THEMATIC REVIEW

Impact of ACE2 on the susceptibility and vulnerability to COVID-19

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Abstract

Angiotensin-converting enzyme 2 (ACE2) is not only the viral receptor for the novel coronavirus severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) but is also classically known as a key carboxypeptidase, which through multiple interacting partners plays vital physiological roles in the heart, kidney, lung, and gastrointestinal tract. An accumulating body of evidence has implicated the dysregulation of ACE2 abundance and activity in the pathophysiology of multiple disease states. ACE2 has recently regained attention due to its evolving role in driving the susceptibility and disease severity of coronavirus disease 2019 (COVID-19). This narrative review outlines the current knowledge of the structure and tissue distribution of ACE2, its role in mediating SARS-CoV-2 cellular entry, its interacting partners, and functions. It also highlights how SARS-CoV-2-mediated dysregulation of membrane-bound and circulating soluble ACE2 during infection plays an important role in the pathogenesis of COVID-19. We explore contemporary evidence for the dysregulation of ACE2 in populations that have emerged as most vulnerable to COVID-19 morbidity and mortality, including the elderly, men, and pregnant women, and draw attention to ACE2 dynamics and discrepancies across the mRNA, protein (membrane-bound and circulating), and activity levels. This review highlights the need for improved understanding of the basic biology of ACE2 in populations vulnerable to COVID-19 to best ensure their clinical management and the appropriate prescription of targeted therapeutics.

Key Words

- angiotensin-converting enzyme 2 (ACE2)
- COVID-19
- age
- sex
- pregnancy

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Introduction

Angiotensin-converting enzyme 2 (ACE2) is a critical regulator of cardiovascular, respiratory, renal, and gastrointestinal physiology and pathology, through its actions as part of the renin–angiotensin system (RAS) and kallikrein–kinin system, among others. The enzymatic activity of ACE2 is protective against cardiovascular and kidney diseases as well as acute respiratory distress syndrome (Oudit *et al.* 2003, Kuba *et al.* 2010, 2013). ACE2 is also the receptor for several coronaviruses, including human coronavirus NL63 (HCoV-NL63), severe acute respiratory syndrome coronavirus (SARS-CoV), and severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) (Li *et al.* 2003, Hoffmann *et al.* 2005, 2020*b*, Zhou *et al.* 2020*a*).

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ACE2 can be found in most tissues and organs of the body, including the lung, cardiovascular system, gastrointestinal tract, renal system, brain and nervous system, liver, pancreas, gallbladder, as well as the male and female reproductive tracts (Donoghue *et al.* 2000, Tipnis *et al.* 2000, Komatsu *et al.* 2002, Hamming *et al.* 2004, Beyerstedt *et al.* 2021), thereby allowing coronaviruses to access a wide variety of tissues and organ systems. A novel short isoform of ACE2, delta ACE2 (dACE2), that is enriched in lung airway and bile duct epithelia in the liver has recently been identified; however, dACE2 lacks SARS-CoV-2 binding sites and is unlikely to directly contribute to host susceptibility to SARS-CoV-2 infection (Onabajo *et al.* 2020, Williams *et al.* 2021). A soluble form of ACE2, sACE2, can also be found in extracellular compartments and may serve as a biomarker of certain disease states, including hypertension and heart failure (Uri *et al.* 2014).

Importantly, binding of the SARS-CoV-2 spike protein to membrane-bound ACE2 reduces surface ACE2 expression, resulting in loss of the protective actions of ACE2. Thus, ACE2 is not only the SARS-CoV-2 receptor but might also play an important role in multiple aspects of coronavirus disease 2019 (COVID-19) pathogenesis and possibly post-COVID-19 syndromes. Furthermore, the relative levels of tissue ACE2 and sACE2 may be a factor in determining the susceptibility to certain COVID-19 pathologies.

In this narrative review we outline the current knowledge surrounding ACE2 as the receptor for SARS-CoV-2, its interacting partners, hormonal regulation, and functions, highlighting the importance of ACE2 dysregulation in the pathogenesis of COVID-19. We also discuss the plethora of evidence demonstrating that ACE2 drives COVID-19 age, sex, and pregnancy-associated vulnerabilities and the culminating need for greater understanding of ACE2 and its pathophysiological role. The objective of this narrative review was to explore changes in the expression and regulation of ACE2 that may drive vulnerability to COVID-19 morbidity. As such, we undertook a comprehensive broad-sweeping literature search through PubMed using key words including ACE2, hormones, sex (or gender), age, pregnancy, COVID-19, and SARS-CoV-2, which were distilled into the following narrative.

ACE2 as the entry receptor for coronaviruses

ACE2 serves as a viral receptor for the SARS-CoV (Li *et al.* 2003), HCoV-NL63 (Hofmann *et al.* 2005), and SARS-CoV-2 (Hoffmann *et al.* 2020*b*, Zhou *et al.* 2020*a*). Both SARS-CoV and SARS-CoV-2 are structurally and genetically highly similar; both are enveloped single-stranded RNA viruses that carry a transmembrane spike protein on their surface. The binding of the spike protein to ACE2 on the host cell surface leads to viral entry into cells, a process that is required to establish infection (Hoffmann *et al.* 2020*b*). The spike protein (S) consists of an S1 region containing the receptor-binding domain (RBD), which binds ACE2, and an S2 region that promotes fusion of the viral membrane with the host cell (Walls *et al.* 2020). Membrane fusion between host cells and the infectious virus particles requires spike protein priming, which involves cleavage at the S1/S2 and the S2′ site by proteases such as furin (Hoffmann *et al.* 2020*a*), transmembrane protease serine 2 (TMPRSS2), and cathepsin (Simmons *et al.* 2005, Matsuyama *et al.* 2010, Shulla *et al.* 2011, Glowacka *et al.* 2011, Hoffmann *et al.* 2020*b*, Shang *et al.* 2020, Peacock *et al.* 2021, Zhao *et al.* 2021, Jackson *et al.* 2022). Spike protein priming allows fusion of viral and cellular membranes, a process driven by the S2 subunit. Although the SARS-CoV-2 RBD binds to ACE2 with a greater affinity than the SARS-CoV RBD, the spike protein itself has an affinity similar to or less than SARS-CoV (Shang *et al.* 2020). The prefusion spike protein trimer fluctuates between the three RBD down (closed) conformations, which enhances its ability to evade immune defence mechanisms, and the one RBD up (open) conformation, which is required to bind to ACE2 (Fan *et al.* 2020, Walls *et al.* 2020). The importance of ACE2 and TMPRSS2 to viral entry has been demonstrated through gene knockout studies. Exposure of *Ace2* null mice to SARS-CoV infection results in lower infectivity and viral copy number in the lungs, and reduced pathological lung injury, compared to wild-type mice (Kuba *et al.* 2005). Similarly, mice lacking *TMPRSS2* show lower viral titre in the lungs, a weaker proinflammatory response, and less severe lung pathology following SARS-CoV infection, compared to wild-type animals (Iwata-Yoshikawa *et al.* 2019).

ACE2 is not only critical to initial viral infection through surface receptor binding but also to COVID-19 progression following changes in membrane-bound and sACE2 action and their interactome. SARS-CoV and SARS-CoV-2 affect ACE2 levels in two ways. SARS-CoV-2 binding to ACE2 causes endocytosis of ACE2 alongside viral particles into endosomes and induces shedding of the ACE2 ectodomain (Haga *et al.* 2008, Wang *et al.* 2008). ACE2 shedding of the catalytically active ectodomain is mediated by A disintegrin and metalloprotease 17

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(ADAM17; also known as TACE (tumour necrosis factor alpha-converting enzyme)) both *in vitro* and *in vivo* (Lambert *et al.* 2005, Jia *et al.* 2009).

In mice, SARS-CoV infection reduces ACE2 protein in the lungs, following spike protein binding and subsequent downregulation of transmembrane ACE2, exposing them to severe lung injury through the pathological impacts of RAS deregulation (Kuba *et al.* 2005). Through gene knockout and recombinant protein rescue studies, downregulation of lung ACE2 following severe acute lung injury has been shown to drive lung disease pathogenesis, promoting oedema and impaired lung function (Imai *et al.* 2005). In turn, ACE2 cleavage from the cell surface by interacting enzymes (e.g. TMPRSS2) and shedding results in increased sACE2 in the plasma (Heurich *et al.* 2014, Kragstrup *et al.* 2021, Daniell *et al.* 2022) and saliva (Daniell *et al.* 2022) of patients with COVID-19. A recent study has shown that sACE2 activity in plasma remains elevated for at least 2–3 months after SARS-CoV-2 infection and is associated with disease severity (Patel *et al.* 2021), suggesting that there is prolonged shedding of ACE2 and potentially prolonged dysregulation of tissue RASs. There is a major gap in the literature surrounding ACE2 changes with long COVID (long COVID/long-hauler COVID/post-acute sequelae of SARS-CoV-2); inevitably, this will be a key research question in time, as more follow-up datasets of patients in recovery and post-recovery from COVID-19 become available to the scientific community. Given the multifaceted downregulation of ACE2 triggered by SARS-CoV-2 infection, ACE2 dysregulation has been proposed to play a central role in the pathogenesis of COVID-19 and in driving vulnerability to severe COVID-19 illness in at-risk populations, likely contributed to by knock-on effects for the ACE2 interactome.

ACE2 and the renin–angiotensin system

ACE2 was first discovered as an enzyme involved in the RAS. Two independent genomic screens led to the discovery, expression, and characterisation of human ACE2 just over 20 years ago (Donoghue *et al.* 2000, Tipnis *et al.* 2000). The predicted ACE2 protein sequence revealed characteristic features of a plasma membrane-bound zinc metallopeptidase with 41.8% similarity to the N-terminal catalytic domain of angiotensin-converting enzyme (ACE) (Donoghue *et al.* 2000, Tipnis *et al.* 2000, Turner & Hooper 2002). Like ACE, ACE2 is a single-span transmembrane protein with an extracellular facing N-terminal catalytic site available to hydrolyse circulating vasoactive and other regulatory peptides. However, in contrast to ACE, ACE2 functions exclusively as a carboxypeptidase, removing a single C-terminal amino acid from angiotensin (Ang) II generating Ang-(1–7) or, much less efficiently, from Ang I forming Ang-(1–9) (Turner 2015) (Fig. 1).

Ang II is the major peptide of the Ang II type 1 receptor (AT_1R) axis of the RAS cascade. Ang II acting via the AT_1R is important in cardiovascular homeostasis but when unbalanced it can cause vasoconstriction, inflammation, oxidative stress, cell proliferation, hypertrophy, fibrosis, and tissue remodelling (Dasgupta & Zhang 2011). ACE2 metabolises and thus limits the abundance of Ang II. It converts Ang II into Ang-(1–7), which counterbalances the actions of Ang II via the Mas receptor (Fig. 1). Therefore, ACE2 protects against organ damage via two processes: (i) by metabolising Ang I and Ang II, so limiting substrate availability to the AT_1R axis, and (ii) by generating Ang-(1–7), which increases the activity of the Mas receptor axis and has protective roles that stimulate vasodilatation and antioxidant pathways and inhibit inflammation, fibrosis, and hypertrophy (Simões e Silva *et al.* 2013). Thus, dysregulation of ACE2, as seen in COVID-19 patients, results in loss of the protective actions of ACE2 in the RAS.

ACE2 and its other regulatory peptides

ACE2 has a diverse substrate specificity and hydrolyses several other regulatory peptides (Fig. 1). These include apelin peptides, des-Arg^9-BK (but not bradykinin (BK) itself), neurotensin metabolites, dynorphin A (1–13), and ghrelin (Donoghue *et al.* 2000, Vickers *et al.* 2002). Whether the cleavage of neurotensin metabolites, dynorphin A (1–13), and ghrelin occurs *in vivo* remains unexplored; thus, the functions of these peptides will not be discussed.

Apelin is an inotropic and cardioprotective peptide. It acts on the apelin receptor (APJ) to regulate vascular homeostasis, angiogenesis, myocardial adaptation to stress, and fluid balance, thereby playing a key role in vascular diseases, such as systemic and pulmonary arterial hypertension, myocardial infarction, and heart failure (Pitkin *et al.* 2010, Wang *et al.* 2016*b*) (Fig. 1). Apelin is synthesised as a pre-pro-hormone, which is processed into a mature peptide, apelin-36, that is further proteolytically cleaved to apelin-13 and apelin-17 (Tatemoto *et al.* 1998, Lee *et al.* 2000, Lee *et al.* 2006, Pitkin *et al.* 2010). ACE2 hydrolyses apelin-13, apelin-17, and apelin-36 peptides with high catalytic efficiency

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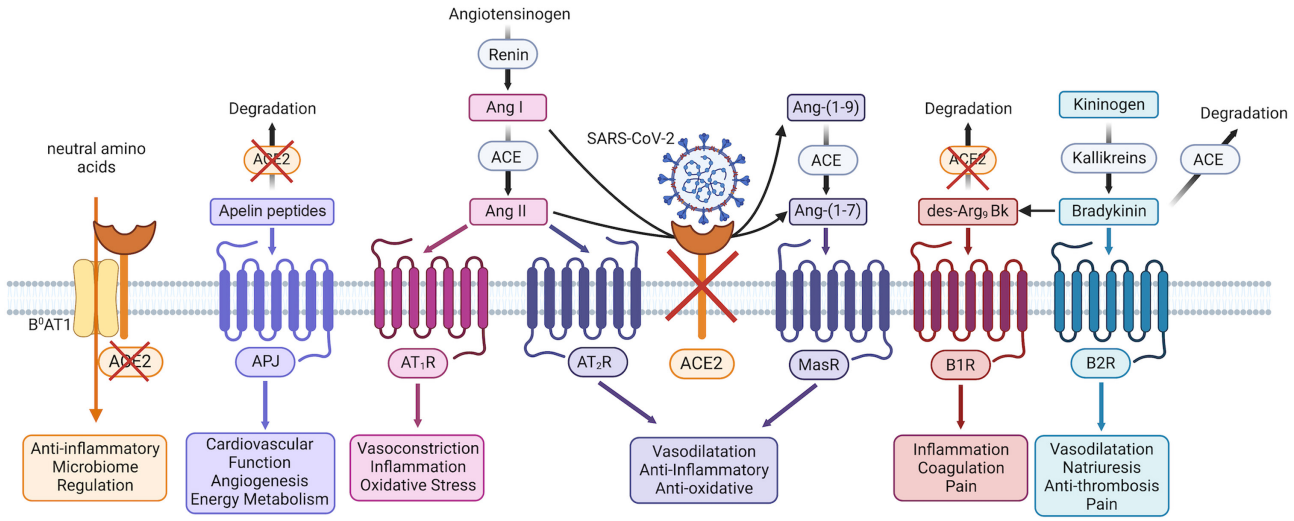

Figure 1

The impact of SARS-CoV-2 on the actions of ACE2 and its interacting partners. Upon entry into cells, SARS-CoV-2 infection causes internalisation and shedding of ACE2 from the cell membrane (indicated by the red crosses). This prevents the conversion of angiotensin (Ang) II to Ang-(1–7) by ACE2 and promotes the actions of Ang II via the angiotensin II type 1 receptor (AT_1R). SARS-CoV-2-mediated downregulation of ACE2 also prevents its degradation of both apelin peptides and des-Arg^9-bradykinin (des-Arg^9-BK), which acting via the bradykinin 1 receptor (B1R) causes inflammation, coagulation, and pain. Finally, loss of ACE2's actions in the intestine may cause diarrhoea, nausea, and vomiting, by promoting inflammation and altering the microbiome. Adapted from *American Journal of Pathology*, volume 191, Ramos SG, Cruz Rattis BA de, Ottaviani G, Nunes Celes MR, Dias EP, ACE2 down-regulation may act as a transient molecular disease causing RAAS dysregulation and tissue damage in the microcirculatory environment among COVID-19 patients, pages 1154–1164, 2021, with permission from Elsevier (Ramos *et al.* 2021). Created with BioRender.com. ACE2, angiotensin-converting enzyme 2; SARS-CoV-2, severe acute respiratory syndrome coronavirus 2.

(Vickers *et al.* 2002, Wang *et al.* 2016*b*, Yang *et al.* 2017) (Fig. 1). Although an initial investigation suggested that cleavage of apelin-13 and -17 by ACE2 partially inactivates these peptides (Wang *et al.* 2016*b*), it was subsequently found that apelin-12, the product of apelin-13 cleavage by ACE2, retains biological activity both *in vitro* and *in vivo* (Yang *et al.* 2017). At present, it is unclear of the significance of the cleavage of apelin peptides by ACE2 for the pathogenesis of COVID-19. Apelin could be protective, as it inhibits pulmonary fibrosis in mice by activating ACE2 (Wang *et al.* 2022*a*). However, like ACE2, apelin is downregulated in SARS-CoV-2-infected cardiomyocytes and COVID-19 patients (Li *et al.* 2021*b*).

ACE2 also plays critical roles in the kinin–kallikrein system, which constitutes the inactive precursor kininogen, the proteolytic kallikrein enzymes, and effector peptides bradykinin and its active metabolite des-Arg^9-BK (Schmaier 2002, Marceau *et al.* 2020). Bradykinin can be degraded by ACE (Ahmad *et al.* 2006), whereas ACE2 hydrolyses des-Arg^9-BK, generating biologically inactive breakdown products (Vickers *et al.* 2002, Sodhi *et al.* 2018) (Fig. 1). Bradykinin and des-Arg^9-BK act on their target organs via two pharmacologically distinct G protein-coupled receptors: the B1 receptor (B1R), whose main agonist is des-Arg^9-BK; and the B2 receptor (B2R), whose ligand is bradykinin (Manning & Snyder 1989, Mantione & Rodriguez 1990, Rhaleb *et al.* 2011). Bradykinin is a potent endothelium-dependent vasodilator that possesses vasorelaxant and natriuretic activity (Hornig & Drexler 1997). Bradykinin is also part of the inflammatory response after injury and acts to induce pain (Dray & Perkins 1993), neutrophil recruitment, and vascular permeability (Araujo *et al.* 2001, Stuardo *et al.* 2004, Choi & Hwang 2018). The B2R is constitutively expressed and is involved in the acute phase of inflammatory and pain responses, whereas the B1R is markedly upregulated during inflammation and tissue injury and is involved in the chronic phase of the inflammatory response (Dray 1997). Activation of B1R by des-Arg^9-BK can stimulate inflammation by modulating leukocyte migration, microglial activation, and cytokine production (Sriramula 2020). Thus, by degrading des-Arg^9-BK, ACE2 prevents this immune response (Fig. 1). Attenuation of ACE2 activity has been shown to impair des-Arg^9-BK inactivation and thus to enhance B1R signalling in the lung (Sodhi *et al.* 2018). This could be a possible mechanism by which SARS-CoV-2, via downregulation of ACE2, could promote chronic inflammation in the lung.

ACE2 also regulates aspects of amino acid transport (Fig. 1) as its carboxy-terminal domain shows 48% sequence identity with collectrin (Zhang *et al.*

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2001). Collectrin regulates B_0AT1 neutral amino acid transporters in the kidney (Danilczyk *et al.* 2006, Malakauskas *et al.* 2007) and pancreatic insulin secretion (Fukui *et al.* 2005). Conversely, ACE2 binds to the B_0AT1 amino acid transporters in the intestine but not kidney (Kowalczuk *et al.* 2008, Camargo *et al.* 2009) and has been implicated in the pathology of Hartnup's disease, a disorder of amino acid homeostasis. Through this process, ACE2 also appears to regulate intestinal inflammation and diarrhoea, hence modulating the gut microbiome (Hashimoto *et al.* 2012). In patients with COVID-19, dysregulation of ACE2 and thus amino acid transport in the gastrointestinal tract may therefore contribute to the diarrhoea, nausea, and vomiting seen in some cases (Mao *et al.* 2020).

ACE2 and ageing

Changes in ACE2 expression with age

Even prior to the COVID-19 pandemic, age-associated changes in ACE2 expression were described in developing rodents. In CD1 mice, Song *et al.* (2012) reported *Ace2* expression in the lung was lowest in the embryo, increasing into early postnatal life and peaking in adulthood (2 months) (Song *et al.* 2012). Alternatively, Inde *et al.* (2021) showed a biphasic age-driven distribution of *Ace2* in murine lungs and trachea across three different gene expression datasets (Inde *et al.* 2021). First, there was a subtle biphasic distribution in two of the three datasets where lung and tracheal *Ace2* expression was lowest in adolescence (<day (d) 15), with ascending expression in the lung from late adolescence through to middle age (Inde *et al.* 2021). In contrast, there was a distinct biphasic distribution of lung *Ace2* in the third dataset encompassing d0–30, where peak *Ace2* mRNA levels were observed at d1 post-partum compared to lower expression in prenatal and d10 postnatal mice, increasing towards late adolescence (d30). Irrespective of the dataset, *Ace2* expression is low in murine lung homogenates (Inde *et al.* 2021), which likely contributes to the enhanced variability seen across individuals and datasets.

In contrast to mRNA observations, at the protein level ACE2 was lower in lung homogenates of adult CD1 mice compared with postnatal and embryonic tissue (Song *et al.* 2012). Similarly, in Sprague–Dawley rats, ACE2 was lowest in lung homogenates of elderly rats (24 months) compared to middle-aged (12-month-old) and young adult (3-month-old) rats, irrespective of gender (Xudong *et al.* 2006). However, Inde and colleagues (2021) demonstrated, by immunofluorescent staining of ACE2 protein, that ACE2 abundance slightly increased immediately after birth, dipped at postnatal d7, and rose into adulthood (3 months) (Inde *et al.* 2021). In contrast, Bengs *et al.* (2021) observed no significant change in lung ACE2 immunoreactivity in FVB/N mice with age (4, 12, and 20–22 months), irrespective of gender (Bengs *et al.* 2021), albeit the spread of immunoreactivity was highly variable across samples.

ACE2 activity was also variable across age, markedly peaking at embryonic d14.5 followed by a second peak at d10 post-partum in CD1 mice (Song *et al.* 2012). Circulating sACE2, however, was lowest in adult mice compared to young postnatal CD1 mice (Song *et al.* 2012). These rodent studies are summarised in Fig. 2 and highlight that ACE2 dynamics with ageing heavily depend on the transcriptional, translational, activity, and soluble read-outs of ACE2. Further to this, significant variability exists between studies and may reflect the differences in study design, including ages of tissue collection, species, and strain. Great care should thus be taken in interpreting contrasting literature demonstrating ACE2 abundance in SARS-CoV-2 patients across risk groups.

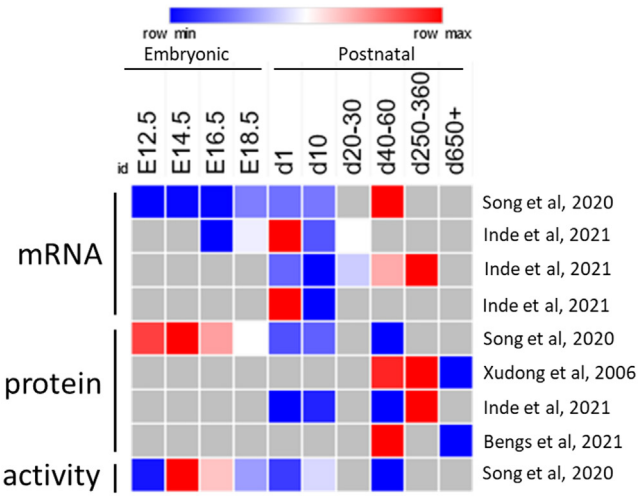

Figure 2
Longitudinal age impact on ACE2 mRNA, protein, and activity in the lungs of rodents. ACE2 mRNA, protein, and activity levels were collated from available rodent studies demonstrating changes with embryonic (embryonic day (E)) and postnatal age (days, d). Morpheus (Broad Institute, https://software.broadinstitute.org/morpheus) and approximate mean ACE2 levels in the lungs were used to construct a longitudinal heat map over age, normalised by row. Data were distilled from Song *et al.* (mouse lung), Inde *et al.* (multiple datasets of mouse lung), Xudong *et al.* (rat lung), and Bengs *et al.* (mouse lung) (Xudong *et al.* 2006, Song *et al.* 2020, Bengs *et al.* 2021, Inde *et al.* 2021).

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Consistent with observations in rodents, adult humans have shown significantly greater *ACE2* expression in nasal and respiratory tissue biopsies than children (Saheb Sharif-Askari *et al.* 2020). *ACE2* mRNA levels were reported to be at their lowest in the nasal epithelium of children <10 years of age, and increased into adulthood (Bunyavanich *et al.* 2020). Leveraging single cell (sc) RNAseq, the percentage of cells expressing *ACE2* transcript was greatest in lung alveolar type 2 (AT2) cells, and a higher proportion of positive cells were observed in adult samples, compared to paediatric lungs (Wang *et al.* 2020). Likewise, aggregated scRNAseq and single nucleus (sn) RNAseq of 1,320,896 cells from human nasal, airway, and lung samples from 228 individuals demonstrated increased *ACE2* expression with age in the AT2 and multi-ciliated cells of the airway (Muus *et al.* 2021). Similarly, in human airway epithelium, *ACE2* mRNA expression increased with increasing age; this increase was also observed at both the mRNA and protein levels in endobronchial biopsies (Wark *et al.* 2021). In human samples from autopsies spanning from the fetus (28 weeks' gestation) to early adulthood (28 years), ACE2 immunostaining increased in the lung with age, plateauing from 5 years of age onwards (Schurink *et al.* 2022). Using lung tissue microarrays immunofluorescently stained for ACE2, Inde and colleagues (2021) also demonstrated a rise in the percentage of ACE2-positive nucleated cells in adults aged ≥40 years, compared to children, adolescents, and young adults (Inde *et al.* 2021). However, in a handful of studies, no age-associated changes in ACE2 were observed (Barker & Parkkila 2020, Lee *et al.* 2020, Li *et al.* 2020*b*, Maremanda *et al.* 2020). Furthermore, in one study, children under 10 years of age exhibited greater ACE2 protein levels than adults (Ortiz *et al.* 2020). The reviewed studies highlight fundamental contradictions across multiple studies, which may be influenced by low sample size and thus power in some studies, but a more profound driver of these conflicting results is the inherent heterogeneity of *ACE2* expression between individuals, and between cell and tissue types within individuals (Inde *et al.* 2021). The diversity of ACE2 through the human respiratory tract was highlighted by Oritz *et al.* (2020), whereby ACE2 was sparse on the surface epithelium of the upper respiratory tract (trachea, bronchi – 12% and 27% of donors, respectively) but more abundant in the distal lung (bronchiolar, alveolar – 36% and 59% of donors) (Ortiz *et al.* 2020). By scRNAseq, alveolar *ACE2* was also heterogeneous across cell types and donors, where 50% of subjects possessed low *ACE2* transcript in AT2 cells, while the remainder had high *ACE2* (Ortiz *et al.* 2020).

ACE2 abundance in the circulation has also been stratified by age in a handful of studies. A weak curvilinear association of sACE2 abundance was observed across age with a positive association in participants below 55 years old and a negative association in those above 55 years (AlGhatrif *et al.* 2021). In healthy human subjects, serum ACE2 was markedly higher in infants (<1 year of age) compared to levels in children (6–10 years), teens (11–15 years), and adults (Gu *et al.* 2021). In stark contrast, ACE2 protein was reported to be significantly more abundant in healthy adults than in infants and toddlers (Pavel *et al.* 2021). Similarly, sACE2 abundance across five longitudinal youth aging assessment points ranging from age ~9.9 years to ~23.5 years showed low sACE2 levels up to age 12 years, with adolescence inducing an increase in sACE2 in young men but not women (Sward *et al.* 2020). In a genome-wide association meta-analysis of 28,000 individuals, however, plasma ACE2 levels were not significantly associated with age (Yang *et al.* 2022*b*). In contrast to sACE2 abundance, even fewer studies have examined sACE2 activity with aging. In one study, patients hospitalised for COVID-19 demonstrated that the activity of serum ACE2 was higher with increasing age, irrespective of disease severity (Fagyas *et al.* 2022). However, when relationships were assessed separately in each disease severity group, no correlation between circulating ACE2 activity and age was detected (Fagyas *et al.* 2022). Like that observed for membrane-bound ACE2, it should be noted that sACE2 levels exhibit a high degree of heterogeneity across human subjects.

Influence of age on the susceptibility to COVID-19

Since the pandemic start, age has been considered a major independent risk factor associated with intensive care unit (ICU) admission and mortality, with the aging population exhibiting more severe COVID-19, especially in contrast to children and youths. Children have consistently been reported as most protected from COVID-19 infection and severe illness and mortality (Wu & McGoogan 2020, Milani *et al.* 2021, Martin *et al.* 2022). Early in the pandemic, across Mainland China, increased disease severity resulting in ICU admission, mechanical ventilation, or death was mostly reported in patients >50 years of age, accounting for 81.5% of cases, in comparison to infants and children (0–15 years), in whom there was zero incidence (Guan *et al.* 2020). In Italy,

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99.1% of mortality cases were in patients >50 years of age, while the aging population was considered a key contributor to the higher mortality rates observed in Italy at the pandemic onset (Onder *et al.* 2020). Across France, of 16,386 SARS-CoV-2 recorded deaths, 81.6% were in patients >70 years old (Raparelli *et al.* 2020). Individuals >70 years of age also contributed to 50% of a total 95,120 hospitalisations, and mathematical modelling predicted an infection fatality ratio of 0.001% in those <20 years of age, compared to 8.3% in those aged >80 years (Raparelli *et al.* 2020). In the UK, increasing age was a strong predictor of mortality across a hospital cohort of 20,133 COVID-19 patients after adjusting for major comorbidities (reference age <50 years; 50–59, 60–69, 70–79, ≥80 years; hazard ratios 2.63, 4.99, 8.51, 11.09 respectively) (Docherty *et al.* 2020). Furthermore, a comprehensive study of US adults hospitalised with COVID-19 ($n=2491$; 154 acute care hospitals, 74 counties, 13 states) showed that adults with the greatest risk of ICU admission included patients aged 50–64, 65–74, 75–84, and >85 years vs 18–39 years (adjusted risk ratio (aRR) 1.53, 1.65, 1.84, and 1.43 respectively) and those at increased risk of mortality were aged 50–64, 65–74, 75–84, and ≥85 years vs 18–39 years (aRR 3.11, 5.77, 7.67, and 10.98, respectively) (Kim *et al.* 2020). The increased vulnerability of the aged population remains evident in today's latest COVID-19 mortality statistics depicted for the United States (CDC 2022) in Fig. 3. This increased risk in aged populations is stark compared with the low risk in children and infants whom typically contract mild disease (Götzinger *et al.* 2020) and as a population have experienced deaths from COVID-19 at a low 0.48% of the estimated total mortality from all causes in a normal year (Bhopal *et al.* 2021).

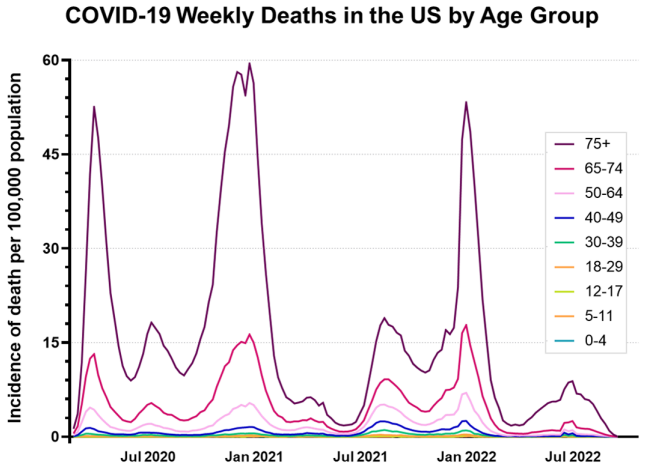

Figure 3
Population statistics for the United States showing the influence of age on SARS-CoV-2 mortality across the COVID-19 pandemic to date. Graph represents COVID-19 incident deaths per 100,000 of the population across the pandemic to date (1 March 2020 to 24 Sept 2022), stratified by age 0–4, 5–11, 12–17, 18–29, 30–39, 4049, 50–64, 65–74, and 74+ years. Analysed from data obtained from the US Centers for Disease Control and Prevention COVID Data Tracker (CDC 2022).

Inherent differences in ACE2 abundance with age may in part underlie the increased morbidity and mortality observed in older patients with COVID-19. In mice, exposing aged animals to three spike proteins representing different SARS-CoV strains resulted in increased viral titre in the lungs post infection, compared to young mice (Rockx *et al.* 2009). In lung epithelial cells, SARS-CoV infection reduced *ACE2* expression 24- and 48-h post infection (Saheb Sharif-Askari *et al.* 2020). Furthermore, in aged mice, *ACE2* mRNA was downregulated up to five-fold 48-h post SARS-CoV infection (Rockx *et al.* 2009). In rhesus macaques, SARS-CoV-2 infection reduced the frequency and density of ACE2-positive cells in both young and old lungs by ~30–50% (Zheng *et al.* 2021). However, in aged but not young macaques, this coincided with increased expression of cellular mediators of apoptosis and autophagy, increased inflammatory marker infiltration, and more severe lung lesions (Zheng *et al.* 2021). ACE2 clearly plays a pivotal role in the susceptibility and vulnerability of the aged population to coronavirus infection; however, a greater understanding of its dynamics across infection is needed, especially in considering ACE2 modulation as a therapeutic target.

While age has been determined to be an independent predictor of poor outcome from SARS-CoV-2 infection, the risk of severe COVID-19 outcomes is increased in those with certain underlying health conditions and especially in those over 65 years of age. Across 1100 studies, it was found that having at least one comorbidity, including hypertension, heart disease, obesity, type 2 diabetes mellitus, chronic lung disease, or cancer, resulted in significantly worse outcomes in COVID-19 hospitalised patients (Mason *et al.* 2021). In fact, in one study, the risk of COVID-19-related death associated with at least one age-related comorbidity combined was 1113 times greater compared to that with no comorbidity (Antos *et al.* 2021). The top four comorbidities observed included hypertension, diabetes, cardiovascular disease, and chronic kidney disease (OR 34.73, $P=0.002$; OR 20.16; $P < 0.00001$; OR 18.91, $P=0.002$; OR 12.34, $P < 0.00001$, respectively) (Antos *et al.* 2021). Circulating ACE2 levels have been shown, even prior to the COVID-19 pandemic, to be dysregulated in patients with these disease states (Uri *et al.* 2016, Chirinos *et al.* 2020,

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Emilsson *et al.* 2021, Arefin *et al.* 2022). Thus, the age-related risks associated with COVID-19 susceptibility and outcomes are likely to be due, at least in part, to the increased number of underlying health conditions in the ageing population as well as changes in ACE2 levels.

Like the age disparity in COVID-19 severity, the gender disparity has shone the spotlight on ACE2 expression and its underlying contribution to severe disease.

ACE2 and biological sex

Changes in ACE2 expression with sex

Localisation of the *Ace2* gene on the X-chromosome (Komatsu *et al.* 2002) has led to speculation for potential X-driven gene dosing and heterozygosity across *ACE2* copies. This may offer SARS-CoV-2 protection in females, but not males, which, in addition to the X-chromosome featuring many immune function-associated genes, has compounded theories of females having greater immunological advantage than males (Wang *et al.* 2016*a*, Gemmati *et al.* 2020). While further research into the genomic impacts of gender-based copy number variations and X-inactivation is required to improve our understanding of ACE2 in human COVID-19 pathophysiology, in mice, renal ACE2 activity was shown to be greater in male kidneys than in female kidneys, with no significant link to X-chromosome gene dosage (Liu *et al.* 2010).

Studies assessing the effect of biological sex, referred to herein as sex, in the rodent respiratory tract have shown a degree of sexual dimorphism across studies (summarised in Table 1). In C57BL6/J mice, females exhibited significantly greater *Ace2* mRNA expression in the lung and oesophagus, and tended to have increased *Ace2* mRNA levels in the trachea, compared to males (Sarver & Wong 2021). Similarly, lung *Ace2* expression was higher in females than males in adult C57BL/6J mice (12 months) but not in young (3-month-old) or aged (18-month-old) mice (Viveiros *et al.* 2022), and in contrast, lung ACE2 protein levels and activity were lower in aged females than males, but again unchanged in young or adult mice. In contrast, *Ace2* mRNA abundance was lower in the mouse airway of females than in males, and concordant results were observed for ACE2 protein (Baratchian *et al.* 2021). While in Sprague–Dawley rats, lung ACE2 protein levels were unchanged by sex in young adult (3-month-old) and middle-aged (12-month-old) rats, in aged females (24 months) the levels were greater than that in males (Xudong *et al.* 2006). ACE2 levels were also unchanged with sex in the lungs and serum (sACE2) of FVB/N mice (Bengs *et al.* 2021), and in the lung (mRNA, protein, and activity) and serum of Wistar rats (Martins *et al.* 2021).

Studies in humans have also reported conflicting findings with regard to sex differences in lung ACE2 levels (Table 1). In humans, *ACE2* transcript expression is increased in lung epithelial cells (Song *et al.* 2020) and lower airway (endobronchial) tissue (Wark *et al.* 2021) of males compared to females; however, other gene expression repositories including the The Genotype-Tissue Expression (Barker & Parkkila 2020, Li *et al.* 2020*b*) and The Cancer Genome Atlas (Li *et al.* 2020*b*) showed no difference in lung *ACE2* expression with sex. Likewise, across the nasal cilia, large airway bronchioles,

Table 1 Number of rodent and human studies that report a sex difference in ACE2 mRNA and protein/activity in lung tissue.

	mRNA			Protein/activity		
Mice	1 (Viveiros *et al.* 2022[df])[a]	2 (Sarver & Wong 2021, Viveiros *et al.* 2022[e])[b]	1 (Baratchian *et al.* 2021)[c]	3 (Baratchian *et al.* 2021, Bengs *et al.* 2021, Viveiros *et al.* 2022[de])[a]	0	2 (Baratchian *et al.* 2021, Viveiros *et al.* 2022[f])[c]
Rats	1 (Martins *et al.* 2021)[a]	0	0	2 (Xudong *et al.* 2006[de], Martins *et al.* 2021)[a]	1 (Xudong *et al.* 2006[f])[b]	0
Humans	6 (Barker & Parkilla 2020, Camiolo *et al.* 2020, Lee *et al.* 2020, Li *et al.* 2020*b*, Zhang *et al.* 2020, Baratchian *et al.* 2021)[a]	0	2 (Song *et al.* 2020, Wark *et al.* 2021)[c]	1 (Li *et al.* 2021*a*)[a]	0	2 (Kalidhini *et al.* 2020, Xie *et al.* 2023)[c]

[a]Depicted are studies that report no sex difference, [b]higher expression/activity in females, or [c]higher expression/activity in males.
[d]Young (3 months); [e]adult (12 months); [f]aged (18–24 months).

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and small airway epithelium and lung parenchyma (Camiolo *et al.* 2020, Lee *et al.* 2020, Zhang *et al.* 2020, Baratchian *et al.* 2021), as well as 22 individual human tissues, *ACE2* mRNA abundance remained unchanged with sex (Li *et al.* 2020*b*). At the protein level, human males have been reported to have an increase in the mean intensity of ACE2 staining in lung tissue compared to females (Xie *et al.* 2023). Furthermore, baseline ACE2 protein abundance in airway smooth muscle cells is greater in males (Kalidhindi *et al.* 2020), but similar in the human airway epithelium, than in females (Li *et al.* 2021*a*).

With regard to levels of soluble ACE2 in the circulation, studies in rodents and human are conflicting. sACE2 levels are reportedly the same in the serum of male and female FVB/N mice (Bengs *et al.* 2021) and Wistar rats (Martins *et al.* 2021). In contrast, human studies consistently report that males have greater plasma levels of ACE2 than females (Zhou *et al.* 2020*b*, Kuznetsova & Cauwenberghs 2021, Xie *et al.* 2023).

Influence of sex hormones on ACE2 expression

Despite unclear baseline differences in ACE2 levels between sexes within different species, what is more well defined is that ACE2 is exquisitely regulated by sex hormones. Exposure of cultured primary differentiated airway smooth muscle cells to female sex hormone 17-beta-oestradiol tended to reduce ACE2 protein levels in cells of both male and female origin. Similarly 17-beta-oestradiol reduced *ACE2* mRNA abundance in differentiated primary bronchial epithelial cells (Stelzig *et al.* 2020) (Fig. 4). In contrast, oestrogen has been shown to upregulate ACE2 in the kidney (Ji *et al.* 2008), adipose tissue (Gupte *et al.* 2012), and heart (Bukowska *et al.* 2017). Treatment of atrial myocardium from elderly men with oestrogen reduced *ACE* mRNA expression and increased the expression of *ACE2* and *MAS* (Bukowska *et al.* 2017). Treatment with an oestrogen receptor alpha (ERα) antagonist reversed this effect (Bukowska *et al.* 2017). A subsequent report has suggested that oestrogen, primarily through its receptors ERα and G protein-coupled oestrogen receptor, affects cardiac ACE2 levels and activity by regulating ACE2 shedding via ADAM-17 and TMPRSS2 (Wang *et al.* 2021). Similarly, exposure of endothelial cells to oestradiol significantly increased *ACE* and *ACE2* mRNA expression, ACE protein levels, and both ACE and ACE2 enzyme activities, resulting in increased Ang-(1–7) levels (Mompeon *et al.* 2016). It was concluded that these effects were mediated through ERα activation, since ER antagonists completely abolished the effect of oestradiol.

In contrast, exposure to the male sex steroid, testosterone, elevated ACE2 protein levels in airway smooth muscle cells, irrespective of sex (Kalidhindi *et al.* 2020). Given that ACE2 is an established direct transcriptional target of androgen receptor (AR) signalling and is repressed in response to AR knockdown, the highly androgen-responsive nature of ACE2 could contribute to the sex disparity observed in COVID-19 severity between men and women (Samuel *et al.* 2020) (Fig. 4). The AR antagonist enzalutamide modestly

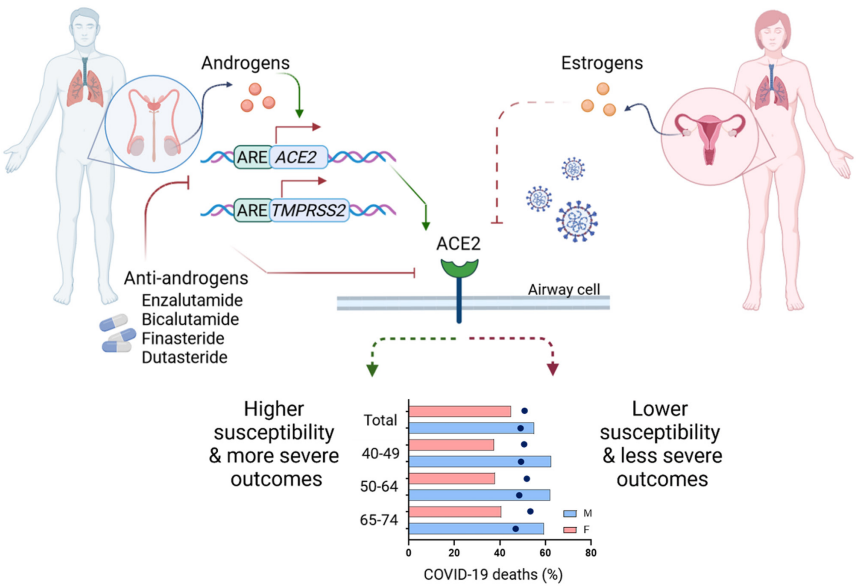

Figure 4
ACE2 – the link between divergent COVID-19 outcomes and sex? Men are more susceptible to SARS-CoV-2 infection, experience more severe disease and are at higher risk of dying from COVID-19. Androgens are described to drive *ACE2* and *TMPRSS2* transcription via an androgen response element in their gene promoter regions, and conversely anti-androgen drugs inhibit *ACE2*. On the other hand, oestrogens have been shown to inhibit *ACE2*. ACE2 deregulation could be driving the more severe disease observed in men. We highlight the latest US statistics, where more men than women have died from COVID-19, especially when stratified by age, over 40 years old (percentage deaths, compared to '●' the percentage of the US population). Analysed from data obtained from the US Centers for Disease Control and Prevention COVID Data Tracker (CDC 2022). Created with BioRender.com. ACE2, angiotensin-converting enzyme 2; COVID-19, coronavirus disease 2019; SARS-CoV-2, severe acute respiratory syndrome coronavirus 2.

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decreased ACE2 levels in males to those similar to that seen in females in the murine lung (Baratchian *et al.* 2021). A study interrogating the role of AR signalling in ACE2 regulation and SARS-CoV-2 infectivity demonstrated that anti-androgens finasteride and dutasteride, which inhibit the conversion of testosterone to potent dihydrotestosterone (DHT), markedly reduced ACE2 immunofluorescence in cardiac cells derived from embryonic stem cells (Samuel *et al.* 2020). On the other hand, dutasteride reduced SARS-CoV-2 virus internalisation in human primary alveolar epithelial cells and anti-androgens reduced ACE2 expression and virus infection of lung epithelial cell-enriched organoids established from differentiated embryonic stem cells (Samuel *et al.* 2020). The interplay between ACE2 and cleavage enzyme TMPRSS2 is also of critical importance in this equation. TMPRSS2, first identified in the prostate, is a long established androgen-regulated protease that is strongly upregulated by androgens and repressed by anti-androgens, exhibiting an androgen response element in its promoter (Lin *et al.* 1999, Afar *et al.* 2001, Wang *et al.* 2007, Leach *et al.* 2021) (Fig. 4). In the lung, TMPRSS2 protein is robustly expressed and by scRNAseq *TMPRSS2* mRNA has been shown to co-localise with the *AR* broadly across lung cell types (Stopsack *et al.* 2020, Leach *et al.* 2021). The androgenicity of TMPRSS2 has prompted suggestion that TMPRSS2 could partly explain the poor outcomes in SARS-CoV-2-infected men. However, baseline *TMPRSS2* mRNA and protein are highly variable between sexes in the human and rodent lung (Stopsack *et al.* 2020). Similarly, in murine lung alveoli and bronchioli, sex did not impact TMPRSS2 protein abundance, while gonadectomy and subsequent hormone replacement had no impact on TMPRSS2 levels in males or females (Bengs *et al.* 2021). These results have been concurred in further studies demonstrating that AR antagonist enzalutamide had no impact on TMPRSS2 in the murine airway (Baratchian *et al.* 2021). In opposition, however, Leach *et al.* (2021) showed significantly higher lung *TMPRSS2* mRNA abundance in human males compared to females, marked *TMPRSS2* upregulation upon DHT exposure, and repression by enzalutamide in human lung A549 and H1944 cells. In contrast, BEAS-2B cells lacked androgen responsiveness (Leach *et al.* 2021). Surgical castration of male mice and enzalutamide treatment also supressed *TMPRSS2* expression in murine lungs (Leach *et al.* 2021). Like ACE2, TMPRSS2 abundance and its regulation are clearly heterogeneous across studies, and further work is required to elucidate its physiological role across sexes.

Irrespective of the unclear baseline landscape between the sexes, patients with acute respiratory infections exhibit increased *ACE2* expression in the (naso)pharynx with SARS-CoV-2, positively correlating with increased viral load (Cai *et al.* 2021). Furthermore, ACE2 protein levels are increased by >two-fold in the lungs of COVID-19 patients compared with non-COVID-19 controls, and positively correlated with inflammatory and coagulation markers CD61 and CD163 (Gheware *et al.* 2022).

Influence of biological sex on the susceptibility to COVID-19

Although several studies report changes in ACE2 abundance across tissues at baseline, with respiratory challenge, and under sex hormone-driven conditions, they also highlight the variability of ACE2 at the mRNA, protein, activity, and circulating levels across studies. Thus, drawing conclusions around the fundamental role of ACE2 in driving health outcomes in vulnerable populations is difficult.

Although country-specific nuances in pandemic response, COVID-19 reporting, and the capture of COVID-19-related deaths have resulted in inherent differences in disease statistics across countries, it quickly became apparent that being male is associated with increased risk of death from COVID-19 (Williamson *et al.* 2020). Through multiple independent studies across the globe, concurred by today's latest disease statistics (Fig. 4) (CDC 2022), men have consistently been identified as most vulnerable to increased disease severity and mortality from COVID-19. An analysis of the characteristics of 1096 SARS-CoV-2 patients hospitalised across 552 sites in Mainland China demonstrated that men were more likely to die from COVID-19 (67.2%), be admitted to hospital (58.1%), and experience more severe disease (57.8%) (Guan *et al.* 2020). Likewise, a retrospective analysis of mortality in Italy in 3517 SARS-CoV-2-positive patients captured a robust sex disparity, with men representing 66.7% of deaths (Raparelli *et al.* 2020). In the UK, a prospective observational study of 20,133 hospital in-patients with COVID-19 revealed that men often entered acute hospital care for COVID-19 (60%), while being female was associated with significantly lower risk of death (Docherty *et al.* 2020). In Australia, despite a starkly different strategic response to pandemic management (Rang *et al.* 2020), longitudinal data have demonstrated an increased incidence of COVID-19-related death in men (56.3%) and a particular prominence of male deaths in the 60–69-year-of-age and

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70–79-year-of-age groups (65.1% and 65.1%, respectively), despite no increased incidence of infection (49% and 52.1%, respectively) (CADHAC 2022). The increased vulnerability of men to more severe COVID-19, resulting in higher ICU admissions and death, has been echoed in many other studies internationally (Chen *et al.* 2020*b*, Grasselli *et al.* 2020, Li *et al.* 2020*a*, Nikpouraghdam *et al.* 2020, Salje *et al.* 2020, Yanez *et al.* 2020, Dalal *et al.* 2021, Lewnard *et al.* 2022).

While sociodemographic research has demonstrated a clear gender disparity in social attributes surrounding risk perception and behaviour, which could act as potential drivers of COVID-19 severity (Alsan *et al.* 2020, Galasso *et al.* 2020), the biological sex differences and underlying hormonal, immunological, and genetic mechanisms driving disparities in disease severity have been a hot topic of research enquiry. Naturally, the sex-linked differences in the expression and hormonal regulation of SARS-CoV-2 viral entry factors have been highlighted. This has not only stemmed from the greater mortality in males but the reported partial protection from SARS-CoV-2 infection of men with prostate cancer receiving androgen deprivation therapy (ADT) (Montopoli *et al.* 2020). In a population-based study of 68 hospitals in Veneto, Italy ($n=4532$ males), men undergoing ADT for prostate cancer had >four-fold decreased risk of SARS-CoV-2 infection than patients not receiving ADT (OR: 4.05), and compared with patients suffering from other cancers (OR: 4.86) (Montopoli *et al.* 2020). This was supported *in vitro* whereby targeting androgen signalling in SARS-CoV-2-infected human lung cells dampened SARS-CoV-2 cellular entry and subsequent infection, using two drugs that block the androgen axis, bicalutamide, an gonadotropin-releasing hormone agonist, and enzalutamide (Leach *et al.* 2021). The exact factors driving this putative sex hormone-mediated response (be it androgens or oestrogen) remain controversial, while a further physiological state with altered sex hormone milieu that also shows changes with COVID-19 susceptibility and severity is pregnancy.

ACE2 and pregnant women

Changes in ACE2 levels during pregnancy

In the maternal circulation of healthy pregnant women, sACE2 levels and activity are increased compared with non-pregnant women and remain high throughout gestation (Tamanna *et al.* 2020). This may be driven by the high levels of oestrogen and progesterone from early in gestation (Schock *et al.* 2016), which could regulate ACE2 levels. Ang-(1–7) levels are also increased in pregnant compared with non-pregnant women (Merrill *et al.* 2002, Tamanna *et al.* 2020) and could counterbalance the high levels of Ang II (Merrill *et al.* 2002) and its vascular actions. Furthermore, Ang-(1–7) levels are positively correlated with sACE2 levels and activity (Tamanna *et al.* 2020), suggesting that the amount and activity of sACE2 in the circulation are rate-limiting in terms of the production of Ang-(1–7).

Locally in intrauterine tissues, the human endometrium (both non-pregnant and pregnant) and placenta express ACE2 (Marques *et al.* 2011, Pringle *et al.* 2011, Wang *et al.* 2015, Delforce *et al.* 2017), and in mice, uterine ACE2 expression has been shown to be stimulated by progesterone (Chadchan *et al.* 2021). In the human placenta, immunohistochemical analysis has shown that ACE2 and Ang-(1–7) are expressed in cytotrophoblasts, syncytiotrophoblasts, and invading and intravascular trophoblasts (Valdes *et al.* 2006, Marques *et al.* 2011). The expression of ACE2 in the syncytiotrophoblast, which is in direct contact with maternal blood, means that placental ACE2 can be accessed by SARS-CoV-2 and affect the balance between Ang II and Ang-(1–7) both locally and in the maternal circulation. In this way, placental ACE2 could play a role in regulating maternal inflammation and vascular tone as well as protecting the placenta through the anti-oxidant and anti-inflammatory properties of the Ang-(1–7)Mas axis (Tamanna *et al.* 2020).

Importantly, placental ACE2 expression is highest in the first trimester and decreases with gestation (Marques *et al.* 2011, Bloise *et al.* 2021, Colson *et al.* 2021). Similarly, expression of TMPRSS2 in the placenta is also reduced with gestation (Colson *et al.* 2021). Therefore, the placenta may be less susceptible to infection by SARS-CoV-2 in the third trimester.

Placental infection and fetal transmission of SARS-CoV-2

Placental infection with SARS-CoV-2 has been confirmed in numerous studies (Hosier *et al.* 2020, Schwartz *et al.* 2020, Sanchez *et al.* 2021, Valdespino-Vazquez *et al.* 2021); however, vertical transmission to the fetus is extremely low and rare (Allotey *et al.* 2022, Ezechukwu *et al.* 2022) (Fig. 5). In a recent systematic review of fetoplacental transmission by Ezechukwu *et al.* (2022), out of 45 eligible studies identified, 53.34% showed no evidence of vertical transmission; 33.33%

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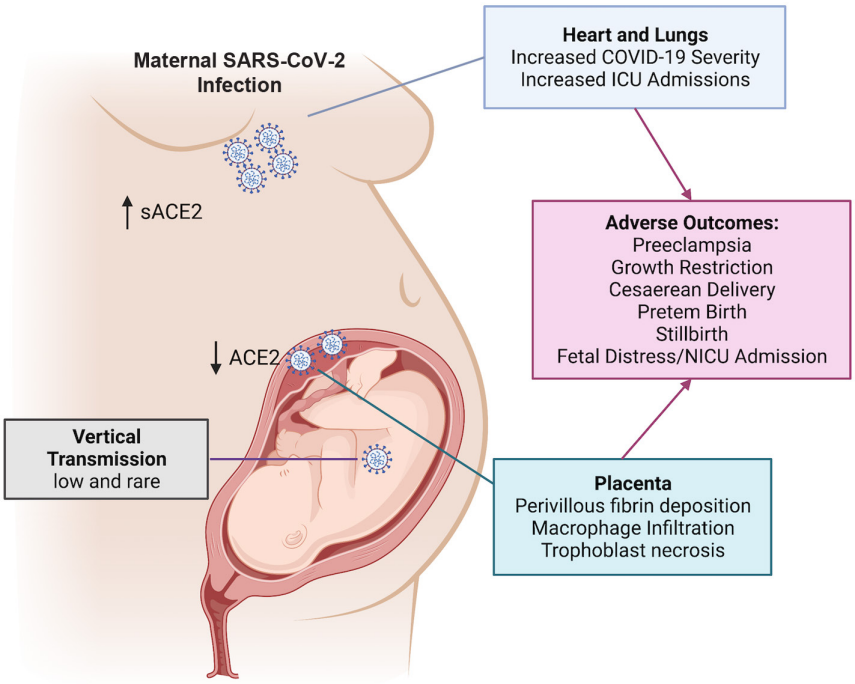

Figure 5
Influence of SARS-CoV-2 infection on pregnant women. Although SARS-CoV-2 infection occurs at similar rates in pregnant and non-pregnant women there is a heightened risk of COVID-19 severity in pregnancy. Placental infection with SARS-CoV-2 is possible, although vertical transmission to the fetus is extremely low and rare. Many placentas, regardless of placental SARS-CoV-2 infection, show signs of chronic histiocytic intervillositis, accompanied by massive infiltration of inflammatory cells, trophoblast necrosis, and fibrin deposition. SARS-CoV-2 infection in pregnant women acutely decreases ACE2 protein levels in the placenta, which likely prevents the protective actions of ACE2 and causes local inflammation and tissue injury. These changes, along with increased severity of COVID-19, are likely to underlie the increased risk of adverse pregnancy outcomes for both the mother and fetus. Created with BioRender.com. ACE2, angiotensin-converting enzyme 2; COVID-19, coronavirus disease 2019; SARS-CoV-2, severe acute respiratory syndrome coronavirus 2.

supported the hypothesis of very rare, low possibility of vertical transmission; and 13.33% were indecisive and had no comment on vertical transmission. Despite this, a systematic review of histological studies of placentas from SARS-CoV-2 positive pregnant women showed that 6% of placentas have signs of chronic histiocytic intervillositis, accompanied by massive infiltration of inflammatory cells, trophoblast necrosis, and fibrin deposition (Ezechukwu *et al.* 2022). They also have signs of maternal vascular malperfusion including decidual vasculopathy, intervillous thrombosis and infarction, as well as signs of fetal vascular malperfusion, which includes thrombotic vasculopathy and chorangiosis (Ezechukwu *et al.* 2022). Thus, SARS-CoV-2 can cause an inflammatory response in placentas, even in the absence of placental infection, which can adversely affect the developing fetus (Fig. 5).

Verma *et al.* (2021) were the first to examine changes in ACE2 expression in the placenta following SARS-CoV-2 infection. They showed that placental ACE2 mRNA and protein levels are reduced in women with COVID-19 infection and, *in vitro*, culture of a human trophoblast cell line with recombinant SARS-CoV-2 spike protein, or a live modified virus expressing spike protein, reduces ACE2 expression (Verma *et al.* 2021). Furthermore, levels of AT_1R and AT_1R autoantibodies, which act as AT_1R agonists, were increased by SARS-CoV-2 spike protein (Verma *et al.* 2021). Subsequently, several other studies have also investigated ACE2 expression in the placenta. In contrast to Verma *et al.* (2021), Lu-Culligan *et al.* (2021) reported that placental ACE2 levels were elevated in mothers with COVID-19 at term. A more recent study suggests that these differences may be due to a previously unrecognised dynamic expression of ACE2 in the human placenta that can be modulated by the timing of maternal SARS-CoV-2 infection in pregnancy relative to delivery (Taglauer *et al.* 2022). Taglauer *et al.* (2022) showed that although villous placental tissues from remote, second-trimester COVID-19 cases had similar ACE2 expression to those of control placental tissues at term, there is increased placental *Ace2* mRNA and decreased ACE2 protein levels in acute SARS-CoV-2 infection in the third trimester. There is also increased sACE2 in maternal plasma (Taglauer *et al.* 2022), which could suggest increased shedding of ACE2 from the placenta. Overall, these data suggest that SARS-CoV-2 infection in pregnant women is likely to acutely decrease ACE2 protein levels in the placenta, preventing the protective actions of ACE2 and causing local inflammation and tissue injury. These changes are likely to underlie the increased risk of adverse pregnancy outcomes for both the mother and fetus in pregnancies affected by COVID-19 (Fig. 5).

Influence of SARS-CoV-2 on pregnant women

Pregnant women were initially identified as a vulnerable population for COVID-19 (Favre *et al.* 2020,

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Schwartz & Graham 2020) as they are at high risk of complications and severe disease from infection with other viruses and coronaviruses (Wong *et al.* 2004, Alfaraj *et al.* 2019). It appears, however, that SARS-CoV-2 infection occurs at similar rates among pregnant women compared with the general population (Kotlar *et al.* 2021, Overton *et al.* 2022). The most commonly reported symptoms are fever (87.5%) and cough (53.8%), followed by fatigue (22.5%), myalgia (16.3%), dyspnoea (11.3%), diarrhoea (8.8%), and sore throat (7.5%) (Yang *et al.* 2022*a*). In terms of the severity of infection, although some studies report that clinical characteristics in pregnant patients are similar to those of non-pregnant adults (Chen *et al.* 2020*a*, Yang *et al.* 2022*a*), several larger studies suggest that there is a heightened risk of COVID-19 severity in pregnant women (Khan *et al.* 2021, Kotlar *et al.* 2021, Lassi *et al.* 2021, Wang *et al.* 2022*b*) (Fig. 5). Specifically, Lassi *et al.* (2021), in a systematic review and meta-analysis of 31,016 pregnant women from 62 studies, report that 16.4% of women developed severe COVID-19, although nearly half were asymptomatic (Lassi *et al.* 2021). About 7% were admitted to the ICU, 8% required mechanical ventilation, and 2% died. Importantly, the risk of severe COVID-19 was significantly higher among women who were >35 years old, obese, had smoked, diabetic, or had preeclampsia (Lassi *et al.* 2021).

COVID-19 infection in pregnancy is also associated with a high risk of adverse pregnancy outcomes (Di Mascio *et al.* 2020, Khan *et al.* 2021, Wei *et al.* 2021, Pathirathna *et al.* 2022) (Fig. 5). A systematic review and meta-analysis by Wei *et al.* (2021) including 438,548 pregnant people from 42 studies, found that COVID-19 was associated with an increased risk of pre-eclampsia (OR: 1.33), preterm birth (OR: 1.82), and stillbirth (OR: 2.11). Similarly, Pathirathna *et al.* (2022) in a meta-analysis of 21 studies that included 14,131 COVID-infected and 585,376 non-infected pregnant women found that the odds of maternal death (OR: 7.05), pre-eclampsia (OR: 1.39), caesarean delivery (OR: 1.67), fetal distress (OR: 1.66), preterm birth (OR: 1.86), low birthweight (OR: 1.69), stillbirth (OR: 1.46), and neonatal ICU admission (OR: 2.12) were higher among COVID-19 infected pregnant women. Compared with mild COVID-19, severe COVID-19 was strongly associated with preeclampsia (OR: 4.16), preterm birth (OR: 4.29), gestational diabetes (OR: 1.99), and low birthweight (OR: 1.9) (Wei *et al.* 2021). Furthermore, symptomatic pregnant women were more likely to have adverse outcomes than asymptomatic pregnant women, with increased risk of caesarean delivery (OR: 1.40), low birthweight (OR: 1.85), preterm birth (OR: 2.10), and neonatal ICU admissions (OR: 1.96) (Khan *et al.* 2021). These studies highlight the vulnerabilities for women infected with COVID-19 in pregnancy. However, much more needs to be done to better understand the role of ACE2 and alterations to its actions locally within the placenta in mediating some of these adverse outcomes.

Conclusion

This review highlights the fundamental role of ACE2 as the gateway receptor for SARS-CoV-2 infection and subsequent driver of COVID-19 disease pathogenesis. Normally, ACE2 plays a multifaceted physiological role throughout the body given its widespread tissue expression and plentiful interactome. However, the active changes in ACE2 expression and activity following SARS-CoV-2 spike protein binding and the deregulation of ACE2 interacting partners are key to driving one's susceptibility to SARS-CoV-2 infection, severity of COVID-19 symptoms across affected organs, and subsequent clinical outcome. Despite a vast body of evidence supporting this critical role of ACE2, when looking across populations most vulnerable to COVID-19, changes in ACE2 become less clear. This review describes the evidence supporting ACE2 in driving COVID-19 age-, sex-, and pregnancy-associated vulnerabilities. Furthermore, it highlights the disparities in ACE2 expression across studies especially those comparing age- and hormone-mediated states (sex, pregnancy), with large variability in reports of ACE2 mRNA, protein (membrane-bound and circulating), and activity levels and cellular pattern of expression. Fundamentally, improved characterisation of ACE2 is needed to gain a better understanding of the basic biology of ACE2 across groups vulnerable to COVID-19. This improved understanding of ACE2 expression, regulation, and function could reveal potential novel treatments strategies for targeting the deregulation of ACE2 and its interactors in the clinical management of COVID-19 but also other ACE2-mediated disease states.

Declaration of interest

The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of this review.

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References

Afar DE, Vivanco I, Hubert RS, Kuo J, Chen E, Saffran DC, Raitano AB & Jakobovits A 2001 Catalytic cleavage of the androgen-regulated TMPRSS2 protease results in its secretion by prostate and prostate cancer epithelia. *Cancer Research* **61** 1686–1692.

Ahmad M, Zeitlin IJ, Parratt JR & Pitt AR 2006 Degradation of bradykinin, a cardioprotective substance, during a single passage through isolated rat-heart. *Archives of Pharmacal Research* **29** 241–248. (https://doi.org/10.1007/BF02969400)

Alfaraj SH, Al-Tawfiq JA & Memish ZA 2019 Middle East Respiratory Syndrome coronavirus (MERS-CoV) infection during pregnancy: report of two cases & review of the literature. *Journal of Microbiology, Immunology, and Infection* **52** 501–503. (https://doi.org/10.1016/j.jmii.2018.04.005)

AlGhatrif M, Tanaka T, Moore AZ, Bandinelli S, Lakatta EG & Ferrucci L 2021 Age-associated difference in circulating ACE2, the gateway for SARS-COV-2, in humans: results from the InCHIANTI study. *GeroScience* **43** 619–627. (https://doi.org/10.1007/s11357-020-00314-w)

Allotey J, Chatterjee S, Kew T, Gaetano A, Stallings E, Fernandez-Garcia S, Yap M, Sheikh J, Lawson H, Coomar D, *et al.* 2022 SARS-CoV-2 positivity in offspring and timing of mother-to-child transmission: living systematic review and meta-analysis. *BMJ* **376** e067696. (https://doi.org/10.1136/bmj-2021-067696)

Alsan M, Stantcheva S, Yang D & Cutler D 2020 Disparities in coronavirus 2019 reported incidence, knowledge, and behavior among US adults. *JAMA Network Open* **3** e2012403. (https://doi.org/10.1001/jamanetworkopen.2020.12403)

Antos A, Kwong ML, Balmorez T, Villanueva A & Murakami S 2021 Unusually high risks of COVID-19 mortality with age-related comorbidities: an adjusted meta-analysis method to improve the risk assessment of mortality using the comorbid mortality data. *Infectious Disease Reports* **13** 700–711. (https://doi.org/10.3390/idr13030065)

Araujo RC, Kettritz R, Fichtner I, Paiva AC, Pesquero JB & Bader M 2001 Altered neutrophil homeostasis in kinin B1 receptor-deficient mice. *Biological Chemistry* **382** 91–95. (https://doi.org/10.1515/BC.2001.014)

Arefin S, Hernandez L, Ward LJ, Schwarz A, Barany P, Stenvinkel P, Kublickiene K, Pilote L, Norris CM, Raparelli V, *et al.* 2022 Angiotensin-converting enzyme 2 and transmembrane protease serine 2 in female and male patients with end-stage kidney disease. *European Journal of Clinical Investigation* **52** e13786. (https://doi.org/10.1111/eci.13786)

Baratchian M, Mcmanus JM, Berk MP, Nakamura F, Mukhopadhyay S, Xu W, Erzurum S, Drazba J, Peterson J, Klein EA, *et al.* 2021 Androgen regulation of pulmonary AR, TMPRSS2 and ACE2 with implications for sex-discordant COVID-19 outcomes. *Scientific Reports* **11** 11130. (https://doi.org/10.1038/s41598-021-90491-1)

Barker H & Parkkila S 2020 Bioinformatic characterization of angiotensin-converting enzyme 2, the entry receptor for SARS-CoV-2. *PLoS One* **15** e0240647. (https://doi.org/10.1371/journal.pone.0240647)

Bengs S, Rossi A, Haberecker M, Mikail N, Meisel A, Haider A, Grämer M, Portmann A, Todorov A, Schönenberger C, *et al.* 2021 Immunoreactivity of the SARS-CoV-2 entry proteins ACE-2 and TMPRSS-2 in murine models of hormonal manipulation, ageing, and cardiac injury. *Scientific Reports* **11** 23993. (https://doi.org/10.1038/s41598-021-03181-3)

Beyerstedt S, Casaro EB & Rangel ÉB 2021 COVID-19: angiotensin-converting enzyme 2 (ACE2) expression and tissue susceptibility to SARS-CoV-2 infection. *European Journal of Clinical Microbiology and Infectious Diseases* **40** 905–919. (https://doi.org/10.1007/s10096-020-04138-6)

Bhopal SS, Bagaria J, Olabi B & Bhopal R 2021 Children and young people remain at low risk of COVID-19 mortality. *Lancet. Child and Adolescent Health* **5** e12–e13. (https://doi.org/10.1016/S2352-4642(21)00066-3)

Bloise E, Zhang J, Nakpu J, Hamada H, Dunk CE, Li S, Imperio GE, Nadeem L, Kibschull M, Lye P, *et al.* 2021 Expression of severe acute respiratory syndrome coronavirus 2 cell entry genes, angiotensin-converting enzyme 2 and transmembrane protease serine 2, in the placenta across gestation and at the maternal-fetal interface in pregnancies complicated by preterm birth or preeclampsia. *American Journal of Obstetrics and Gynecology* **224** 298.e1–298.e8. (https://doi.org/10.1016/j.ajog.2020.08.055)

Bukowska A, Spiller L, Wolke C, Lendeckel U, Weinert S, Hoffmann J, Bornfleth P, Kutschka I, Gardemann A, Isermann B, *et al.* 2017 Protective regulation of the ACE2/ACE gene expression by estrogen in human atrial tissue from elderly men. *Experimental Biology and Medicine* **242** 1412–1423. (https://doi.org/10.1177/1535370217718808)

Bunyavanich S, Do A & Vicencio A 2020 Nasal gene expression of angiotensin-converting enzyme 2 in children and adults. *JAMA* **323** 2427–2429. (https://doi.org/10.1001/jama.2020.8707)

CADHAC 2022 Coronavirus (COVID-19) case numbers and statistics. *Commonwealth of Australia Department of Health and Aged Care*. (available at: https://www.health.gov.au/health-alerts/covid-19/case-numbers-and-statistics).

Cai G, Du M, Bossé Y, Albrecht H, Qin F, Luo X, Androulakis XM, Cheng C, Nagarkatti M, Nagarkatti P, *et al.* 2021 SARS-CoV-2 impairs dendritic cells and regulates DC-SIGN gene expression in tissues. *International Journal of Molecular Sciences* **22** 9228. (https://doi.org/10.3390/ijms22179228)

Camargo SM, Singer D, Makrides V, Huggel K, Pos KM, Wagner CA, Kuba K, Danilczyk U, Skovby F, Kleta R, *et al.* 2009 Tissue-specific amino acid transporter partners ACE2 and collectrin differentially interact with hartnup mutations. *Gastroenterology* **136** 872–882. (https://doi.org/10.1053/j.gastro.2008.10.055)

Camiolo M, Gauthier M, Kaminski N, Ray A & Wenzel SE 2020 Expression of SARS-CoV-2 receptor ACE2 and coincident host response signature varies by asthma inflammatory phenotype. *Journal of Allergy and Clinical Immunology* **146** 315–324.e7. (https://doi.org/10.1016/j.jaci.2020.05.051)

CDC 2022 *COVID data Tracker*. United States Department of Health and Human Services, Centers for Disease Control and Prevention. (available at: https://covid.cdc.gov/covid-data-tracker)

Chadchan SB, Popli P, Maurya VK & Kommagani R 2021 The SARS-CoV-2 receptor, angiotensin-converting enzyme 2, is required for human endometrial stromal cell decidualization. *Biology of Reproduction* **104** 336–343. (https://doi.org/10.1093/biolre/ioaa211)

Chen L, Li Q, Zheng D, Jiang H, Wei Y, Zou L, Feng L, Xiong G, Sun G, Wang H, *et al.* 2020*a* Clinical characteristics of pregnant women with Covid-19 in Wuhan, China. *New England Journal of Medicine* **382** e100. (https://doi.org/10.1056/NEJMc2009226)

Chen T, Wu D, Chen H, Yan W, Yang D, Chen G, Ma K, Xu D, Yu H, Wang H, *et al.* 2020*b* Clinical characteristics of 113 deceased patients with coronavirus disease 2019: retrospective study. *BMJ* **368** m1091. (https://doi.org/10.1136/bmj.m1091)

Chirinos JA, Cohen JB, Zhao L, Hanff T, Sweitzer N, Fang J, Corrales-Medina V, Anmar R, Morley M, Zamani P, *et al.* 2020 Clinical and proteomic correlates of plasma ACE2 (angiotensin-converting enzyme 2) in human heart failure. *Hypertension* **76** 1526–1536. (https://doi.org/10.1161/HYPERTENSIONAHA.120.15829)

Choi SI & Hwang SW 2018 Depolarizing effectors of bradykinin signaling in nociceptor excitation in pain perception. *Biomolecules and Therapeutics* **26** 255–267. (https://doi.org/10.4062/biomolther.2017.127)

Colson A, Depoix CL, Dessilly G, Baldin P, Danhaive O, Hubinont C, Sonveaux P & Debieve F 2021 Clinical and in vitro evidence against placenta infection at term by severe acute respiratory syndrome coronavirus 2. *American Journal of Pathology* **191** 1610–1623. (https://doi.org/10.1016/j.ajpath.2021.05.009)

Dalal J, Triulzi I, James A, Nguimbis B, Dri GG, Venkatasubramanian A, Noubi Tchoupopnou Royd L, Botero Mesa S, Somerville C, Turchetti G, *et al.* 2021 COVID-19 mortality in women and men in sub-Saharan Africa: a cross-sectional study. *BMJ Global Health* **6** e007225. (https://doi.org/10.1136/bmjgh-2021-007225)

Daniell H, Nair SK, Shi Y, Wang P, Montone KT, Shaw PA, Choi GH, Ghani D, Weaver J, Rader DJ, *et al.* 2022 Decrease in angiotensin-converting enzyme activity but not concentration in plasma/lungs in COVID-19 patients offers clues for diagnosis/treatment. *Molecular Therapy. Methods and Clinical Development* **26** 266–278. (https://doi.org/10.1016/j.omtm.2022.07.003)

Danilczyk U, Sarao R, Remy C, Benabbas C, Stange G, Richter A, Arya S, Pospisilik JA, Singer D, Camargo SM, *et al.* 2006 Essential role for collectrin in renal amino acid transport. *Nature* **444** 1088–1091. (https://doi.org/10.1038/nature05475)

Dasgupta C & Zhang L 2011 Angiotensin II receptors and drug discovery in cardiovascular disease. *Drug Discovery Today* **16** 22–34. (https://doi.org/10.1016/j.drudis.2010.11.016)

Delforce SJ, Lumbers ER, Corbisier De Meaultsart C, Wang Y, Proietto A, Otton G, Scurry J, Verrills NM, Scott RJ & Pringle KG 2017 Expression of renin-angiotensin system (RAS) components in endometrial cancer. *Endocrine Connections* **6** 9–19. (https://doi.org/10.1530/EC-16-0082)

Di Mascio D, Khalil A, Saccone G, Rizzo G, Buca D, Liberati M, Vecchiet J, Nappi L, Scambia G, Berghella V, *et al.* 2020 Outcome of coronavirus spectrum infections (SARS, MERS, COVID-19) during pregnancy: a systematic review and meta-analysis. *American Journal of Obstetrics and Gynecology MFM* **2** 100107. (https://doi.org/10.1016/j.ajogmf.2020.100107)

Docherty AB, Harrison EM, Green CA, Hardwick HE, Pius R, Norman L, Holden KA, Read JM, Dondelinger F, Carson G, *et al.* 2020 Features of 20 133 UK patients in hospital with Covid-19 using the ISARIC WHO Clinical Characterisation Protocol: prospective observational cohort study. *BMJ* **369** m1985. (https://doi.org/10.1136/bmj.m1985)

Donoghue M, Hsieh F, Baronas E, Godbout K, Gosselin M, Stagliano N, Donovan M, Woolf B, Robison K, Jeyaseelan R, *et al.* 2000 A novel angiotensin-converting enzyme-related carboxypeptidase (ACE2) converts angiotensin I to angiotensin 1–9. *Circulation Research* **87** E1–E9. (https://doi.org/10.1161/01.res.87.5.e1)

Dray A & Perkins M 1993 Bradykinin and inflammatory pain. *Trends in Neurosciences* **16** 99–104. (https://doi.org/10.1016/0166-2236(93)90133-7)

Dray A 1997 Kinins and their receptors in hyperalgesia. *Canadian Journal of Physiology and Pharmacology* **75** 704–712. (https://doi.org/10.1139/Y97-068)

Emilsson V, Gudmundsson EF, Aspelund T, Jonsson BG, Gudjonsson A, Launer LJ, Lamb JR, Gudmundsdottir V, Jennings LL & Gudnason V 2021 Serum levels of ACE2 are higher in patients with obesity and diabetes. *Obesity Science and Practice* **7** 239–243. (https://doi.org/10.1002/osp4.472)

Ezechukwu HC, Shi J, Fowora MA, Diya CA, Elfaki F & Adegboye OA 2022 Fetoplacental transmission and placental response to SARS-CoV-2: evidence from the literature. *Frontiers in Medicine* **9** 962937. (https://doi.org/10.3389/fmed.2022.962937)

Fagyas M, Fejes Z, Suto R, Nagy Z, Szekely B, Pocsi M, Ivady G, Biro E, Beko G, Nagy A, *et al.* 2022 Circulating ACE2 activity predicts mortality and disease severity in hospitalized COVID-19 patients. *International Journal of Infectious Diseases* **115** 8–16. (https://doi.org/10.1016/j.ijid.2021.11.028)

Fan X, Cao D, Kong L & Zhang X 2020 Cryo-EM analysis of the post-fusion structure of the SARS-CoV spike glycoprotein. *Nature Communications* **11** 3618. (https://doi.org/10.1038/s41467-020-17371-6)

Favre G, Pomar L, Musso D & Baud D 2020 2019-nCoV epidemic: what about pregnancies? *Lancet* **395** e40. (https://doi.org/10.1016/S0140-6736(20)30311-1)

Fukui K, Yang Q, Cao Y, Takahashi N, Hatakeyama H, Wang H, Wada J, Zhang Y, Marselli L, Nammo T, *et al.* 2005 The HNF-1 target collectrin controls insulin exocytosis by SNARE complex formation. *Cell Metabolism* **2** 373–384. (https://doi.org/10.1016/j.cmet.2005.11.003)

Galasso V, Pons V, Profeta P, Becher M, Brouard S & Foucault M 2020 Gender differences in COVID-19 attitudes and behavior: panel evidence from eight countries. *Proceedings of the National Academy of Sciences of the United States of America* **117** 27285–27291. (https://doi.org/10.1073/pnas.2012520117)

Gemmati D, Bramanti B, Serino ML, Secchiero P, Zauli G & Tisato V 2020 COVID-19 and individual genetic susceptibility/receptivity: role of ACE1/ACE2 genes, immunity, inflammation and coagulation. Might the double X-chromosome in females be protective against SARS-CoV-2 compared to the single X-chromosome in males? *International Journal of Molecular Sciences* **21** 3474. (https://doi.org/10.3390/ijms21103474)

Gheware A, Ray A, Rana D, Bajpai P, Nambirajan A, Arulselvi S, Mathur P, Trikha A, Arava S, Das P, *et al.* 2022 ACE2 protein expression in lung tissues of severe COVID-19 infection. *Scientific Reports* **12** 4058. (https://doi.org/10.1038/s41598-022-07918-6)

Glowacka I, Bertram S, Muller MA, Allen P, Soilleux E, Pfefferle S, Steffen I, Tsegaye TS, He Y, Gnirss K, *et al.* 2011 Evidence that TMPRSS2 activates the severe acute respiratory syndrome coronavirus spike protein for membrane fusion and reduces viral control by the humoral immune response. *Journal of Virology* **85** 4122–4134. (https://doi.org/10.1128/JVI.02232-10)

Götzinger F, Santiago-García B, Noguera-Julián A, Lanaspa M, Lancella L, Calò Carducci FI, Gabrovska N, Velizarova S, Prunk P, Osterman V, *et al.* 2020 COVID-19 in children and adolescents in Europe: a multinational, multicentre cohort study. *Lancet. Child and Adolescent Health* **4** 653–661. (https://doi.org/10.1016/S2352-4642(20)30177-2)

Grasselli G, Greco M, Zanella A, Albano G, Antonelli M, Bellani G, Bonanomi E, Cabrini L, Carlesso E, Castelli G, *et al.* 2020 Risk factors associated with mortality among patients with COVID-19 in intensive care units in Lombardy, Italy. *JAMA Internal Medicine* **180** 1345–1355. (https://doi.org/10.1001/jamainternmed.2020.3539)

Gu J, Yin J, Zhang M, Li J, Wu Y, Chen J & Miao H 2021 Study on the clinical significance of ACE2 and its age-related expression. *Journal of Inflammation Research* **14** 2873–2882. (https://doi.org/10.2147/JIR.S315981)

Guan WJ, Ni ZY, Hu Y, Liang WH, Ou CQ, He JX, Liu L, Shan H, Lei CL, Hui DSC, *et al.* 2020 Clinical characteristics of coronavirus disease 2019 in China. *New England Journal of Medicine* **382** 1708–1720. (https://doi.org/10.1056/NEJMoa2002032)

Gupte M, Thatcher SE, Boustany-Kari CM, Shoemaker R, Yiannikouris F, Zhang X, Karounos M & Cassis LA 2012 Angiotensin converting enzyme 2 contributes to sex differences in the development of obesity hypertension in C57BL/6 mice. *Arteriosclerosis, Thrombosis, and Vascular Biology* **32** 1392–1399. (https://doi.org/10.1161/ATVBAHA.112.248559)

Haga S, Yamamoto N, Nakai-Murakami C, Osawa Y, Tokunaga K, Sata T, Yamamoto N, Sasazuki T & Ishizaka Y 2008 Modulation of TNF-alpha-converting enzyme by the spike protein of SARS-CoV and ACE2 induces TNF-alpha production and facilitates viral entry. *Proceedings of the National Academy of Sciences of the United States of America* **105** 7809–7814. (https://doi.org/10.1073/pnas.0711241105)

Hamming I, Timens W, Bulthuis ML, Lely AT, Navis G & Van Goor H 2004 Tissue distribution of ACE2 protein, the functional receptor for SARS coronavirus. A first step in understanding SARS pathogenesis. *Journal of Pathology* **203** 631–637. (https://doi.org/10.1002/path.1570)

Hashimoto T, Perlot T, Rehman A, Trichereau J, Ishiguro H, Paolino M, Sigl V, Hanada T, Hanada R, Lipinski S, *et al.* 2012 ACE2 links amino acid malnutrition to microbial ecology and intestinal inflammation. *Nature* **487** 477–481. (https://doi.org/10.1038/nature11228)

Heurich A, Hofmann-Winkler H, Gierer S, Liepold T, Jahn O & Pöhlmann S 2014 TMPRSS2 and ADAM17 cleave ACE2 differentially and only proteolysis by TMPRSS2 augments entry driven by the severe acute respiratory syndrome coronavirus spike protein. *Journal of Virology* **88** 1293–1307. (https://doi.org/10.1128/JVI.02202-13)

Hofmann H, Pyrc K, Van Der Hoek L, Geier M, Berkhout B & Pohlmann S 2005 Human coronavirus NL63 employs the severe acute respiratory syndrome coronavirus receptor for cellular entry. *Proceedings of the National Academy of Sciences of the United States of America* **102** 7988–7993. (https://doi.org/10.1073/pnas.0409465102)

Hoffmann M, Kleine-Weber H & Pohlmann S 2020*a* A multibasic cleavage site in the spike protein of SARS-CoV-2 is essential for infection of human lung cells. *Molecular Cell* **78** 779–784.e5. (https://doi.org/10.1016/j.molcel.2020.04.022)

Hoffmann M, Kleine-Weber H, Schroeder S, Krüger N, Herrler T, Erichsen S, Schiergens TS, Herrler G, Wu NH, Nitsche A, *et al.* 2020*b* SARS-CoV-2 cell entry depends on ACE2 and TMPRSS2 and is blocked by a clinically proven protease inhibitor. *Cell* **181** 271–280.e8. (https://doi.org/10.1016/j.cell.2020.02.052)

Hornig B & Drexler H 1997 Endothelial function and bradykinin in humans. *Drugs* **54**(Supplement 5) 42–47. (https://doi.org/10.2165/00003495-199700545-00007)

Hosier H, Farhadian SF, Morotti RA, Deshmukh D, Lu-Culligan A, K, C, Yasumoto Y, Vogels C, Casanovas-Massana A, *et al.* 2020 SARS-CoV-2 infection of the placenta. *Journal of Clinical Investigation* **130** 4947–4953. (https://doi.org/10.1172/JCI139569)

Imai Y, Kuba K, Rao S, Huan Y, Guo F, Guan B, Yang P, Sarao R, Wada T, Leong-Poi H, *et al.* 2005 Angiotensin-converting enzyme 2 protects from severe acute lung failure. *Nature* **436** 112–116. (https://doi.org/10.1038/nature03712)

Inde Z, Croker BA, Yapp C, Joshi GN, Spetz J, Fraser C, Qin X, Xu L, Deskin B, Ghelfi E, *et al.* 2021 Age-dependent regulation of SARS-CoV-2 cell entry genes and cell death programs correlates with COVID-19 severity. *Science Advances* **7** eabf8609. (https://doi.org/10.1126/sciadv.abf8609)

Iwata-Yoshikawa N, Okamura T, Shimizu Y, Hasegawa H, Takeda M & Nagata N 2019 TMPRSS2 contributes to virus spread and immunopathology in the airways of murine models after coronavirus infection. *Journal of Virology* **93** e01815-18. (https://doi.org/10.1128/JVI.01815-18)

Jackson CB, Farzan M, Chen B & Choe H 2022 Mechanisms of SARS-CoV-2 entry into cells. *Nature Reviews. Molecular Cell Biology* **23** 3–20. (https://doi.org/10.1038/s41580-021-00418-x)

Ji H, Menini S, Zheng W, Pesce C, Wu X & Sandberg K 2008 Role of angiotensin-converting enzyme 2 and angiotensin(1–7) in 17beta-oestradiol regulation of renal pathology in renal wrap hypertension in rats. *Experimental Physiology* **93** 648–657. (https://doi.org/10.1113/expphysiol.2007.041392)

Jia HP, Look DC, Tan P, Shi L, Hickey M, Gakhar L, Chappell MC, Wohlford-Lenane C & Mccray PB 2009 Ectodomain shedding of angiotensin converting enzyme 2 in human airway epithelia. *American Journal of Physiology. Lung Cellular and Molecular Physiology* **297** L84–L96. (https://doi.org/10.1152/ajplung.00071.2009)

Kalidhindi RSR, Borkar NA, Ambhore NS, Pabelick CM, Prakash YS & Sathish V 2020 Sex steroids skew ACE2 expression in human airway: a contributing factor to sex differences in COVID-19? *American Journal of Physiology-Lung Cellular and Molecular Physiology* **319** L843–L847. (https://doi.org/10.1152/ajplung.00391.2020)

Khan DSA, Hamid LR, Ali A, Salam RA, Zuberi N, Lassi ZS & Das JK 2021 Differences in pregnancy and perinatal outcomes among symptomatic versus asymptomatic COVID-19-infected pregnant women: a systematic review and meta-analysis. *BMC Pregnancy and Childbirth* **21** 801. (https://doi.org/10.1186/s12884-021-04250-1)

Kim L, Garg S, O'Halloran A, Whitaker M, Pham H, Anderson EJ, Armistead I, Bennett NM, Billing L, Como-Sabetti K, *et al.* 2020 Risk factors for Intensive Care Unit admission and in-hospital mortality among hospitalized adults identified through the US coronavirus disease 2019 (COVID-19)-associated hospitalization surveillance network (COVID-NET). *Clinical Infectious Diseases* **72** e206–e214. (https://doi.org/10.1093/cid/ciaa1012)

Komatsu T, Suzuki Y, Imai J, Sugano S, Hida M, Tanigami A, Muroi S, Yamada Y & Hanaoka K 2002 Molecular cloning, mRNA expression and chromosomal localization of mouse angiotensin-converting enzyme-related carboxypeptidase (mACE2). *DNA Sequence* **13** 217–220. (https://doi.org/10.1080/1042517021000021608)

Kotlar B, Gerson EM, Petrillo S, Langer A & Tiemeier H 2021 The impact of the COVID-19 pandemic on maternal and perinatal health: a scoping review. *Reproductive Health* **18** 10. (https://doi.org/10.1186/s12978-021-01070-6)

Kowalczuk S, Broer A, Tietze N, Vanslambrouck JM, Rasko JE & Broer S 2008 A protein complex in the brush-border membrane explains a Hartnup disorder allele. *FASEB Journal* **22** 2880–2887. (https://doi.org/10.1096/fj.08-107300)

Kragstrup TW, Singh HS, Grundberg I, Nielsen AL-L, Rivellese F, Mehta A, Goldberg MB, Filbin MR, Qvist P & Bibby BM 2021 Plasma ACE2 predicts outcome of COVID-19 in hospitalized patients. *PLoS One* **16** e0252799. (https://doi.org/10.1371/journal.pone.0252799)

Kuba K, Imai Y, Rao S, Gao H, Guo F, Guan B, Huan Y, Yang P, Zhang Y, Deng W, *et al.* 2005 A crucial role of angiotensin converting enzyme 2 (ACE2) in SARS coronavirus-induced lung injury. *Nature Medicine* **11** 875–879. (https://doi.org/10.1038/nm1267)

Kuba K, Imai Y, Ohto-Nakanishi T & Penninger JM 2010 Trilogy of ACE2: a peptidase in the renin-angiotensin system, a SARS receptor, and a partner for amino acid transporters. *Pharmacology and Therapeutics* **128** 119–128. (https://doi.org/10.1016/j.pharmthera.2010.06.003)

Kuba K, Imai Y & Penninger JM 2013 Multiple functions of angiotensin-converting enzyme 2 and its relevance in cardiovascular diseases. *Circulation Journal* **77** 301–308. (https://doi.org/10.1253/circj.cj-12-1544)

Kuznetsova T & Cauwenberghs N 2021 Determinants of circulating angiotensin-converting enzyme 2 protein levels in the general population. *European Journal of Internal Medicine* **84** 104–105. (https://doi.org/10.1016/j.ejim.2020.10.012)

Lambert DW, Yarski M, Warner FJ, Thornhill P, Parkin ET, Smith AI, Hooper NM & Turner AJ 2005 Tumor necrosis factor-alpha convertase (ADAM17) mediates regulated ectodomain shedding of the severe-acute respiratory syndrome-coronavirus (SARS-CoV) receptor, angiotensin-converting enzyme-2 (ACE2). *Journal of Biological Chemistry* **280** 30113–30119. (https://doi.org/10.1074/jbc.M505111200)

Lassi ZS, Ana A, Das JK, Salam RA, Padhani ZA, Irfan O & Bhutta ZA 2021 A systematic review and meta-analysis of data on pregnant women with confirmed COVID-19: clinical presentation, and pregnancy and perinatal outcomes based on COVID-19 severity. *Journal of Global Health* **11** 05018. (https://doi.org/10.7189/jogh.11.05018)

Leach DA, Mohr A, Giotis ES, Cil E, Isac AM, Yates LL, Barclay WS, Zwacka RM, Bevan CL & Brooke GN 2021 The antiandrogen enzalutamide downregulates TMPRSS2 and reduces cellular entry of SARS-CoV-2 in human lung cells. *Nature Communications* **12** 4068. (https://doi.org/10.1038/s41467-021-24342-y)

Lee DK, Cheng R, Nguyen T, Fan T, Kariyawasam AP, Liu Y, Osmond DH, George SR & O'dowd BF 2000 Characterization of apelin, the ligand for the APJ receptor. *Journal of Neurochemistry* **74** 34–41. (https://doi.org/10.1046/j.1471-4159.2000.0740034.x)

Lee DK, George SR & O'dowd BF 2006 Unravelling the roles of the apelin system: prospective therapeutic applications in heart failure and obesity. *Trends in Pharmacological Sciences* **27** 190–194. (https://doi.org/10.1016/j.tips.2006.02.006)

Lee IT, Nakayama T, Wu CT, Goltsev Y, Jiang S, Gall PA, Liao CK, Shih LC, Schürch CM, Mcilwain DR, *et al.* 2020 ACE2 localizes to the respiratory cilia and is not increased by ACE inhibitors or ARBs. *Nature Communications* **11** 5453. (https://doi.org/10.1038/s41467-020-19145-6)

Lewnard JA, Mahmud A, Narayan T, Wahl B, Selvavinayagam TS, Mohan BC & Laxminarayan R 2022 All-cause mortality during the

COVID-19 pandemic in Chennai, India: an observational study. *Lancet. Infectious Diseases* **22** 463–472. (https://doi.org/10.1016/S1473-3099(21)00746-5)

Li W, Moore MJ, Vasilieva N, Sui J, Wong SK, Berne MA, Somasundaran M, Sullivan JL, Luzuriaga K, Greenough TC, *et al.* 2003 Angiotensin-converting enzyme 2 is a functional receptor for the SARS coronavirus. *Nature* **426** 450–454. (https://doi.org/10.1038/nature02145)

Li LQ, Huang T, Wang YQ, Wang ZP, Liang Y, Huang TB, Zhang HY, Sun W & Wang Y 2020*a* COVID-19 patients' clinical characteristics, discharge rate, and fatality rate of meta-analysis. *Journal of Medical Virology* **92** 577–583. (https://doi.org/10.1002/jmv.25757)

Li MY, Li L, Zhang Y & Wang XS 2020*b* Expression of the SARS-CoV-2 cell receptor gene ACE2 in a wide variety of human tissues. *Infectious Diseases of Poverty* **9** 45. (https://doi.org/10.1186/s40249-020-00662-x)

Li K, Wohlford-Lenane C, Bartlett JA & Mccray PB 2021*a* Intersubject variation in ACE2 protein expression in human airway epithelia and its relationship to severe acute respiratory syndrome coronavirus 2. *Journal of Infectious Diseases* **224** 1357–1361. (https://doi.org/10.1093/infdis/jiab383)

Li XT, Zhang MW, Zhang ZZ, Cao YD, Liu XY, Miao R, Xu Y, Song XF, Song JW, Liu Y, *et al.* 2021*b* Abnormal apelin-ACE2 and SGLT2 signaling contribute to adverse cardiorenal injury in patients with COVID-19. *International Journal of Cardiology* **336** 123–129. (https://doi.org/10.1016/j.ijcard.2021.05.029)

Lin B, Ferguson C, White JT, Wang S, Vessella R, True LD, Hood L & Nelson PS 1999 Prostate-localized and androgen-regulated expression of the membrane-bound serine protease TMPRSS21. *Cancer Research* **59** 4180–4184.

Liu J, Ji H, Zheng W, Wu X, Zhu JJ, Arnold AP & Sandberg K 2010 Sex differences in renal angiotensin converting enzyme 2 (ACE2) activity are 17β-oestradiol-dependent and sex chromosome-independent. *Biology of Sex Differences* **1** 6. (https://doi.org/10.1186/2042-6410-1-6)

Lu-Culligan A, Chavan AR, Vijayakumar P, Irshaid L, Courchaine EM, Milano KM, Tang Z, Pope SD, Song E, Vogels CBF, *et al.* 2021 Maternal respiratory SARS-CoV-2 infection in pregnancy is associated with a robust inflammatory response at the maternal-fetal interface. *Med* **2** 591–610.e10. (https://doi.org/10.1016/j.medj.2021.04.016)

Malakauskas SM, Quan H, Fields TA, Mccall SJ, Yu MJ, Kourany WM, Frey CW & Le TH 2007 Aminoaciduria and altered renal expression of luminal amino acid transporters in mice lacking novel gene collectrin. *American Journal of Physiology. Renal Physiology* **292** F533–F544. (https://doi.org/10.1152/ajprenal.00325.2006)

Manning DC & Snyder SH 1989 Bradykinin receptors localized by quantitative autoradiography in kidney, ureter, and bladder. *American Journal of Physiology* **256** F909–F915. (https://doi.org/10.1152/ajprenal.1989.256.5.F909)

Mantione CR & Rodriguez R 1990 A bradykinin (BK)1 receptor antagonist blocks capsaicin-induced ear inflammation in mice. *British Journal of Pharmacology* **99** 516–518. (https://doi.org/10.1111/j.1476-5381.1990.tb12960.x)

Mao R, Qiu Y, He JS, Tan JY, Li XH, Liang J, Shen J, Zhu LR, Chen Y, Iacucci M, *et al.* 2020 Manifestations and prognosis of gastrointestinal and liver involvement in patients with COVID-19: a systematic review and meta-analysis. *Lancet. Gastroenterology and Hepatology* **5** 667–678. (https://doi.org/10.1016/S2468-1253(20)30126-6)

Marceau F, Bachelard H, Bouthillier J, Fortin JP, Morissette G, Bawolak MT, Charest-Morin X & Gera L 2020 Bradykinin receptors: agonists, antagonists, expression, signaling, and adaptation to sustained stimulation. *International Immunopharmacology* **82** 106305. (https://doi.org/10.1016/j.intimp.2020.106305)

Maremanda KP, Sundar IK, Li D & Rahman I 2020 Age-dependent assessment of genes involved in cellular senescence, telomere, and mitochondrial pathways in human lung tissue of smokers, COPD, and IPF: associations with SARS-CoV-2 COVID-19 ACE2-TMPRSS2-Furin-DPP4 axis. *Frontiers in Pharmacology* **11** 584637. (https://doi.org/10.3389/fphar.2020.584637)

Marques FZ, Pringle KG, Conquest A, Hirst JJ, Markus MA, Sarris M, Zakar T, Morris BJ & Lumbers ER 2011 Molecular characterization of renin-angiotensin system components in human intrauterine tissues and fetal membranes from vaginal delivery and cesarean section. *Placenta* **32** 214–221. (https://doi.org/10.1016/j.placenta.2010.12.006)

Martin B, Dewitt PE, Russell S, Anand A, Bradwell KR, Bremer C, Gabriel D, Girvin AT, Hajagos JG, Mcmurry JA, *et al.* 2022 Characteristics, outcomes, and severity risk factors associated with SARS-CoV-2 infection among children in the US national COVID cohort collaborative. *JAMA Network Open* **5** e2143151. (https://doi.org/10.1001/jamanetworkopen.2021.43151)

Martins FL, Tavares CAM, Malagrino PA, Rentz T, Benetti A, Rios TMS, Pereira GMD, Caramelli B, Teixeira SK, Krieger JE, *et al.* 2021 Sex differences in the lung ACE/ACE2 balance in hypertensive rats. *Bioscience Reports* **41**. (https://doi.org/10.1042/BSR20211201)

Mason KE, Maudsley G, Mchale P, Pennington A, Day J & Barr B 2021 Age-adjusted associations between comorbidity and outcomes of COVID-19: a review of the evidence from the early stages of the pandemic. *Frontiers in Public Health* **9** 584182. (https://doi.org/10.3389/fpubh.2021.584182)

Matsuyama S, Nagata N, Shirato K, Kawase M, Takeda M & Taguchi F 2010 Efficient activation of the severe acute respiratory syndrome coronavirus spike protein by the transmembrane protease TMPRSS2. *Journal of Virology* **84** 12658–12664. (https://doi.org/10.1128/JVI.01542-10)

Merrill DC, Karoly M, Chen K, Ferrario CM & Brosnihan KB 2002 Angiotensin-(1–7) in normal and preeclamptic pregnancy. *Endocrine* **18** 239–245. (https://doi.org/10.1385/ENDO:18:3:239)

Milani GP, Bottino I, Rocchi A, Marchisio P, Elli S, Agostoni C & Costantino G 2021 Frequency of children vs adults carrying severe acute respiratory syndrome coronavirus 2 asymptomatically. *JAMA Pediatrics* **175** 193–194. (https://doi.org/10.1001/jamapediatrics.2020.3595)

Mompeon A, Lazaro-Franco M, Bueno-Beti C, Perez-Cremades D, Vidal-Gomez X, Monsalve E, Gironacci MM, Hermenegildo C & Novella S 2016 Estradiol, acting through ERalpha, induces endothelial non-classic renin-angiotensin system increasing angiotensin 1–7 production. *Molecular and Cellular Endocrinology* **422** 1–8. (https://doi.org/10.1016/j.mce.2015.11.004)

Montopoli M, Zumerle S, Vettor R, Rugge M, Zorzi M, Catapano CV, Carbone GM, Cavalli A, Pagano F, Ragazzi E, *et al.* 2020 Androgen-deprivation therapies for prostate cancer and risk of infection by SARS-CoV-2: a population-based study ($N = 4532$). *Annals of Oncology* **31** 1040–1045. (https://doi.org/10.1016/j.annonc.2020.04.479)

Muus C, Luecken MD, Eraslan G, Sikkema L, Waghray A, Heimberg G, Kobayashi Y, Vaishnav ED, Subramanian A, Smillie C, *et al.* 2021 Single-cell meta-analysis of SARS-CoV-2 entry genes across tissues and demographics. *Nature Medicine* **27** 546–559. (https://doi.org/10.1038/s41591-020-01227-z)

Nikpouraghdam M, Jalali Farahani A, Alishiri G, Heydari S, Ebrahimnia M, Samadinia H, Sepandi M, Jafari NJ, Izadi M, Qazvini A, *et al.* 2020 Epidemiological characteristics of coronavirus disease 2019 (COVID-19) patients in IRAN: a single center study. *Journal of Clinical Virology* **127** 104378. (https://doi.org/10.1016/j.jcv.2020.104378)

Onabajo OO, Banday AR, Stanifer ML, Yan W, Obajemu A, Santer DM, Florez-Vargas O, Piontkivska H, Vargas JM, Ring TJ, *et al.* 2020 Interferons and viruses induce a novel truncated ACE2 isoform and not the full-length SARS-CoV-2 receptor. *Nature Genetics* **52** 1283–1293. (https://doi.org/10.1038/s41588-020-00731-9)

Onder G, Rezza G & Brusaferro S 2020 Case-fatality rate and characteristics of patients dying in relation to COVID-19 in Italy. *JAMA* **323** 1775–1776. (https://doi.org/10.1001/jama.2020.4683)

Ortiz ME, Thurman A, Pezzulo AA, Leidinger MR, Klesney-Tait JA, Karp PH, Tan P, Wohlford-Lenane C, Mccray PB & Meyerholz DK 2020 Heterogeneous expression of the SARS-Coronavirus-2 receptor ACE2 in the human respiratory tract. *EBioMedicine* **60** 102976. (https://doi.org/10.1016/j.ebiom.2020.102976)

Oudit GY, Crackower MA, Backx PH & Penninger JM 2003 The role of ACE2 in cardiovascular physiology. *Trends in Cardiovascular Medicine* **13** 93–101. (https://doi.org/10.1016/s1050-1738(02)00233-5)

Overton EE, Goffman D & Friedman AM 2022 The epidemiology of COVID-19 in pregnancy. *Clinical Obstetrics and Gynecology* **65** 110–122. (https://doi.org/10.1097/GRF.0000000000000674)

Patel SK, Juno JA, Lee WS, Wragg KM, Hogarth PM, Kent SJ & Burrell LM 2021 Plasma ACE2 activity is persistently elevated following SARS-CoV-2 infection: implications for COVID-19 pathogenesis and consequences. *European Respiratory Journal* **57**. (https://doi.org/10.1183/13993003.03730-2020)

Pathirathna ML, Samarasekara BPP, Dasanayake TS, Saravanakumar P & Weerasekara I 2022 Adverse perinatal outcomes in COVID-19 infected pregnant women: a systematic review and meta-analysis. *Healthcare (Basel)* **10**. (https://doi.org/10.3390/healthcare10020203)

Pavel AB, Wu J, Renert-Yuval Y, Del Duca E, Glickman JW, Miller RL, Paller AS, Krueger JG & Guttman-Yassky E 2021 SARS-CoV-2 receptor ACE2 protein expression in serum is significantly associated with age. *Allergy* **76** 875–878. (https://doi.org/10.1111/all.14522)

Peacock TP, Goldhill DH, Zhou J, Baillon L, Frise R, Swann OC, Kugathasan R, Penn R, Brown JC, Sanchez-David RY, *et al.* 2021 The furin cleavage site in the SARS-CoV-2 spike protein is required for transmission in ferrets. *Nature Microbiology* **6** 899–909. (https://doi.org/10.1038/s41564-021-00908-w)

Pitkin SL, Maguire JJ, Bonner TI & Davenport AP 2010 International union of basic and Clinical Pharmacology. LXXIV. Apelin receptor nomenclature, distribution, pharmacology, and function. *Pharmacological Reviews* **62** 331–342. (https://doi.org/10.1124/pr.110.002949)

Pringle KG, Tadros MA, Callister RJ & Lumbers ER 2011 The expression and localization of the human placental prorenin/renin-angiotensin system throughout pregnancy: roles in trophoblast invasion and angiogenesis? *Placenta* **32** 956–962. (https://doi.org/10.1016/j.placenta.2011.09.020)

Ramos SG, Rattis BADC, Ottaviani G, Celes MRN & Dias EP 2021 ACE2 down-regulation may act as a transient molecular disease causing RAAS dysregulation and tissue damage in the microcirculatory environment among COVID-19 patients. *American Journal of Pathology* **191** 1154–1164. (https://doi.org/10.1016/j.ajpath.2021.04.010)

Rang C, Cheng A, Kelly P & Kotsimbos T 2020 COVID-19 from the land "Down Under" in an upside-down world: an Australian perspective. *European Respiratory Journal* **56** 2001844. (https://doi.org/10.1183/13993003.01844-2020)

Raparelli V, Palmieri L, Canevelli M, Pricci F, Unim B, Lo Noce C, Villani ER, Rochon PA, Pilote L, Vanacore N, *et al.* 2020 Sex differences in clinical phenotype and transitions of care among individuals dying of COVID-19 in Italy. *Biology of Sex Differences* **11** 57. (https://doi.org/10.1186/s13293-020-00334-3)

Rhaleb NE, Yang XP & Carretero OA 2011 The kallikrein-kinin system as a regulator of cardiovascular and renal function. *Comprehensive Physiology* **1** 971–993. (https://doi.org/10.1002/cphy.c100053)

Rockx B, Baas T, Zornetzer GA, Haagmans B, Sheahan T, Frieman M, Dyer MD, Teal TH, Proll S, Brand JVD, *et al.* 2009 Early upregulation of acute respiratory distress syndrome-associated cytokines promotes lethal disease in an aged-mouse model of severe acute respiratory syndrome coronavirus infection. *Journal of Virology* **83** 7062–7074. (https://doi.org/10.1128/JVI.00127-09)

Saheb Sharif-Askari N, Saheb Sharif-Askari F, Alabed M, Temsah MH, Al Heialy S, Hamid Q & Halwani R 2020 Airways expression of SARS-CoV-2 receptor, ACE2, and TMPRSS2 is lower in children than adults and increases with smoking and COPD. *Molecular Therapy. Methods and Clinical Development* **18** 1–6. (https://doi.org/10.1016/j.omtm.2020.05.013)

Salje H, Tran Kiem C, Lefrancq N, Courtejoie N, Bosetti P, Paireau J, Andronico A, Hozé N, Richet J, Dubost CL, *et al.* 2020 Estimating the burden of SARS-CoV-2 in France. *Science* **369** 208–211. (https://doi.org/10.1126/science.abc3517)

Samuel RM, Majd H, Richter MN, Ghazizadeh Z, Zekavat SM, Navickas A, Ramirez JT, Asgharian H, Simoneau CR, Bonser LR, *et al.* 2020 Androgen signaling regulates SARS-CoV-2 receptor levels and is associated with severe COVID-19 symptoms in men. *Cell Stem Cell* **27** 876–889.e12. (https://doi.org/10.1016/j.stem.2020.11.009)

Sanchez J, Vigil-De Gracia P, Guerrero E, Gaitan M, Fu C, Chen-German M, Villalobos R, Coronado L, Martinez AA, Arauz D, *et al.* 2021 Severe acute respiratory syndrome coronavirus 2 detected in placentas of 2 coronavirus disease 2019-positive asymptomatic pregnant women-case report. *AJOG Global Reports* **1** 100001. (https://doi.org/10.1016/j.xagr.2020.100001)

Sarver DC & Wong GW 2021 Obesity alters Ace2 and Tmprss2 expression in lung, trachea, and esophagus in a sex-dependent manner: implications for COVID-19. *Biochemical and Biophysical Research Communications* **538** 92–96. (https://doi.org/10.1016/j.bbrc.2020.10.066)

Schmaier AH 2002 The plasma kallikrein-kinin system counterbalances the renin-angiotensin system. *Journal of Clinical Investigation* **109** 1007–1009. (https://doi.org/10.1172/JCI15490)

Schock H, Zeleniuch-Jacquotte A, Lundin E, Grankvist K, Lakso HÅ, Idahl A, Lehtinen M, Surcel HM & Fortner RT 2016 Hormone concentrations throughout uncomplicated pregnancies: a longitudinal study. *BMC Pregnancy and Childbirth* **16** 146. (https://doi.org/10.1186/s12884-016-0937-5)

Schurink B, Roos E, Vos W, Breur M, Van Der Valk P & Bugiani M 2022 ACE2 protein expression during childhood, adolescence, and early adulthood. *Pediatric and Developmental Pathology* **25** 404–408. (https://doi.org/10.1177/10935266221075312)

Schwartz DA, Baldewijns M, Benachi A, Bugatti M, Collins RRJ, De Luca D, Facchetti F, Linn RL, Marcelis L, Morotti D, *et al.* 2020 Chronic histiocytic intervillositis with trophoblast necrosis are risk factors associated with placental infection from coronavirus disease 2019 (COVID-19) and intrauterine maternal-fetal severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) transmission in liveborn and stillborn infants. *Archives of Pathology and Laboratory Medicine* **145** 517–528. (https://doi.org/10.5858/arpa.2020-0771-sa)

Schwartz DA & Graham AL 2020 Potential maternal and infant outcomes from (wuhan) coronavirus 2019-nCoV infecting pregnant women: lessons from SARS, MERS, and other human coronavirus infections. *Viruses* **12**. (https://doi.org/10.3390/v12020194)

Shang J, Wan Y, Luo C, Ye G, Geng Q, Auerbach A & Li F 2020 Cell entry mechanisms of SARS-CoV-2. *Proceedings of the National Academy of Sciences of the United States of America* **117** 11727–11734. (https://doi.org/10.1073/pnas.2003138117)

Shulla A, Heald-Sargent T, Subramanya G, Zhao J, Perlman S & Gallagher T 2011 A transmembrane serine protease is linked to the severe acute respiratory syndrome coronavirus receptor and activates virus entry. *Journal of Virology* **85** 873–882. (https://doi.org/10.1128/JVI.02062-10)

Simmons G, Gosalia DN, Rennekamp AJ, Reeves JD, Diamond SL & Bates P 2005 Inhibitors of cathepsin L prevent severe acute respiratory syndrome coronavirus entry. *Proceedings of the National Academy of Sciences of the United States of America* **102** 11876–11881. (https://doi.org/10.1073/pnas.0505577102)

Simões e Silva AC, Silveira KD, Ferreira AJ & Teixeira MM 2013 ACE2, angiotensin-(1-7) and Mas receptor axis in inflammation and fibrosis. *British Journal of Pharmacology* **169** 477–492. (https://doi.org/10.1111/bph.12159)

Sodhi CP, Wohlford-Lenane C, Yamaguchi Y, Prindle T, Fulton WB, Wang S, Mccray PB, Chappell M, Hackam DJ & Jia H 2018 Attenuation of pulmonary ACE2 activity impairs inactivation of des-Arg(9) bradykinin/BKB1R axis and facilitates LPS-induced neutrophil infiltration. *American Journal of Physiology. Lung Cellular and Molecular Physiology* **314** L17–L31. (https://doi.org/10.1152/ajplung.00498.2016)

Song R, Preston G & Yosypiv IV 2012 Ontogeny of angiotensin-converting enzyme 2. *Pediatric Research* **71** 13–19. (https://doi.org/10.1038/pr.2011.7)

Song H, Seddighzadeh B, Cooperberg MR & Huang FW 2020 Expression of ACE2, the SARS-CoV-2 receptor, and TMPRSS2 in prostate epithelial

cells. *European Urology* **78** 296–298. (https://doi.org/10.1016/j.eururo.2020.04.065)
Sriramula S 2020 Kinin B1 receptor: a target for neuroinflammation in hypertension. *Pharmacological Research* **155** 104715. (https://doi.org/10.1016/j.phrs.2020.104715)
Stelzig KE, Canepa-Escaro F, Schiliro M, Berdnikovs S, Prakash YS & Chiarella SE 2020 Estrogen regulates the expression of SARS-CoV-2 receptor ACE2 in differentiated airway epithelial cells. *American Journal of Physiology-Lung Cellular and Molecular Physiology* **318** L1280–L1281. (https://doi.org/10.1152/ajplung.00153.2020)
Stopsack KH, Mucci LA, Antonarakis ES, Nelson PS & Kantoff PW 2020 TMPRSS2 and COVID-19: serendipity or opportunity for intervention? *Cancer Discovery* **10** 779–782. (https://doi.org/10.1158/2159-8290.CD-20-0451)
Stuardo M, Gonzalez CB, Nualart F, Boric M, Corthorn J, Bhoola KD & Figueroa CD 2004 Stimulated human neutrophils form biologically active kinin peptides from high and low molecular weight kininogens. *Journal of Leukocyte Biology* **75** 631–640. (https://doi.org/10.1189/jlb.1103546)
Sward P, Edsfeldt A, Reepalu A, Jehpsson L, Rosengren BE & Karlsson MK 2020 Age and sex differences in soluble ACE2 may give insights for COVID-19. *Critical Care* **24** 221. (https://doi.org/10.1186/s13054-020-02942-2)
Taglauer ES, Wachman EM, Juttukonda L, Klouda T, Kim J, Wang Q, Ishiyama A, Hackam DJ, Yuan K & Jia H 2022 Acute severe acute respiratory syndrome coronavirus 2 infection in pregnancy is associated with placental angiotensin-converting enzyme 2 shedding. *American Journal of Pathology* **192** 595–603. (https://doi.org/10.1016/j.ajpath.2021.12.011)
Tamanna S, Clifton VL, Rae K, Van Helden DF, Lumbers ER & Pringle KG 2020 Angiotensin converting enzyme 2 (ACE2) in pregnancy: preeclampsia and small for gestational age. *Frontiers in Physiology* **11** 590787. (https://doi.org/10.3389/fphys.2020.590787)
Tatemoto K, Hosoya M, Habata Y, Fujii R, Kakegawa T, Zou MX, Kawamata Y, Fukusumi S, Hinuma S, Kitada C, *et al.* 1998 Isolation and characterization of a novel endogenous peptide ligand for the human APJ receptor. *Biochemical and Biophysical Research Communications* **251** 471–476. (https://doi.org/10.1006/bbrc.1998.9489)
Tipnis SR, Hooper NM, Hyde R, Karran E, Christie G & Turner AJ 2000 A human homolog of angiotensin-converting enzyme. Cloning and functional expression as a captopril-insensitive carboxypeptidase. *Journal of Biological Chemistry* **275** 33238–33243. (https://doi.org/10.1074/jbc.M002615200)
Turner AJ & Hooper NM 2002 The angiotensin-converting enzyme gene family: genomics and pharmacology. *Trends in Pharmacological Sciences* **23** 177–183. (https://doi.org/10.1016/s0165-6147(00)01994-5)
Turner AJ 2015 ACE2 cell biology, regulation, and physiological functions. In *The Protective Arm of the Renin Angiotensin System (RAS)*. T Unger, UM Steckelings & RAS Dos Santos Eds. Amsterdam: Academic Press. (https://doi.org/10.1016/B978-0-12-801364-9.00025-0)
Uri K, Fagyas M, Manyine Siket I, Kertesz A, Csanadi Z, Sandorfi G, Clemens M, Fedor R, Papp Z, Edes I, *et al.* 2014 New perspectives in the renin-angiotensin-aldosterone system (RAAS) IV: circulating ACE2 as a biomarker of systolic dysfunction in human hypertension and heart failure. *PLoS One* **9** e87845. (https://doi.org/10.1371/journal.pone.0087845)
Uri K, Fagyas M, Kertesz A, Borbely A, Jenei C, Bene O, Csanadi Z, Paulus WJ, Edes I, Papp Z, *et al.* 2016 Circulating ACE2 activity correlates with cardiovascular disease development. *Journal of the Renin-Angiotensin-Aldosterone System* **17**. (https://doi.org/10.1177/1470320316668435)
Valdes G, Neves LA, Anton L, Corthorn J, Chacon C, Germain AM, Merrill DC, Ferrario CM, Sarao R, Penninger J, *et al.* 2006 Distribution of angiotensin-(1–7) and ACE2 in human placentas of normal and pathological pregnancies. *Placenta* **27** 200–207. (https://doi.org/10.1016/j.placenta.2005.02.015)
Wang Y, Lumbers ER, Sykes SD & Pringle KG 2015 Regulation of the renin-angiotensin system pathways in the human decidua. *Reproductive Sciences* **22** 865–872. (https://doi.org/10.1177/1933719114565029)
Valdespino-Vazquez MY, Helguera-Repetto CA, Leon-Juarez M, Villavicencio-Carrisoza O, Flores-Pliego A, Moreno-Verduzco ER, Diaz-Perez DL, Villegas-Mota I, Carrasco-Ramirez E, Lopez-Martinez IE, *et al.* 2021 Fetal and placental infection with SARS-CoV-2 in early pregnancy. *Journal of Medical Virology* **93** 4480–4487. (https://doi.org/10.1002/jmv.26965)
Verma S, Joshi CS, Silverstein RB, He M, Carter EB & Mysorekar IU 2021 SARS-CoV-2 colonization of maternal and fetal cells of the human placenta promotes alteration of local renin-angiotensin system. *Med* **2** 575–590.e5. (https://doi.org/10.1016/j.medj.2021.04.009)
Vickers C, Hales P, Kaushik V, Dick L, Gavin J, Tang J, Godbout K, Parsons T, Baronas E, Hsieh F, *et al.* 2002 Hydrolysis of biological peptides by human angiotensin-converting enzyme-related carboxypeptidase. *Journal of Biological Chemistry* **277** 14838–14843. (https://doi.org/10.1074/jbc.M200581200)
Viveiros A, Gheblawi M, Aujla PK, Sosnowski DK, Seubert JM, Kassiri Z & Oudit GY 2022 Sex- and age-specific regulation of ACE2: insights into severe COVID-19 susceptibility. *Journal of Molecular and Cellular Cardiology* **164** 13–16. (https://doi.org/10.1016/j.yjmcc.2021.11.003)
Walls AC, Park YJ, Tortorici MA, Wall A, Mcguire AT & Veesler D 2020 Structure, function, and antigenicity of the SARS-CoV-2 spike glycoprotein. *Cell* **181** 281–292.e6. (https://doi.org/10.1016/j.cell.2020.02.058)
Wang Q, Li W, Liu XS, Carroll JS, Jänne OA, Keeton EK, Chinnaiyan AM, Pienta KJ & Brown M 2007 A hierarchical network of transcription factors governs androgen receptor-dependent prostate cancer growth. *Molecular Cell* **27** 380–392. (https://doi.org/10.1016/j.molcel.2007.05.041)
Wang S, Guo F, Liu K, Wang H, Rao S, Yang P & Jiang C 2008 Endocytosis of the receptor-binding domain of SARS-CoV spike protein together with virus receptor ACE2. *Virus Research* **136** 8–15. (https://doi.org/10.1016/j.virusres.2008.03.004)
Wang J, Syrett CM, Kramer MC, Basu A, Atchison ML & Anguera MC 2016*a* Unusual maintenance of X chromosome inactivation predisposes female lymphocytes for increased expression from the inactive X. *Proceedings of the National Academy of Sciences of the United States of America* **113** E2029–E2038. (https://doi.org/10.1073/pnas.1520113113)
Wang W, Mckinnie SM, Farhan M, Paul M, Mcdonald T, Mclean B, Llorens-Cortes C, Hazra S, Murray AG, Vederas JC, *et al.* 2016*b* Angiotensin-converting enzyme 2 metabolizes and partially inactivates Pyr-Apelin-13 and Apelin-17: physiological effects in the cardiovascular system. *Hypertension* **68** 365–377. (https://doi.org/10.1161/HYPERTENSIONAHA.115.06892)
Wang A, Chiou J, Poirion OB, Buchanan J, Valdez MJ, Verheyden JM, Hou X, Kudtarkar P, Narendra S, Newsome JM, *et al.* 2020 Single-cell multiomic profiling of human lungs reveals cell-type-specific and age-dynamic control of SARS-CoV2 host genes. *eLife* **9** e62522. (https://doi.org/10.7554/eLife.62522)
Wang H, Sun X, L Voncannon J, Kon ND, Ferrario CM & Groban L 2021 Estrogen receptors are linked to angiotensin-converting enzyme 2 (ACE2), ADAM metallopeptidase domain 17 (ADAM-17), and transmembrane protease serine 2 (TMPRSS2) expression in the human atrium: insights into COVID-19. *Hypertension Research* **44** 882–884. (https://doi.org/10.1038/s41440-021-00626-0)
Wang H, Cong L, Yin X, Zhang N, Zhu M, Sun T, Fan J, Xue F, Fan X & Gong Y 2022*a* The apelin-APJ axis alleviates LPS-induced pulmonary fibrosis and endothelial mesenchymal transformation in mice by promoting angiotensin-converting enzyme 2. *Cellular Signalling* **98** 110418. (https://doi.org/10.1016/j.cellsig.2022.110418)
Wang H, Li N, Sun C, Guo X, Su W, Song Q, Liang Q, Liang M, Ding X, Lowe S, *et al.* 2022*b* The association between pregnancy and COVID-19: a systematic review and meta-analysis. *American Journal*

of Emergency Medicine **56** 188–195. (https://doi.org/10.1016/j.ajem.2022.03.060)

Wark PAB, Pathinayake PS, Kaiko G, Nichol K, Ali A, Chen L, Sutanto EN, Garratt LW, Sohal SS, Lu W, *et al.* 2021 ACE2 expression is elevated in airway epithelial cells from older and male healthy individuals but reduced in asthma. *Respirology* **26** 442–451. (https://doi.org/10.1111/resp.14003)

Wei SQ, Bilodeau-Bertrand M, Liu S & Auger N 2021 The impact of COVID-19 on pregnancy outcomes: a systematic review and meta-analysis. *CMAJ* **193** E540–E548. (https://doi.org/10.1503/cmaj.202604)

Williams TL, Strachan G, Macrae RGC, Kuc RE, Nyimanu D, Paterson AL, Sinha S, Maguire JJ & Davenport AP 2021 Differential expression in humans of the viral entry receptor ACE2 compared with the short deltaACE2 isoform lacking SARS-CoV-2 binding sites. *Scientific Reports* **11** 24336. (https://doi.org/10.1038/s41598-021-03731-9)

Williamson EJ, Walker AJ, Bhaskaran K, Bacon S, Bates C, Morton CE, Curtis HJ, Mehrkar A, Evans D, Inglesby P, *et al.* 2020 Factors associated with COVID-19-related death using OpenSAFELY. *Nature* **584** 430–436. (https://doi.org/10.1038/s41586-020-2521-4)

Wong SF, Chow KM, Leung TN, Ng WF, Ng TK, Shek CC, Ng PC, Lam PW, Ho LC, To WW, *et al.* 2004 Pregnancy and perinatal outcomes of women with severe acute respiratory syndrome. *American Journal of Obstetrics and Gynecology* **191** 292–297. (https://doi.org/10.1016/j.ajog.2003.11.019)

Wu Z & McGoogan JM 2020 Characteristics of and important lessons from the coronavirus disease 2019 (COVID-19) outbreak in China: summary of a report of 72 314 cases from the Chinese Center for Disease Control and Prevention. *JAMA* **323** 1239–1242. (https://doi.org/10.1001/jama.2020.2648)

Xie J, Huang QF, Zhang Z, Dong Y, Xu H, Cao Y, Sheng CS, Li Y, Wang C, Wang X, *et al.* 2023 Angiotensin-converting enzyme 2 in human plasma and lung tissue. *Blood Pressure* **32** 6–15. (https://doi.org/10.1080/08037051.2022.2154745)

Xudong X, Junzhu C, Xingxiang W, Furong Z & Yanrong L 2006 Age- and gender-related difference of ACE2 expression in rat lung. *Life Sciences* **78** 2166–2171. (https://doi.org/10.1016/j.lfs.2005.09.038)

Yanez ND, Weiss NS, Romand JA & Treggiari MM 2020 COVID-19 mortality risk for older men and women. *BMC Public Health* **20** 1742. (https://doi.org/10.1186/s12889-020-09826-8)

Yang P, Kuc RE, Brame AL, Dyson A, Singer M, Glen RC, Cheriyan J, Wilkinson IB, Davenport AP & Maguire JJ 2017 Pyr(1). *Frontiers in Neuroscience* **11** 92. (https://doi.org/10.3389/fnins.2017.00092)

Yang J, D'souza R, Kharrat A, Fell DB, Snelgrove JW, Murphy KE & Shah PS 2022*a* Coronavirus disease 2019 pandemic and pregnancy and neonatal outcomes in general population: a living systematic review and meta-analysis (updated Aug 14, 2021). *Acta Obstetricia et Gynecologica Scandinavica* **101** 7–24. (https://doi.org/10.1111/aogs.14277)

Yang Z, Macdonald-Dunlop E, Chen J, Zhai R, Li T, Richmond A, Klaric L, Pirastu N, Ning Z, Zheng C, *et al.* 2022*b* Genetic landscape of the ACE2 coronavirus receptor. *Circulation* **145** 1398–1411. (https://doi.org/10.1161/CIRCULATIONAHA.121.057888)

Zhang H, Rostami MR, Leopold PL, Mezey JG, O'beirne SL, Strulovici-Barel Y & Crystal RG 2020 Expression of the SARS-CoV-2 ACE2 receptor in the human airway epithelium. *American Journal of Respiratory and Critical Care Medicine* **202** 219–229. (https://doi.org/10.1164/rccm.202003-0541OC)

Zhang H, Wada J, Hida K, Tsuchiyama Y, Hiragushi K, Shikata K, Wang H, Lin S, Kanwar YS & Makino H 2001 Collectrin, a collecting duct-specific transmembrane glycoprotein, is a novel homolog of ACE2 and is developmentally regulated in embryonic kidneys. *Journal of Biological Chemistry* **276** 17132–17139. (https://doi.org/10.1074/jbc.M006723200)

Zhao MM, Yang WL, Yang FY, Zhang L, Huang WJ, Hou W, Fan CF, Jin RH, Feng YM, Wang YC, *et al.* 2021 Cathepsin L plays a key role in SARS-CoV-2 infection in humans and humanized mice and is a promising target for new drug development. *Signal Transduction and Targeted Therapy* **6** 134. (https://doi.org/10.1038/s41392-021-00558-8)

Zheng H-Y, He X-Y, Li W, Song T-Z, Han J-B, Yang X, Liu F-L, Luo R-H, Tian R-R & Feng X-L, *et al.* 2021 Pro-inflammatory microenvironment and systemic accumulation of CXCR3+ cell exacerbate lung pathology of old rhesus macaques infected with SARS-CoV-2. *Signal Transduction and Targeted Therapy* **6** 328. (https://doi.org/10.1038/s41392-021-00734-w)

Zhou P, Yang XL, Wang XG, Hu B, Zhang L, Zhang W, Si HR, Zhu Y, Li B, Huang CL, *et al.* 2020*a* A pneumonia outbreak associated with a new coronavirus of probable bat origin. *Nature* **579** 270–273. (https://doi.org/10.1038/s41586-020-2012-7)

Zhou X, Zhang P, Liang T, Chen Y, Liu D & Yu H 2020*b* Relationship between circulating levels of angiotensin-converting enzyme 2-angiotensin-(1–7)-MAS axis and coronary heart disease. *Heart and Vessels* **35** 153–161. (https://doi.org/10.1007/s00380-019-01478-y)

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