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REVIEW ARTICLE

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Genetic and epigenetic control of *ACE2* expression and its possible role in COVID-19

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Coronavirus disease 2019 (COVID-19), caused by severe acute respiratory syndrome coronavirus-2 (SARS-CoV-2), is a pandemic that is claiming hundreds of thousands of lives around the world. Angiotensin-converting enzyme-2 (ACE2) is a key player in COVID-19 due to its pivotal role in the SARS-CoV-2 infection. This enzyme is expressed throughout the body and the studies conducted so far have shown that its expression varies according to several factors, including cell type, sex, age, disease states and probably SARS-CoV-2 infection. Single-nucleotide polymorphisms (SNPs) and epigenetic mechanisms, including DNA methylation, histone post-translational modifications and microRNAs, impact *ACE2* expression and may explain structural variation. The understanding of how genetic variants and epigenetic markers act to control *ACE2* expression in health and disease states may contribute to comprehend several aspects of COVID-19 that are puzzling researchers and clinicians. This review collects and appraises the literature regarding some aspects in the ACE2 biology, the expression patterns of this molecule, SNPs of the *ACE2* gene and epigenetic mechanisms that may impact *ACE2* expression in the context of COVID-19.

KEYWORDS
ACE2, COVID-19, epigenetic, gene expression, SNPs

1 | INTRODUCTION

Coronavirus disease 2019 (COVID-19), caused by severe acute respiratory syndrome coronavirus-2 (SARS-CoV-2), is a disease that spread quickly from its epicentre in Wuhan, a city located in the Chinese province of Hubei, reaching 1 million confirmed cases worldwide on 2 April 2020, with an estimated death rate of 5.2%.[1] One of the key features of the infection - and that explains its widespread - is its high interpersonal transmissibility. Interhuman transmission has been identified via droplets, direct contact and indirect contact through surfaces.[2] Moreover, the virus has been identified in blood, saliva and stool.[3–5] Another important characteristic of the virus is its resistance in the external environment: it can remain viable on plastic surfaces for up to 3 days.[6]

Once in contact with healthy cells, SARS-CoV-2 can use the renin-angiotensin system enzyme angiotensin-converting enzyme-2 (ACE2) along with the transmembrane protease serine type-2 (TMPRSS2), which are anchored on the cell membrane, to enter and continue the infection.[7] Because not every cell and tissue express these enzymes, the virus cannot attack all tissues or organs indistinctly. In addition to this difference in susceptibility in the context of the organs of the same individual, there is a marked discrepancy in the susceptibility levels of different patients: men, elders and patients with prior history of cardiovascular, pulmonary and metabolic diseases constitute the population with a higher risk of severe and lethal disease.[8,9] On the other extreme, a large proportion of the individuals - perhaps the great majority of infected subjects - are asymptomatic.[10] The factors that account for this differential susceptibility are not fully understood yet. Nevertheless, the study of ACE2 is undoubtfully of great importance to shed light on some of the open questions.

Given the importance of ACE2 for the present pandemic, this review will explore the characteristics of ACE2, the genetic and epigenetic control of the *ACE2* gene and its possible impact in the context of COVID-19. Our intent in the elaboration of this review was to

better comprehend some features of ACE2 and point out new and promising research fields in COVID-19.

2 | BIOLOGY OF ACE2

ACE2 was discovered in 2000, as two independent groups characterized a carboxypeptidase homologue of the well-known ACE that could cleave angiotensin I and angiotensin II and was not inhibited by conventional ACE inhibitors, such as captopril, lisinopril and enalaprilat.[11,12] The *ACE2* gene is located in chromosome X position p22 and comprises approximately 40 kb, containing 18 exons intercalated with 17 introns.[12] Interestingly, it was reported that this region could escape X inactivation in female cells, although higher expression was seen in males in some tissues.[13] In addition to this, several single-nucleotide polymorphisms (SNPs) have been found in *ACE2*.[14,15]

ACE2 is a type I transmembrane, 805 amino acids-long glycoprotein that contains a short C-terminal intracellular tail and an N-terminal that extends to the extracellular space and contains the peptidase domain (PD) of the enzyme.[11,12] ACE2 and the testicular ACE share 33% of the amino acids and both enzymes have a conserved region in the active site: the zinc-binding motif His-Glu-Met-Gly-His.[11] ACE2 is a strict monocarboxypeptidase and contains a single active site.[11,16] ACE2 can also be detached from the plasma membrane through the action of disintegrin and metalloproteinase domain-containing protein 17 (ADAM-17), induced by angiotensin II.[17] ACE2 cleaves angiotensin II and angiotensin I - the latter much less efficiently than angiotensin II - into angiotensin (1-7) and angiotensin (1-9), respectively.[11,16] Through its action, ACE2 reduces the availability of angiotensin II and produces angiotensin (1-7), which acts on the Mas receptor (MasR) inhibiting diuresis and inducing vasodilation and vasoprotection.[18,19] Given the cardiovascular protective properties of the peptides produced through the action of ACE2, especially angiotensin (1-7), it may be important to maintain ACE2 expression or activity in the context of COVID-19.

In addition to counterbalancing the action of ACE and angiotensin II, ACE2 has two other physiological roles: the cleavage of apelin peptides and the formation of an amino acid transporter along with SLC6A19.[20] Apelin is a peptide, comprised of 77 amino acids, that is cleaved by endopeptidases to give rise to several smaller peptides whose activity through the G protein-coupled receptor angiotensin II protein J receptor (APJR) mediates important protective actions in the cardiovascular system.[21,22] ACE2 is able to remove the C-terminal amino acids from these peptides and, at least, partially inactivate them given the lower hypotensive action in the presence of ACE2, whereas the ACE2 deficiency or inhibition or the introduction of apelin-resistant analogs rescued the cardioprotective effects of the peptides.[23] However, the metabolized apelin peptides may also be biologically relevant.[24] Remarkably, apelin peptides specifically induce ACE2 expression through the action of APJR.[25] Given its actions, it has been hypothesized that apelin, and possibly its ACE2-metabolized peptides, may be protective in COVID-19 through their cardioprotective mechanisms.[26,27] ACE2 has been found associated with SLC6A19 in the brush border of epithelial intestinal cells and the proximal kidney tubules.[28,29] SLC6A19 is expressed in the kidney and in the small intestine, where it mediates the transport of neutral amino acids from the lumen to the interstice.[29] Whereas it partners with collectrin and ACE2 in the kidney, it relies exclusively on ACE2 in the intestine, forming a dimer of heterodimers.[30,31] Although SARS-CoV-2 can bind to ACE2 in the presence of SLC6A19 when the complex is in a closed state,[31] it was not determined yet if the presence of the amino acid transporter alters the dynamics of interaction or internalization.

Following the onset of the COVID-19 pandemic, ACE2 was readily recognized as the receptor for SARS-CoV-2 entry.[7,32] Like SARS-CoV, SARS-CoV-2 has a spike (S) protein, which is a trimeric protein composed of S1 and S2 domains both with high homology, mainly in the receptor-binding domain (RBD) - 73.9% of the residues are similar between the viruses.[33,34] Before interacting with ACE2, the priming of the S protein is necessary and achieved by proteases, like TMPRSS2 and cathepsin L/B.[7] The S protein of SARS-CoV-2, specifically, is also cleaved by furin at the S1/S2 boundary, which enhances the virus entry in susceptible cells if compared to SARS-CoV, which does not rely on this mechanism.[35,36] After cleavage, the C-terminal domain (CTD) of S1 is exposed and ready to make contact with the outer surface of the PD of ACE2.[33,37] Both SARS-CoV and SARS-CoV-2 have a CTD structure composed of a core subdomain bearing five antiparallel β-strands and an external subdomain composed of two β-strands connected by a flexible loop.[33,38] The contact of the CTD of S1 and ACE2 is mediated mainly by the external subdomain and is driven by polar interactions - H bonds - between the viral and the human proteins.[33,38] Despite the similarities between the RBDs of the two viruses, ACE2 has a stronger affinity for that of SARS-CoV-2 due to key substitutions in its RBD.[31,33] Moreover, the epitopes displayed in the RBDs of the two proteins are different, which could explain why antibodies targeting the RBD of one virus do not block the infection caused by the other.[33,39] Bioinformatics predictions have pointed to different directions: while it was reported that some variants of ACE2 could bind to the RBD of SARS-CoV-2 with varying affinities and thereby be protective against the infection or favour it,[40,41] another study did not find any difference the interaction considering eight variants found in European non-Finnish and African populations.[42] An in vitro study testing ACE2 variants did not found any evidence for their role in modulating the interaction with SARS-CoV-2, except for one that reduced the expression of ACE2.[43] The relevance of these results in vivo, however, remains to be studied.

3 | EXPRESSION PATTERNS OF ACE2

In the seminal papers characterizing ACE2, its expression determined by Northern-blotting was tracked in higher levels in the kidney, heart and testis, but lower levels were detected in the colon, small intestine and ovary.[12,13] Later, the mRNA expression was determined by qRT-PCR across 72 tissues from three donors. It was shown that *ACE2* is ubiquitously expressed, but higher levels were seen in the testis, kidney, digestive tract and cardiovascular tissues. Interestingly, the

highest expression was observed in the ileum.[44] Following the SARS epidemic, immunohistochemical localization of the ACE2 protein revealed specificity in the localization of the protein within several tissues. Positive staining was found in endothelial cells and smooth muscle in the cardiovascular system; epithelial cells from the proximal tubules of the kidney; in alveolar type 1 and 2 (AT1 and AT2, respectively) epithelial cells in the lungs; in the smooth muscle and enterocytes in the gut; in the basal layer of the skin and oral and nasal mucosa and marginal staining was described in the brain.[45]

Recent studies employing single-cell transcriptome analysis and looking at transcriptome databases have described interesting results that could help to explain the entry and tropism of SARS-CoV-2. Consistent with prior literature, epithelial and endothelial cells of the intestine are the main cell types expressing *ACE2* mRNA, with the ileum and colon presenting the highest proportion of positive cells.[46–49] Considering the oral cavity, which is an important entry site for the virus, the tongue holds epithelial cells, lymphocytes and fibroblasts expressing *ACE2*.[48] In the superior airways, *ACE2* expression was observed in secretory epithelial cells of the nasal and sinus mucosa.[50] Tracheal epithelial cells, large airway epithelial cells and, in a smaller proportion, small airway epithelial cells (SAE) express *ACE2* mRNA.[51] In the lungs, AT2 cells are the main cell type expressing both *ACE2* and *TMPRSS2*.[51,52] In addition to these, transient secretory cells, a cell type that expresses markers of Goblet and ciliated cells, also presented high expression of *ACE2* and *TMPRSS2*.[52] In addition, *FURIN* expression was observed in lung cells, often being expressed in the $ACE2^{+}$ and $TMPRSS2^{+}$ cells.[52] In the heart, pericytes are the main cells expressing *ACE2*. It was shown that these cells establish interactions with endothelial cells, which could promote microcirculatory dysfunctions in the heart if the virus is present.[46] In the urinary tract, epithelial cells of the proximal tubules and the urothelial cells of the bladder were observed expressing the enzyme.[49] In testes, particularly, spermatogonia, leydig and sertoli cells - these two cell populations could not be separated in this study - were shown expressing *ACE2*, with spermatogonia also expressing *TMPRSS2*.[53] Neuronal expression was also reported based on data from transcriptome databases and animal studies.[54–56]

Considering the data presented, it could be hypothesized that the organs with the highest expression could be the most affected by the disease and the infection routes comprise the oral and nasal mucosa. Indeed, it is known that gastrointestinal, pulmonary, cardiovascular and renal symptoms are common in COVID-19 patients and the use of face masks greatly reduces transmission.[57,58] However, the scenario seems to be more nuanced, as multi-organ failure and multisystem inflammatory syndrome in children in the context of COVID-19 reveal that, in addition to cytopathic effects directly attributable to the virus infecting the cells through ACE2, immune activation, cytokine storm, endothelial dysfunction and the coagulopathy that follows the infection contributes to damage and its manifestations.[59–61]

In addition to the cell type, several factors have been described to modulate the expression of ACE2. Studies observed modulation of ACE2 expression by age and sex in animal models and humans. Elder rats (24-month old) presented lower protein expression compared to young and middle-aged (3- and 12-month old) in the lungs. Moreover, elder females showed higher expression than males.[62] In sheep, males experienced increased ACE2 activity from birth to the first year and 1-year-old animals of both sexes displayed increased protein and mRNA expression compared to newborn lambs.[63] In mice, gonadectomy increased the activity of renal ACE2 in females only, and treatment with 17β-estradiol decreased the activity of the enzyme in both males and females regardless of the sex chromosome complement.[64] Normal human bronchial epithelial cells grown at an air-liquid interface treated with 17β-estradiol also displayed lower *ACE2* mRNA expression than vehicle-treated controls.[65] In humans, the analysis of transcriptomic datasets has revealed that *ACE2* is differentially expressed in the nasal and bronchial cells between children and adults and in some tissues between men and women, with adults and men having higher expression.[13,66] Using qPCR, it was also shown that age is positively correlated with *ACE2* mRNA expression in a cohort including patients with respiratory disease and that sex and age are significantly associated with *ACE2* expression in a multivariate analysis.[67] In combination with sex, smoking was reported to increase *ACE2* and *TMPRSS2* mRNA in SAE cells, with male smokers having a higher expression of *ACE2* compared to female smokers.[51] These data suggest that older people, males and smokers are at higher risk for SARS-CoV-2 infection and mortality. Several meta-analyses revealed that males, and particularly older patients are at higher risk for infection and mortality regardless of ethnicity.[68–72] However, the evidence for smoking is controversial, although it favours the case of smoking as a risk factor.[73–75]

Hypoxia is also a factor that modulates ACE2 expression and may be an important risk factor for complications following COVID-19. HIF-1α, a key transcription factor involved in the hypoxia response and that was found to be upregulated in the peripheral blood of COVID-19 patients.[76] HIF-1α has been observed to downregulate *ACE2* mRNA and protein in airway smooth muscle and umbilical vein endothelial cells.[77,78] Consistently with these findings, patients with lung fibrosis, a condition that is associated with the hypoxic state, display lower *ACE2* expression.[79] This ACE2 downregulation promoted by HIF-1α may cause dysregulation of the concentration of the protective peptides derived from the action of this enzyme.[80] Moreover, HIF-1 upregulation following hypoxia may trigger inflammatory signalling.[76] However, *ACE2* increased expression was observed in the culture of hepatocarcinoma cells under prolonged hypoxia and asthmatic patients have no difference in *ACE2*, *TMPRSS2* and *FURIN* expression in the epithelial brush border cells compared to healthy controls, which suggests that hypoxia and pulmonary diseases may induce different responses according to the cell type and the duration of the stimulus.[81,82] Hyperoxia, in its turn, was involved in ACE2 activity downregulation through its shedding promoted by ADAM-17.[83]

The metabolic status also has an influence on ACE2, since type I and II diabetes mellitus were shown to modulate ACE2 activity and expression in humans and mice.[84–88] Inflammation may also play a role in ACE2 expression, since NF-κB, IFN-γ and TNF-α, IL-1β, IL-4 and TGF-β may regulate it.[82,89–91] Conversely, ACE2 may also regulate inflammation and thrombus formation, particularly in the context

of SARS-CoV-2 infection.[92] Heart failure has been associated with increased ACE2 activity and expression, perhaps due to a compensatory mechanism. While angiotensin II may induce further cardiac damage, increased ACE2 means higher levels of angiotensin (1–7), which is cardioprotective.[18,93–95] The pharmacological induction of *ACE2* expression was also reported. Agents that can modulate ACE2 are ACE inhibitors (ACEI), angiotensin II AT1 receptor blockers (ARBs), statins, mineralocorticoid antagonists and rosiglitazone.[96–100] Nevertheless, the discontinuation of these drugs in patients infected with SARS-CoV-2 is a matter of debate.[101,102]

In the SARS-CoV-2 infection, it is possible that ACE2 protein expression is downregulated, as is the case in the SARS-CoV infection.[103] Particularly in the latter, the viral S protein alone was able to decrease the levels of ACE2 protein expressed in the cell membrane, probably due to the internalization of the molecule, and worsen acute lung injury in acid-challenged mice.[103] Indeed, a recent study has shown that platelets reduce their ACE2 membrane expression due to internalization when facing the virus or its S protein. Moreover, virus-activated platelets exhibit higher aggregatory capacity and inflammatory cytokine release, which could explain some of the thromboembolic events in COVID-19.[104] It was also reported that SARS-CoV-2 infection in the lung cancer cell line Calu-3 slightly decreased *ACE2* mRNA expression through the upregulation of the transcriptional repressor zinc finger E-box-binding homeobox 1 (ZEB1).[105] As Verdecchia et al. (2020) discuss in their review, this ACE2 downregulation may have adverse cardiopulmonary effects in COVID-19, since Angiotensin II would accumulate, and Angiotensin 1-7 levels would be decreased.[106] A potentially beneficial approach to rescue ACE2 loss in infected patients would be the treatment with recombinant human ACE2 protein (rhACE2) or an antibody targeting the S protein.[104,107] Currently, there is one completed clinical trial studying rhACE2 as a treatment for COVID-19 (NCT04335136). Additionally, a case report observed decreased concentration of inflammatory cytokines, reduced the presence of SARS-CoV-2 in plasma and airways, and clinical recovery following the administration of soluble rhACE2.[108]

4 | POLYMORPHISM OF *ACE2*

Polymorphic variants may be responsible for altering levels and patterns of expression in different tissues. Also, they can modify the composition and conformation of the protein.[109] The *ACE2* gene is one of the genes with the most genetic variants, and some studies have evaluated its relationship with diseases.[109,110] In the current pandemic, the variability in susceptibility to COVID-19 depends on several factors, and genetic polymorphisms can be one of them. Differences in the spread of the virus, as well as in the development and worsening of symptoms, may be related to the polymorphic genetic variability of the *ACE2* gene.[109,111]

The *ACE2* gene is located on the chromosome X: 15 561 033-15 602 069, reverse strand, and comprises ~41 036 nitrogenous bases. It produces 5 transcripts – two encoding a protein – and is composed of 19 exons and 18 introns. The *ACE2* gene has ~14 194 allelic variants that include ~12 000 intronic variants, ~260 exonic variants. Also, we can distinguish 660 missense and synonymous variants.[112] This gene has great potential for genetic variability and this is observed in different populations worldwide.[111]

Change of only one nucleotide in the base sequence, in at least 1% of the population, is called SNP.[113] SNPs are common changes that occur in DNA with the potential to modify the observed phenotype and can have an important impact on the epidemiology of some diseases.[114,115] However, finding this relationship is not an easy task. Usually, diseases have a multifactorial character and there is the possibility that the assessed SNP is not the variant responsible for the alteration.

The SNPs of the *ACE2* gene have been studied for more than 17 years in different populations and some studies show their relationship with cardiovascular diseases, diabetes, pulmonary alterations, and other comorbidities. The most studied population is the Chinese, and their diverse ethnicities, such as Han and Dongxiang. Interestingly, even before the COVID-19 pandemic affected the world in 2020, studies have pointed to the influence of the presence of *ACE2* polymorphisms in diseases related to organs such as the heart, lungs and kidneys.[116] These organs have also been described as the most affected in hospitalized patients due to COVID-19.[117]

The literature is scarce in studies that evaluated the distribution of SNPs and the expression, or bioavailability, of the protein in diseases. However, many studies assessed the distribution of SNPs in different populations and found associations with diseases. Most published studies evaluated SNPs and variants in the intronic region with an association of alleles and genotypes. Regarding COVID-19, the SNPs of *ACE2* can influence the interaction of the virus with the cell due to alteration of the receptor conformation.[118] Another point is concerning the damage caused to the organism in individuals who already had a genotype susceptible to diseases. These are some important questions that are being raised in current studies.

The cardiovascular system is affected by changes in the renin-angiotensin system in several ways. The change in blood pressure, vasoconstriction, proliferation, inflammation, increased heart rate are some changes that can cause comorbidities or aggravate pre-established situations in the cardiac system.[119,120] The rs6632677 variant has been linked to hypertrophy and dilation in cardiomyopathies in studies with the population of India and China.[114,121] Some variants have been associated with chronic conditions such as hypertension (rs1978124, rs879922, rs714205, rs4646176 and rs2074192), and also myocardial infarction (rs1978124, rs228566 and rs4646142).[114,119] The pre-existence of any disease or cardiovascular alteration worsens the prognosis of a patient affected by COVID-19. A 30%-40% of the patients who presented with the severe form of the disease had some cardiovascular alteration, even if controlled or in the mild forms.[122]

Few studies have related to the allelic frequency of ACE2 variants with specific changes in the lungs, despite the high levels of expression of this enzyme in alveolar cells.[123] The published works refer to the great susceptibility of the structures that make up the airways to

express high levels of ACE2 and consequently increase the risk of infection by SARS-CoV.[14,124] Some variants, like rs2285666, are related to this higher probability in some Asian populations, even so, the studies found it difficult to relate this finding in other populations.[125,126] Despite this, it has been published on the influence of Angiotensin II bioavailability on pulmonary fibrosis, inflammation and cytokine production, clinical findings commonly seen in patients with COVID-19.[127] Changes in the serum level of angiotensin (1-7) were related to the rs2074192 and rs2106809 variants in the Han population in hypertensive patients, which can influence the involvement of the lungs in patients affected by COVID-19.

The cytokine storm is the most important factor in the worsening and widespread involvement of the organism in the disease. The presence of chronic and acute diseases and comorbidities increases the risk of morbidity and mortality in these patients.[128] Obesity, liver disease and kidney disease are those that stand out in terms of fatality related to COVID-19, followed by chronic obstructive pulmonary disease, cardiovascular diseases, diabetes and hypertension.[122] Some of the effects of these comorbidities have been related to polymorphic variants in some populations. The variants rs1978124, rs2236306, rs233575, rs4646188, rs879922, rs2106809, rs2285666, rs4646142, rs4646155 and rs4646188 were related to changes in low-density and high-density lipoprotein cholesterol levels, and rs2106809 and rs2285666 were related to type 2 diabetes in the Chinese population. Also, rs2285666, rs4240157, s4646142, rs4646155 and rs4830542 were related to tissue damage in organs like the heart.

Another important factor about *ACE2* polymorphisms would be the impact on dissemination and susceptibility to infection. This information is perhaps the most important and difficult to obtain, due to the difficulty of analysing the genotype of a population during a pandemic.[129] Even so, some incipient findings give an idea of the SNPs involved. rs4646127, rs2158082, rs5936011, rs6629110, rs4830983 and rs5936029 are related to greater ACE2 expression and greater susceptibility to COVID-19 in East Asian, European, African, South Asian and mixed American.[130]

Besides that, allelic changes in this gene are distributed differently between men and women due to their location on the X chromosome and can interfere with COVID-19 infection.[131,132] Epidemiological data worldwide show that men are most affected by the pandemic. They have the highest rates of infection and also the highest rates of morbidity and mortality.[133] Many factors can contribute to this finding, such as cultural behaviour of exposure and self-care, pre-existing diseases, hormonal factors, genetic and phenotypic factors.[134] However, the genetic basis of *ACE2* can contribute to this scenario, such as the presence of variants related to diseases or related to increased expression. It was seen that there is a relationship between the increase in the expression of ACE2 and the risk of infection. But once infected, the higher expression of ACE2 may be a factor that contributes to a better prognosis.[135] Although many of the changes related to polymorphic variants are detected in women (Table S1 in Data S1), there is evidence that points to a greater expression of ACE2 in the female gender.[135,136] This increase in expression may be related to some polymorphic variants.

Evaluations of exon changes are still very incipient, especially with respect to SARS-CoV-2. The most recent publications bring an analysis of bioinformatics about the possibilities of exonic variants.[40,42] Some candidates have shown to be potential modifiers of the interaction of the viral protein and the ACE2 receptor, but have not yet assessed this change in the field. The data for the variants and polymorphisms can be seen in Table S1 in Data S1.

5 | EPIGENETIC REGULATION OF ACE2 EXPRESSION

Epigenetics is the study of the mitotically heritable changes in the gene function that cannot be explained by the changes in the DNA molecule.[137] One important feature of the epigenetic mechanisms is the malleability in face of an environmental change: through the cellular sensors, changes can be transmitted to the machinery involved in writing, reading and erasing these marks. In this way, the cell can adapt its functions to cope with a particular stimulus.[138,139]

DNA methylation is an epigenetic mechanism characterized by the addition of a methyl group in the 5′ carbon of the cytosine base followed by a guanine (CpG) promoted by the enzymes DNA methyltransferases.[140] Mukerjee et al have shown that the offspring of female mice fed with high-fat high-sucrose during gestation and weaning presented lower methylation of *ACE2* promoter in the brainstem, kidney and cecum at 3 months of age and higher levels of the enzyme in the hypothalamus at 7 months of age compared to the offspring of dams fed with the control diet.[141] Fan et al analysed the methylation of peripheral blood in 5 CpGs in the *ACE2* promoter (ChrX:15,621,790-15,621,942) in the context of essential hypertension and observed that the CpGs located at ChrX:15,621,822 and ChrX:15,621,814 had higher methylation in the cases than in controls.[142] Additionally, they found that control men and women differed in the methylation levels in the CpGs located at ChrX:15621814 and ChrX:15,621,846.[142] Differential *ACE2* methylation and expression were also observed in $CD4^+$ T cells of lupus patients.[143,144] Finally, it was determined that cancer may modulate *ACE2* expression through *ACE2* promoter methylation since methylation changes are concordant with the expression in several cancer types.[145] Taken together, these results suggest that *ACE2* methylation and expression may be regulated by the intrauterine life, by autoimmune, neoplastic and metabolic diseases, and by sexual factors. Concurrently, with the epidemiological data of the COVID-19 pandemics, these findings may provide some evidence to explain the differences in susceptibility and severity between the sexes and point out diseases like lupus and cancer to be risk factors for the disease.[146,147]

Histone PTMs correspond to the covalent attachment of several organic radicals mainly to the residues of the N-terminal tails that protrude from the octamer of histones that compose the nucleosome.[137] The result of the PTMs can be either repress transcription or allow it depending on the nature of the radical that is added and the residue where it occurs.[139] Rabbits treated with a high-cholesterol diet (HCD) experienced in the heart global reductions of H3S10 phosphorylation

TABLE 1 Studied miRNAs that target ACE2 in humans, cell lines and animals

miRNA	Sample type	Main findings	References
mi-200c-3p	Cell culture (HEK293T, THP1 and A549), pneumonia and control patients, C57Bl/6 mice	miR-200c-3p is upregulated in cells exposed to H5N1 virus, dsRNA, LPS and LTAvia NF-κB and in patients with severe pneumonia of various causes. miR-200c-3p inhibition improves ACE2 expression, survival and lung injury score in H5N1-infected mice	[152]
	Cell culture: neonatal rat cardiomyocytes (NRCMs), neonatal rat cardiac fibroblasts (NRCFs), human primary cardiac fibroblasts (HCFs), human umbilical vein endothelial cells (HUVECs), human-induced pluripotent stem cell-derived cardiomyocyte (hiPSC-CM)	miR-200c-3p downregulates *ACE2* mRNA and protein particularly in NRCMs and hiPSC-CM	[153]
miR-let-7b	Cell culture: pulmonary artery smooth muscle cells (PASMCs) isolated from Sprague–Dawley rats, and C57Bl/6 mice	Hypoxia upregulates miR-let-7b, which decreases *ACE2* expression in cultured cells. miR-let-7b expression depends on HIF-1α, as its inhibition partially prevents miR-let-7b upregulation. miR-let-7b induces cell migration and proliferation, whereas miR-let-7b knockout mice under hypoxic conditions presented higher ACE2 histochemical staining in the pulmonary artery wall, concomitant with thickened artery wall and higher Fulton's index	[154]
miR-1246	Cell culture (PMVEC), C57Bl/6mice	LPS treatment induces miR-1246 upregulation, which downregulates *ACE2* expression. LPS upregulates apoptosis, IL-1β and TNF-α through miR-1246, while ACE2 overexpression represses apoptosis. Anti-miR-1246-injected mice presented improved lung injury score, *ACE2* expression, vascular permeability and decreased inflammation following intrathecal LPS instillation	[155]
	Small airway epithelial (SAE) cells from smokers and nonsmoker individuals	Microarray and qRT-PCR analyses showed lower expression of miR-1246 in smoker individuals compared to nonsmokers. In the same study, *ACE2* mRNA was upregulated in smokers	[51]
miR-125b	Cell culture (HK-2 and HEK-293T)	Cells treated with high concentrations of glucose (30 mM) had higher expression of miR-125b and lower *ACE2* expression. ROS production and apoptosis in high glucose conditions are partially dependent on miR-125b expression, as its inhibition caused lower ROS production and fewer apoptotic cells	[156]
miR-421	Cell culture (HEK293T, Huh7, isolated cardiac myofibroblasts)	miR-421 upregulation caused specific ACE2 protein downregulation. miR-421 is highly expressed in the kidney; HEK293T cells have higher expression than Huh7 or isolated cardiac myofibroblasts	[157]
	Chronic kidney disease, haemodialysis and control patients; cell culture (THP1)	miR-421 levels are higher in the serum from patients with kidney disease. miR-421 serum levels are inversely correlated with *ACE2* expression in the leukocytes. THP1 cells exposed to uremic toxins increase miR-421 and decrease ACE2 expression	[158]
miR-143	Female Wistar rats	Rats subjected to mild- and high-intensity training presented higher left ventricle expression of *ACE2*, Ang (1–7), and Ang (1–7)/AngII ratio, while decreased miR-143 was observed in the left ventricle of the heart of the high-intensity group compared to the sedentary and mild-intensity groups	[159]
	Male SHR and Wistar-Kyoto rats	SHR rats subjected to moderate-intensity training showed higher ACE2 protein expression and decreased mi-143 in the aorta	[160]

TABLE 1 (Continued)

miRNA	Sample type	Main findings	References
miR-483-3p	Cell culture (HEK-293T)	miR-483-3p was predicted to target *ACE2* mRNA, besides other components of the renin-angiotensin system. Reporter assay containing the 3′ UTR of *ACE2*revealed lower luciferase expression. *ACE2* mRNA levels were not altered in cells that constitutively express miR-483-3p. The protein levels were not determined	[161]
	Cell culture (HTR-8/SVNeo)	Cell proliferation was decreased following transfection with miR-483-3p mimic	[162]
miR-429	FVB/NJ mice	Female offspring of maternal low-protein diet (MLPD) fed mice presented higher miR-429, while the male offspring presented the inverse trend compared to the offspring of normal diet-fed mice. ACE2 protein expression was lower in the MLPD female offspring	[163]

and H3 acetylation concomitantly with reduced H3 acetylation in the *ACE2* promoter.[148] Atorvastatin treatment of animals that received HCD reverted the effect of HCD on global acetylation and phosphorylation, *ACE2* promoter H3 acetylation and the protein expression in the heart.[148] High-fat diet (HFD) fed mice treated with rhACE2 showed higher H3K9 dimethylation, H3K9 acetylation and H3K27 acetylation in the subcutaneous white adipose tissue than mice of the HFD group. Moreover, the treatment with rhACE2 regulated the levels of the histone modifiers euchromatin histone lysine methyltransferase 1 (EHMT1), histone acetyltransferase GCN5 (GCN5), P300/CBP-associated factor (PCAF), and histone deacetylase 3 (HDAC3).[149] In human embryonic stem cells, knockout of the histone methyltransferase enhancer of zeste homologue 2 (EHZ2) resulted in lower levels of the repressive histone marker H3K27 trimethylation and concomitant higher ACE2 expression.[150] Pinto et al analysing publicly available transcriptomes of the lung in pulmonary diseases revealed that the expression of several histone-modifying enzymes positively correlated with *ACE2* expression.[151] In addition, epigenetic analyses found that several of the genes positively associated with *ACE2* expression, and possibly *ACE2* itself, could be regulated by the lysine demethylase 5B (KDM5B) and by the histone marks H3K27 acetylation, H3K4 methylation and H3K4 trimethylation.[151] In general, the results considering histone PTMs involved in *ACE2* regulation reveal that the chromatin around the *ACE2* locus is actively regulated by several enzymes in response to environmental cues, which may be important in the context of COVID-19. It may be particularly relevant the regulation promoted by atorvastatin in rabbits, which enhanced H3 methylation in the *ACE2* promoter and could therefore be used to increase *ACE2* expression in infected patients. It could also be relevant the modulation promoted by rhACE2 in several histone modifiers, pointing out rhACE2 - or its cleavage products - as a promising therapy in COVID-19 with effects beyond its immediate catalytic actions (see Section 5).

Non-coding RNAs are a class of RNAs that do not code a protein. Some members of this class - including microRNAs (miRNAs), small-interfering RNAs (siRNAs), long-noncoding RNAs (lncRNAs) and piwi-associated RNAs (piRNAs) - are able to regulate gene expression through various mechanisms.[138] miRNAs are small RNAs that can bind to the 3′ UTR of mRNAs and target them for degradation or impede their translation.[138] Several miRNAs have been observed to target *ACE2* mRNA (Table 1). Of note, miR-200c-3p is upregulated in patients with pneumonia of various causes. It was shown that H5N1, synthetic viral RNA, LPS and LTA can upregulate this miRNA through NF-κB.[152] This miRNA has also been observed downregulating *ACE2* mRNA in neonatal rat cardiomyocytes and human cardiomyocytes derived from induced pluripotent stem cells.[153] Taken together, these results indicate that miR-200c-3p may be a potential study target in COVID-19. Other miRNAs of the miR-200 family have been proposed to target ACE2 and might be important in COVID-19 given that they are expressed in respiratory cells.[164] miR-1246 and miR-let-7b, involved in the regulation of *ACE2*, may also be good targets in the context of the infection caused by SARS-CoV-2. miR-1246, induced by LPS, was shown to increase apoptosis of pulmonary microvascular endothelial cells, the expression of IL-1β and TNF-α and pulmonary damage in LPS-challenged mice.[155] In humans, this miRNA was reported to be downregulated in SAE cells of smoker individuals compared to nonsmokers.[51] miR-let-7b is upregulated by hypoxia factor HIF-1α and induces thickening of the wall of the pulmonary artery, increased Fulton's index and right ventricle systolic pressure following hypoxic stress in rats.[154] Although the action of these miRNAs may extend beyond inhibiting *ACE2*, some responses are dependent on the inhibition of *ACE2*, which points to this enzyme having essential roles in hypoxia and respiratory diseases.[152,154,155] Finally, the lncRNA ALT1 would also regulate ACE2 in hypoxic conditions. ALT1 was observed to co-precipitate with ACE2 protein in human umbilical vein endothelial cells. Hypoxia, HIF-1α expression, ALT1 knockdown and contact inhibition of cell proliferation inhibited the expression of ACE2; while ACE2 superexpression decreased HIF-1α, suggesting a feedback loop that could be controlled by epigenetic factors.[77] Given that hypoxia and HIF-1α upregulation are a common phenomenon in COVID-19,[80] the non-coding RNAs induced by hypoxia may be relevant targets in the disease. In addition to the miRNAs described above

and in Table 1, in silico studies conducted after the onset of the COVID-19 have proposed several other miRNAs that could target ACE2 mRNA and therefore be important in the disease pathogenesis.[165–167]

6 | POSSIBLE ROLE OF GENETIC AND EPIGENETIC CONTROL OF *ACE2* IN COVID-19

The degree of susceptibility to COVID-19 may be related to the bioavailability of ACE2 in specific organs and tissues, as is suggested by a number of transcriptomic studies.[46,49–51] Since polymorphisms and epigenetic mechanisms may impact *ACE2* expression, it is of high importance to determine these variables in the context of the infection by SARS-CoV-2.

ACE2 stood out in the context of the new pandemic due to its role in the entry of the virus into the host cells.[7,124] This knowledge came mainly from bioinformatics studies on biocompatibility between viral proteins and receptors in human cells, and previous studies on the pathogenesis of the SARS pandemic in 2002-04.[124,126,168] However, through clinical and laboratory studies during the COVID-19 pandemic, it was observed that comorbidities on patients were, in a way, related to the deregulation of the renin-angiotensin system.[121,169] This highlighted ACE2, which could be participating both in the infection process and in the progression of observed comorbidities.

Another point to consider about the role of ACE2 in COVID-19 would be the diversity of the disease's involvement between individuals and between different populations.[121,127,170] This question arouses interest in whether this variability is related to changes in bioavailability, molecular and structural composition, expression and genetic variants of the molecule.[14,118,171]

The *ACE2* gene has many genetic variants, including introns and exons, that can modify the expression process and the structure of the protein. Therefore, the presence of *ACE2* polymorphic variants in different populations can be critical for understanding the range of COVID-19 on the planet.[119] However, the evaluation of this polymorphic characteristic is still incipient in COVID-19. Some studies show that there is no association between genetic polymorphisms and SARS-CoV infection. The presence of SNPs was verified among people who developed SARS and those SARS-CoV positive without developing the disease. No differences were observed between the groups, which shows that this characteristic may not be important for the infection process.[14,172]

On the other hand, once the SARS-CoV-2 infection is installed, the evolution of the disease to symptomatic condition, and the degree of involvement of the organism may be related to the *ACE2* genetic polymorphisms. These polymorphisms have been associated in several populations with hypertension, stroke, diabetes, cardiac alterations and lesions and the involvement of related organs, such as lungs and kidneys. It has been reported that patients with a higher degree of impairment have cardiac, pulmonary and renal changes, in addition to changes in blood pressure.[173–175] Possibly, patients who have polymorphic genetic variations related to these conditions may suffer from such comorbidities due to a functional overload on ACE2, whose function is impaired by virus interference.[118] Such relationships remain in the scope of hypotheses since there are no studies in the literature that correlate the distribution of SNPs in critically ill patients affected by COVID-19 and the comorbidities presented.

Among the epigenetic mechanisms regulating *ACE2* expression, miRNAs are the most studied at the present and have shown results that might be important in the context of SARS-CoV-2 infection. Hypoxia and its regulated miRNA and lncRNA, for example, maybe clinically relevant in the infection, as important features of pulmonary function that could be affected by the virus are dependent on the regulation of these molecules.[77,154] Smoking may also be relevant due to miRNA modulation.[51] Inflammation, important in SARS-CoV-2 infection, influences ACE2 expression as previously described.[82,89–91,176] It was shown that NF-κB activation due to viral and bacterial stimuli impairs *ACE2* expression via miRNA regulation.[152] The same miRNA - miR-200c-3p - was shown to be increased in patients with pneumonia of various etiologies and to regulate inflammation in the lungs of H5N1-infected mice, making this miRNA a potential candidate in the context of SARS-CoV-2.[152] Studies focusing on DNA methylation have shown that sex, diet and underlying diseases can change this epigenetic mark and *ACE2* expression accordingly.[141,142,144,145] Recent articles have proposed that *ACE2* methylation and expression changes in lupus and cancer may be important in the context of COVID-19.[143,145] Histone PTMs are also being investigated and the studies have observed that diet, pharmacologic agents and lung disease can regulate histone PTMs in *ACE2*.[148,149,151] Atorvastatin, which increased H3 acetylation in the *ACE2* locus and its expression in rabbits, could be a promising drug to help treat COVID-19.[148] There is a registered clinical trial (NCT04380402) to study the effects of atorvastatin administration in COVID-19 patients but no results were published yet. rhACE2 is also promising since it could, in addition to potentially rescue ACE2 activity loss in the SARS-CoV-2 infection, regulate the levels of histone modifiers and promote beneficial metabolic outcomes.[149] However, there is not a conclusive study evaluating whether variations in non-coding RNAs, DNA methylation and histone PTMs targeting *ACE2* are important in COVID-19 patients.

In addition to underlying conditions playing a role in *ACE2* epigenetic regulation, it was shown that coronaviruses are able to induce expression changes through epigenetic mechanisms in cell culture.[177] Remarkably, interferon-stimulated genes (ISGs) – *ACE2* is an ISG - are differentially modulated by respiratory viruses through histone PTMs and DNA methylation.[50,177,178] Indeed, a mice model expressing human ACE2 (hACE2) in its membranes infected with SARS-CoV-2 revealed that genes related to the cellular response to type I interferon and type I interferon signalling pathway are upregulated as short as 3 days after infection.[179] This study also showed that the infection is able to regulate DNA methylation in an organ-specific manner. However, it remains to be determined the factors and pathways that modulate the epigenetic mechanisms in viral infections and whether *ACE2* is epigenetically regulated by SARS-CoV-2 in humans.

7 | CONCLUSION

The current pandemic of COVID-19 demands new approaches in the diagnosis and treatment of the most severely affected patients. To face the drastic consequences of this disease, detailed knowledge about the pathogenesis, and the processes that involve the molecular activity of targets such as ACE2 is essential. ACE2 has vast variability in the way it can be bioavailable in different organs and tissues. Part of this variability may be due to epigenetic mechanisms. Although DNA methylation, histone PTMs and, particularly, miRNAs have been implicated in *ACE2* expression regulation, no comprehensive study determining these markers has been conducted in COVID-19 patients yet. Similarly, the studies investigating *ACE2* polymorphisms have not been extensive enough to determine the variants of this gene that correlate with higher susceptibility or disease severity. In addition to this, finding ways to maintain ACE2 expression in face of infection may be important, given the protective actions peptides converted by this enzyme promote. Thus, the careful study of the forms of genetic and epigenetic regulation of this protein, such as SNPs and miRNAs, can be of great importance.

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CONFLICT OF INTEREST

The authors declare that they have no conflict of interest.

DATA AVAILABILITY STATEMENT

Data sharing is not applicable to this article as no new data were created.

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SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section at the end of this article.

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