to Ang 1–7. The C-terminal domain of ACE2, which has no similarity with ACE, is a homolog of a renal protein, collectrin, which regulates the trafficking of amino acid transporters to the cell surface, endowing ACE2 with multiple and distinctive physiological functions. It is the multiplicity of physiological roles that ACE2 plays that has allowed it to be hijacked by SARS-CoV-2 as a receptor, resulting in the COVID-19 pandemic **(4)**.

The renin-angiotensin-aldosterone system (RAAS)

The renin–angiotensin–aldosterone system (RAAS) is a signaling pathway that acts as a homeostatic regulator of vascular function, as reviewed elsewhere. It has dynamic control over systemic and local blood flow, blood pressure, natriuresis, and trophic responses to a wide range of stimuli. Therefore, macula densa releases renin within the kidneys upon low intra-tubular sodium concentration and sympathetic nervous stimulation, and then renin converts angiotensinogen into angiotensin I in the liver. Next, the angiotensin-converting enzyme (ACE) converts angiotensin I (Ang I) into Ang II, predominantly in the lungs. Then, Ang II stimulates the release of aldosterone from the adrenal cortex **(Figure .1) (5)**.

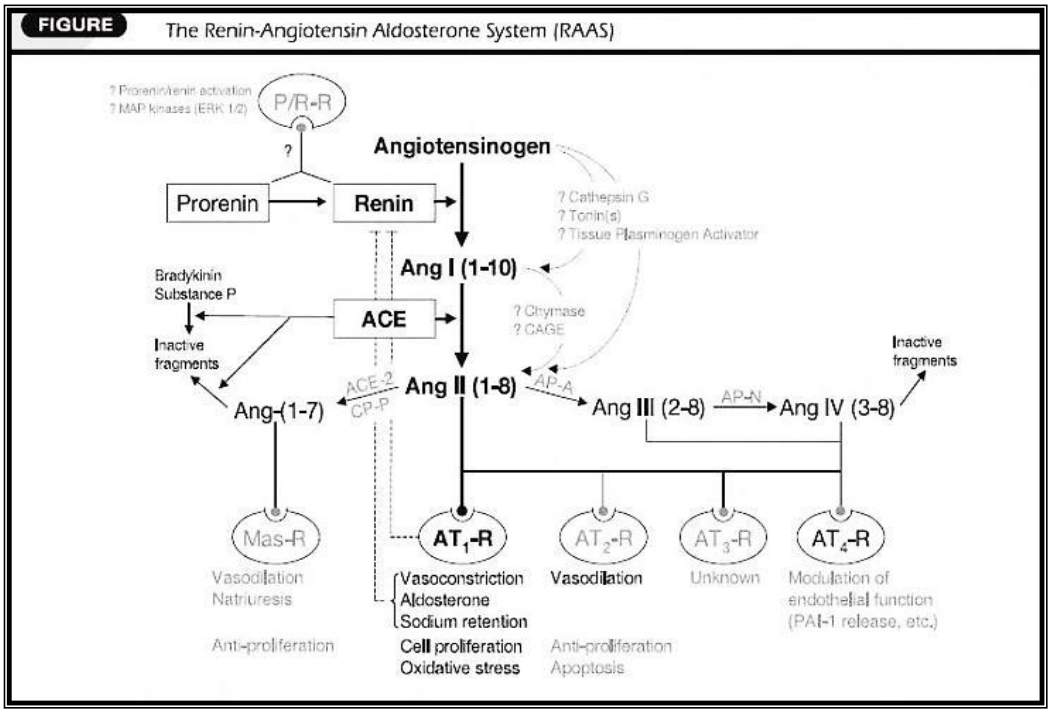

Figure (1): The renin–angiotensin–aldosterone system (RAAS) **(5)**.

ACE2 sites and function

Human ACE2 acted as a functional receptor and binds to spike (S) protein of SARS-CoV with high affinity. ACE2 is found in the epithelia of the lung and intestine, In addition to this, predominant expression was seen in the apical than the basolateral surface which suggests the availability of enzyme for cleavage of peptides at mucosal surfaces of the airway **(Figure. 2A) (6).**

ACE2 is expressed in the vascular system (endothelial cells, migratory angiogenic cells, and vascular smooth muscle cells), heart (cardiofibroblasts, cardiomyocytes, endothelial cells, pericytes, and epicardial adipose cells) and kidneys (glomerular endothelial cells, podocytes and proximal tubule epithelial cells). ACE2 is also expressed and functions in the local RAS of the liver (cholangiocytes and hepatocytes), retina (pigmented epithelial cells, rod and cone photoreceptor cells and Müller glial cells), enterocytes of the intestines, circumventricular organs of the central nervous system, upper airway (goblet and ciliated epithelial cells), and alveolar (Type II) epithelial cells of the lungs and pulmonary vasculature **(Figure. 2A) (4)**.

The C-terminal domain of ACE2 is a homolog of a renal protein, collectrin, which regulates the trafficking of amino acid transporters to the cell surface, endowing ACE2 with multiple and distinctive physiological functions. It is the multiplicity of physiological roles that ACE2 plays that has allowed it to be hijacked by SARS-CoV-2 as a receptor, resulting in the COVID-19 pandemic **(Figure. 2B) (4)**.

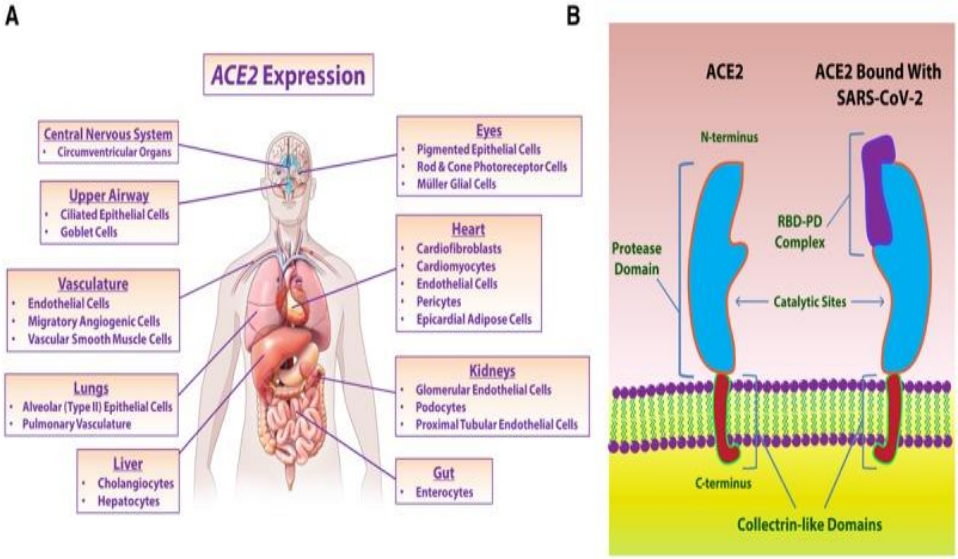

Figure (2A): ACE2 (angiotensin-converting enzyme 2) expression throughout the body and schematic of ACE2 primary domains.

Figure (2B): ACE 2 binding with SARS-Cov -2 (4). ACE2 level and COVID-19 pathogenesis

Since the beginning of the COVID-19 pandemic, hypertension and diabetes have been correlated with a higher risk of mortality, and initial reports speculated that angiotensin-converting enzyme inhibitors (ACEi) and angiotensin receptor blockers (ARBs), which are commonly used therapeutic agents for these conditions, would up-regulate ACE2 expression, thus increasing the risk of severe illness. Recent evidence has challenged this hypothesis, demonstrating both mechanistically and in large cohort studies that ACEi and ARBs do not up-regulate ACE2 and are not associated with increased mortality **(7).**

The significance of ACE2 shedding has not been fully elucidated, but in the context of the COVID-19 pandemic, the comprehension of the mechanism leading to ACE2 shedding, sACE2 function, and its plasma level can lead to the development of better therapies and diagnostic tools to follow disease progression and severity. For example, it has been shown that the binding of SARS-CoV with ACE2 induced ADAM17-dependent shedding, promoting SARSCoV uptake into the cells **(8).**

ACE gene polymorphism and COVID–19 pathogenesis

Angiotensin-converting enzyme (ACE) insertion (I) / deletion (D) polymorphisms

Plasma ACE levels are stable when measured repeatedly in the same individual, whereas large inter-individual differences are observed. This suggests strong long-term control of plasma levels, possibly with genetic origins. This polymorphism is detected using a polymerase chain reaction (PCR) method **(3).**

The protective effect of ACE-2 in acute pulmonary syndrome has been shown in experimental studies, and angiotensin-2 stimulation could be an

important mechanism that could be used for the management of acute lung injuries. Likewise, the demonstration of 30-day mortality in ARDS patients with the ACE DD genotype, as opposed to the ID or II genotypes, may be conceived as a clinical implication of this model **(9).**

Most of the studies conducted to investigate the role of ACE receptor gene polymorphisms are epidemiological studies. There is no study investigating the relationship between ACE I/D and ACE2 receptor gene rs2106809 and rs2285666 polymorphism and COVID-19 severity.

Other mutations:

There are others mutations such as AT1 and AT2 receptors polymorphisms can be detected by Duplex Polymerase chain reaction- restriction fragment length polymorphism (PCR- RFLP) assay. The PCR product was digested by enzyme HaeIII (Haemophilus aegypticus); which cuts the AT1 receptor gene polymorphic site) and EcoRI (Escherichia coli); which cuts the AT2 receptor gene polymorphic site. Genotyping of AT1 receptor polymorphism was as a 233 bp band for the C allele, a 256 bp band for the A allele and the AT 2 receptor was genotyped as a 91 and 29 bp for G allele and a 120 bp for A allele **(10)**.

A1166C is a single nucleotide polymorphism (SNP) in which there is an A to C transversion at position 1166 in the 30 untranslated region of the AT1R gene. (AC and CC) genotypes of AT1R A1166C SNP together with obesity can be considered as significant predictors for breast cancer risk among post menopausal females and pregnancy-induced hypertension (PIH) **(11)**.

ACE2 Polymorphisms and Susceptibility to SARS-CoV-2 Infection

Due to the main role of ACE2 in mediating SARS-CoV-2 entry into cells, many studies have speculated whether ACE2 expression and polymorphism and serum sACE2 levels could explain why some people are more prone to experience a severe phenotype while others remain asymptomatic. Previous studies have demonstrated that specific residues in the ACE2 protein are essential for binding with SARS CoV **(12).**

Angiotensin-converting enzyme (ACE) insertion (I)/ deletion (D) polymorphisms are one of the most frequently defined human polymorphisms. (D) and (I) polymorphisms in the ACE gene in populations may result in differences in ACE levels. For instance, the ACE D allele causes an increase in ACE-1 level and a decrease in ACE-2 level, causing an increased level of angiotensin-2 and progression of pulmonary edema, through increased microvascular permeability. That phenomenon further worsens the clinical course and prognosis of the diseases such as acute respiratory distress syndrome (ARDS) **(9).**

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