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SHORT COMMUNICATION

Acute Severe Acute Respiratory Syndrome Coronavirus 2 Infection in Pregnancy Is Associated with Placental Angiotensin-Converting Enzyme 2 Shedding

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While the human placenta may be infected by severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), the rate of fetal transmission is low, suggesting a barrier at the maternal-fetal interface. Angiotensin-converting enzyme (ACE)2, the main receptor for SARS-CoV-2, is regulated by a metalloprotease cleavage enzyme, a disintegrin and metalloprotease domain 17 (ADAM17). ACE2 is expressed in the human placenta, but its regulation in relation to maternal SARS-CoV-2 infection in pregnancy is not well understood. This study evaluated ACE2 expression, ADAM17 activity, and serum ACE2 abundance in a cohort of matched villous placental and maternal serum samples from control pregnancies (SARS-CoV-2 negative, $n = 8$) and pregnancies affected by symptomatic maternal SARS-CoV-2 infections in the second trimester [2nd Tri coronavirus disease (COVID), $n = 8$] and third trimester (3rd Tri COVID, $n = 8$). In 3rd Tri COVID compared with control and 2nd Tri COVID villous placental tissues, ACE2 mRNA expression was remarkably elevated; however, ACE2 protein expression was significantly decreased with a parallel increase in ADAM17 activity. Soluble ACE2 was also significantly increased in the maternal serum from 3rd Tri COVID infections compared with control and 2nd Tri COVID pregnancies. These data suggest that in acute maternal SARS-CoV-2 infections, decreased placental ACE2 protein may be the result of ACE2 shedding and highlights the importance of ACE2 for studies on SARS-CoV-2 responses at the maternal-fetal interface. *(Am J Pathol 2022, 192: 595–603; https://doi.org/10.1016/j.ajpath.2021.12.011)*

The coronavirus disease 2019 (COVID-19) pandemic, caused by severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), has resulted in >100 million infections and claimed >3 million lives worldwide (Johns Hopkins University Dashboard, *https://coronavirus.jhu.edu/map.html*, last accessed June 13, 2021). Despite effective vaccination and relatively rapid and accurate detection of the virus, the pandemic slowdown is hindered by frequent mutations, which led to new outbreaks in certain areas that spread globally.[1–5] Therefore, further understanding the pathogenesis of and the host defense mechanisms to the viral infection is key in developing novel therapies to curb the spread of COVID-19.

Angiotensin-converting enzyme 2 (ACE2) is the major receptor for SARS-CoV-2.[6,7] This receptor's inducibility

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and variability are proposed to be essential for viral tropism, infectivity, and COVID-19 disease progression and outcomes.[8–13] ACE2, a vital member of the renin-angiotensin system, is a monocarboxyl peptidase and type I transmembrane protein.[14] As a proteinase, ACE2 cleaves one amino acid from carboxyl terminals of its substrates, such as Angiotensin II (Ang II), to generate the bioactive peptide, Ang 1-7, which counters the action of Ang II in many physiological processes, including host defense and inflammatory responses.[15] Therefore, the ACE2/Ang 1-7 axis is a negative regulatory mechanism of renin-angiotensin system to alleviate detrimental effects of the overactivated ACE/Ang II/angiotensin receptor 1 axis.

Most ACE2, after post-translational modification, migrates to the cell membrane. As a type I transmembrane protein, the anchored ACE2 contains a large ectodomain, in which the SARS-CoV-1 and SARS-CoV-2 binding domain and enzymatic active domain separate distally, so the respective functions are not interfered with by the other.[16] Notably, the ectodomain of ACE2 undergoes shedding constitutively, which is inducible in response to various stimuli, including bacteria and viruses. The shed or soluble ACE2 is both enzymatically active and capable of binding to existing viral particles, such as SARS-CoV-2.[17] The soluble ACE2 has been proposed as a decoy receptor to trap the SARS-CoV-2 virus as a promising therapy for COVID-19, and several clinical trials are underway to test the possibility.[16] However, controversy regarding such a strategy remains, because a report indicates that soluble ACE2 can facilitate viral entry via alternative routes.[18]

ACE2 shedding predominantly depends on the activity of a disintegrin and metalloprotease domain 17 (ADAM17) or tumor necrosis factor-α converting enzyme (TACE).[17,19] ADAM17 is 1 of 13 genes in the family that encode functional proteases and are involved in ectodomain shedding of an array of growth factors, cytokines, receptors, and adhesion molecules, including tumor necrosis factor-α and ACE2.[19,20] ADAM17 enzymatic activity is controlled by post-transcriptional tissue inhibitors of metalloprotease. However, its gene expression is affected by many stimuli, and the sex hormonal regulation of ADAM17 is not well understood.[21,22]

ACE2 is found primarily in the outer trophoblast epithelial cell layers of the villous placenta, directly juxtaposed with maternal blood at the main functional interface between mother and fetus.[23] Placental expression levels of ACE2 (and its partner components of the renin-angiotensin system) are prominent in early pregnancy and gradually decrease throughout gestation, implicating a role for ACE2 in key events of placentation.[23] Several groups have identified ACE2 expression in the villous placenta primarily in trophoblast epithelial cells with a lack of expression among other stromal cells and fetal endothelium.[24–26] ADAM17 is also found within the villous placental trophoblast cells, and its expression and activity in the placenta has primarily been linked to tumor necrosis factor-α production in inflammatory pregnancy states.[27–29]

To date, most studies evaluating ACE2 in pregnancies affected by COVID-19 have focused on maternal SARS-CoV-2 infections in the third trimester.[25,26,30] As the pandemic has ensued, women have gradually experienced COVID-19 disease throughout their pregnancies, with consistent reports of low SARS-CoV-2 fetal transmission. Yet, ACE2 expression relative to gestational stage of maternal SARS-CoV-2 infection remains unknown. Furthermore, although the phenomena of ACE2 shedding, ADAM17 activity, and serum ACE2 levels have been characterized in multiple tissue types and COVID-19 patient populations,[31] they are not well defined in the human placenta or in relation to the timing of maternal COVID-19 infections in pregnancy.

Given the dynamic nature of ACE2 and ADAM17 activity among multiple tissues impacted by SARS-CoV-2 infection and COVID-19 disease evolution,[16,31] we hypothesized that ACE2 expression and ADAM17 activity in the placenta are affected by the timing of maternal SARS-CoV-2 infection in pregnancy relative to delivery. In the present study, ACE2 expression/ADAM17 activity were evaluated in placental villous tissues and maternal serum ACE2 levels in a cohort of maternal-fetal dyads with second- and third-trimester maternal SARS-CoV-2 infections in comparison with control pregnancies (Figure 1A). This study design allowed for analysis of ACE2 placental expression dynamics in states of acute versus remote SARS-CoV-2 infections in pregnancy to further understand the trajectory of responses to COVID-19 at the maternal-fetal interface.

Materials and Methods

Study Enrollment

The current study was approved by the Boston University Medical School Institutional Review Board, and written informed consent was obtained from all subjects. Prospective mother-infant dyads were enrolled at Boston Medical Center between July 2020 and April 2021. Eligibility criteria were as follows: aged ≥18 years, documented symptomatic SARS-CoV-2 infection during the second or third trimester of pregnancy (testing via nasal swab PCR; COVID groups) or no documented SARS-CoV-2 infection during pregnancy (testing via nasal swab PCR; control group), singleton gestation pregnancy, and English or Spanish speaking. Exclusion criteria included inability to provide informed consent.

Sample Collection and Processing

Maternal blood samples were collected in EDTA collection tubes by trained staff within 24 hours before or after

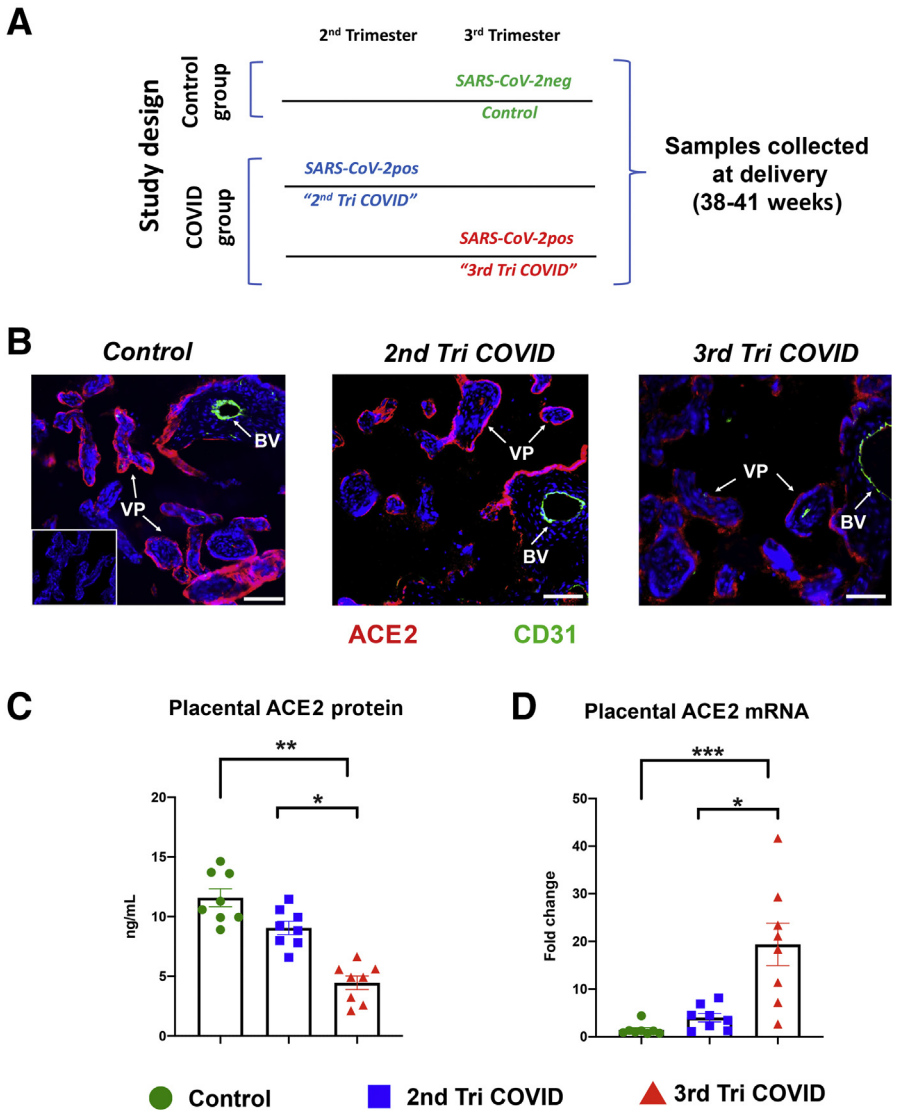

Figure 1 Villous placental angiotensin-converting enzyme 2 (ACE2) expression in acute versus remote SARS-CoV-2 infections in pregnancy. **A:** Study design. Control group: women with no report of SARS-CoV-2 infection or COVID-19 symptoms during pregnancy and SARS-CoV-2 negative (neg) via universal screening at time of admission to labor and delivery. COVID group: women with documented COVID-19 symptoms and a SARS-CoV-2–positive (pos) test during their second trimester (2nd Tri COVID) or third trimester (3rd Tri COVID) of pregnancy. Schematic shows timing of maternal SARS-CoV-2 infection relative to sample collection at delivery. **B:** Representative images from immunohistochemical survey of ACE2 in villous placental tissues from each patient group. Red, ACE2; green, CD31; blue, DAPI nuclear stain. **Inset:** Antibody-negative control. **C:** ACE2 expression in villous placental tissue homogenates, as assayed by human ACE2 enzyme-linked immunosorbent assay. **D:** Quantitative RT-PCR analysis of ACE2 mRNA expression in villous placental tissues. Error bars: ±SEM. $n = 8$ per group (**B**). $^{*}P < 0.05$, $^{**}P < 0.01$, and $^{***}P < 0.001$. Scale bars = 25 mm (**B**). BV, fetal blood vessel; VP, villous placenta.

delivery. Following collection, blood samples were then stored at 4°C and centrifuged within 6 hours of collection. Plasma was then extracted and frozen at −80°C until analysis.

For placental collection, all tissues were collected within an average time of 3.1 hours (SEM, ±0.14 hours) following delivery, and all samples were taken midway between umbilical cord insertion site and edge of placental disk. For histologic analysis, full-thickness placental biopsies (1 × 3 cm) were collected and fixed in 10% neutral-buffered formalin (ThermoFisher, Waltham, MA) for 72 hours, soaked in 18% sucrose for 24 hours, embedded in Tissue-Plus OCT compound (ThermoFisher), and frozen at −80°C. For villous placental protein and RNA analysis, triplicate 0.5-cm pieces of villous placental tissue (obtained from a depth midway between decidua basalis and chorionic plate) were dissected, flash frozen neat or in RNA*later* (ThermoFisher), and stored at −80°C.

Immunohistochemistry

Fixed/frozen placental tissue blocks were cryosectioned at 15 μm. Slides were then washed with 1× phosphate-buffered saline with Tween 20 and then blocked with

donkey blocking serum for 1 hour. Primary antibodies of interest, including sheep anti-CD31 (1:100; AF806; R&D Systems, Minneapolis, MN), rabbit anti−SARS-N protein (1:500; 200-401-A50; Rockland, Pottstown, PA), and goat anti-ACE2 (1:50; AF933; R&D Systems), were then incubated at 4°C overnight. The next day, samples were washed five times for 10 minutes each in phosphate-buffered saline with Tween 20. After blocking with donkey serum at room temperature for 1 hour, secondary conjugate antibodies, donkey anti-sheep Alexa Fluor 488 (1:250; 713-545-003; Jackson Immunoresearch, West Grove, PA), donkey anti-rabbit Alex Fluor 555 (1:250; 711-165-152; Jackson Immunoresearch), and donkey anti-goat Alexa Fluor 647 (1:250; 705-605-147; Jackson Immunoresearch), were applied for 6 hours at room temperature. Slides were then washed five times with phosphate-buffered saline with Tween 20 again for 10 minutes each and mounted with DAPI (AF806; Vector Laboratories, Burlingame, CA). Sections were imaged on a Nikon deconvolution wide-field epifluorescence microscope and processed using NIS-Elements Software F Ver4.60.00 (Nikon, Melville, NY).

Quantitative RT-PCR Analysis

RNA was extracted from fresh frozen villous placental tissue biopsies using an RNAqueous kit (Invitrogen, Waltham, MA), per manufacturer's protocol. RNA transcripts were subsequently evaluated with TaqMan probes and primers (forward: 5′-TAATGCTGGGGACAAATGGT-3′; reverse: 5′-CAGCTGAAGCTTGACTGTGAG-3′; probe: 5′-TCCACACTTGCCCAAATGTA-3′) for ACE2. Target expression was normalized to the transcript for glyceraldehyde-3-phosphate dehydrogenase, and relative expression was quantified via fold change in COVID samples relative to control using $2^{-\Delta\Delta CT}$ calculations.

ELISA Assays

Villous Placental Tissue ACE2 ELISA

Fresh frozen dissected placental tissue (300 to 500 mg) was homogenized with protein lysis buffer [50 mmol/L Tris-HCL, pH 8.0, 150 mmol/L NaCl, 1% nonyl phenoxypolyethoxylethanol-40 (NP-40), 0.5 mmol/L phenylmethylsulfonyl fluoride (Sigma, St. Louis, MO), and cOmplete protease inhibitor (Roche, Sigma). Tissue lysates were then centrifuged at 20,000 × g × 30 minutes at 4°C, followed by collection of sample supernatants. Protein concentration for each sample was determined using a Micro BCA Protein Assay Reagent Kit (ThermoFisher), per manufacturer's instructions. Samples were assayed into a Human ACE2 DuoSet enzyme-linked immunosorbent assay (ELISA; R&D Systems), loading equal amounts of protein in duplicate for all samples.

Serum ACE2 and Estradiol ELISA

Frozen maternal serum aliquots were quick thawed and immediately assayed using either a Human ACE2 DuoSet ELISA assay (R&D Systems) or a Parameter Estradiol assay, per manufacturer's instructions. All samples were assayed in duplicate. Absorbance for all ELISA assays was evaluated using a Molecular Devices SpectraMax i3x Multi-Mode Microplate Reader (Molecular Devices, San Jose, CA).

ADAM17 Activity Assay

ADAM17 (tumor necrosis factor-α converting enzyme) activity was assayed in fresh frozen villous placental tissue homogenates using a SensoLyte(R)520 Fluorometric TACE Activity Assay kit (VWR, Radnor, PA). For sample normalization, equal amounts (300 mg) of fresh frozen villous placental tissue samples were homogenized into the assay lysis buffer containing 0.1% Triton-X 100 (Sigma) and further processed, per manufacturer's instructions. Tissue homogenates were run in duplicate for each placental sample. Fluorescence intensity values (excitation/emission = 490/520 nm) were then measured using a Molecular Devices SpectraMax i3x Multi-Mode Microplate Reader every 5 minutes for a total of 40 minutes.

Statistical Analysis

Patient demographic characteristics and maternal and neonatal outcomes were reported as follows: Continuous variables were reported as either median with interquartile ranges or means with SDs, and categorical variables were reported as percentages, as indicated for each parameter (Table 1). P values for continuous variables were generated using a one-way analysis of variance test with Tukey post hoc analysis. P values for categorical variables were generated using the Fisher exact tests. Differences were considered significant at $\alpha = 0.05$. Statistical analysis for tissue and serum ACE2 ELISA, serum estradiol ELISA, and ACE2 quantitative RT-PCR were evaluated using a one-way analysis of variance test with Tukey post hoc analysis for multiple comparisons. The significance of ADAM17 activity values over time and per experimental group were evaluated using a two-way analysis of variance test with Tukey post hoc analysis. All statistical analyses were conducted using Prism 7 software (GraphPad, San Diego, CA).

Results

To evaluate ACE2 expression dynamics at the maternal-fetal interface in varied types of SARS-CoV-2 infections during pregnancy, a cohort of matched villous placental tissues and maternal serum samples was collected at the time of delivery. SARS-CoV-2 infections in pregnancy were classified according to timing relative to delivery: remote

Table 1 Patient Demographics

Demographic	Control ($N = 8$)	2nd Tri COVID ($N = 8$)	3rd Tri COVID ($N = 8$)	P value
Gestation age at SARS-CoV-2 infection, mean (SD), weeks	N/A	18.7 (4.1)	30.0 (3.1)	N/A
Maternal COVID severity, *n* (%)	N/A			N/A
Hospitalized		0	1 (12.5)	
ICU		0	0	
Maternal age, mean (SD), years	30.4 (6.6)	30.0 (5.0)	30.9 (5.1)	0.95*
Maternal race, *n* (%)				0.89†
Black	0	1 (12.5)	1 (12.5)	
White	2 (25)	3 (37.5)	2 (25)	
Other	6 (75)	4 (50)	5 (62.5)	
Maternal chronic health conditions, *n* (%)‡	5 (62.5)	3 (37.5)	4 (50)	0.87†
Pregnancy complications, *n* (%)§	5 (62.5)	7 (87.5)	8 (100)	0.27†
Gestational age at birth, mean (SD), weeks	38.6 (0.9)	39.9 (1.1)	40.0 (1.9)	0.09*
Infant sex, *n* (%)				1†
Male	3 (37.5)	5 (62.5)	2 (25)	
Female	5 (62.5)	3 (37.5)	6 (75)	
Birth weight, mean (SD), g	3353 (459)	3453 (327)	3446 (411)	0.86*
5-minute Apgar score, median (IQR)	9 (8.75–9.00)	9 (9–9)	9 (9–9)	0.12¶
Required NICU admission, *n* (%)	1 (12.5)	1 (12.5)	0	1†
Infant SARS-CoV-2–positive results	0	0	0	N/A

*P values for continuous variables were generated using analysis of variance test.

†P values for categorical variables were generated using Fisher exact tests.

‡Chronic health conditions included autoimmune disease, diabetes, hepatitis B, hepatitis C, herpes simplex virus, HIV, hypertension, obesity, thyroid disease, substance use disorder, or other.

§Pregnancy complications included chorioamnionitis, gestational diabetes, hypertensive disorder of pregnancy, fetal growth restriction, placenta previa, preterm labor, unexplained vaginal bleeding, or other.

¶P values for continuous variables were generated by Kruskal Wallis rank test.

2nd Tri COVID, SARS-CoV-2 infections in the second trimester; 3rd Tri COVID, SARS-CoV-2 infections in the third trimester; ICU, intensive care unit; IQR, interquartile range; N/A, not applicable; NICU, newborn ICU.

infections in the second trimester (2nd Tri COVID) or acute infections in the third trimester (3rd Tri COVID) (Figure 1A).

All SARS-CoV-2–positive patients (COVID group) were symptomatic at the time of testing (specified as with fever or reports of respiratory or gastrointestinal symptoms and/or loss of smell/taste documented in the medical chart). All cases were classified as mild-moderate infections, with only one case requiring hospital admission due to COVID-19 and no patients requiring respiratory support or intensive care unit care during the symptomatic phase of their illness (Table 1). The SARS-CoV-2–negative cohort (control) was tested via universal screening procedures at the time of admission to Boston Medical Center labor and delivery. There was no significant difference in patient race, ethnicity, mode of delivery, or gestational age at delivery between groups. In addition, infant birth outcomes (ie, Apgar scores and birth weight) and infant sex were also not statistically different between groups. Finally, within the COVID pregnancy group, all infants who met the criteria for testing were negative for SARS-CoV-2.

Villous Placental ACE2 Expression Is Decreased in Acute Maternal SARS-CoV-2 Infections in Pregnancy

To evaluate the abundance of ACE2 in placental tissues from our patient cohorts, ACE2 expression was first examined in full-thickness placental biopsies using immunohistochemistry. Placental tissues were first screened for the presence of SARS-N protein. Only 3 of the 16 placental tissues within our COVID group showed the presence of SARS-N expression ($n = 1$ in second-trimester cohort; $n = 2$ in third-trimester cohort; data not shown), a frequency similar to previously published placental tissue cohorts.[30,32] First, ACE2 expression was identified in villous trophoblast epithelial cells (but not fetal blood vessel endothelium), consistent with previous publications[24–26] (Figure 1B). Villous placental tissues from remote, 2nd Tri COVID had similar ACE2 expression intensity to those of control placental tissues. However, ACE2 expression appeared to be decreased in placentas from acute, 3rd Tri COVID (Figure 1B) compared with both control and the 2nd Tri COVID group.

To further quantify ACE2 expression in these placenta tissues, protein was isolated from dissected villous

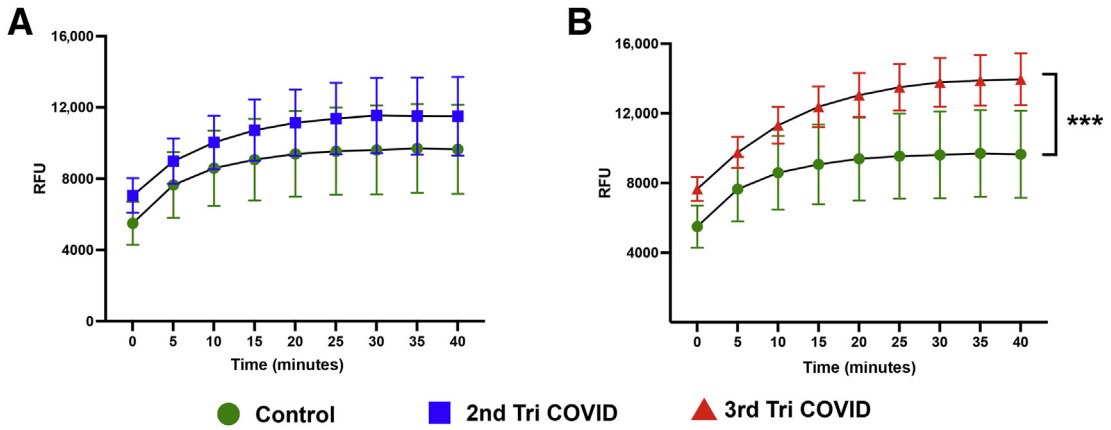

Figure 2 Increased placental a disintegrin and metalloprotease domain 17 (ADAM17) activity in acute maternal SARS-CoV-2 infections during pregnancy. ADAM17 activity over time in villous placental tissue homogenates in placental tissues from patient groups, as described in Figure 1. **A:** Control group versus 2nd Tri COVID. **B:** Control group versus 3rd Tri COVID. Error bars: SEM. ***$P < 0.001$. RFU, relative fluorescent unit.

placental tissue lysates and ACE2 expression was quantified through ELISA assay analysis. Similar to the trend noted in the immunohistochemical analysis, ACE2 in acute third-trimester infections was significantly decreased in comparison with control and 2nd Tri COVID groups (Figure 1C). Thus, through immunohistochemical and quantitative protein analysis in our tissue cohort, ACE2 expression in villous epithelial tissues appeared to be decreased in acute, but not remote, SARS-CoV-2 infections in pregnancy compared with controls. Interestingly, quantitative RT-PCR analysis of ACE2 from villous samples of the same placental tissues showed a significant up-regulation of ACE2 mRNA expression in acute 3rd Tri COVID (Figure 1D), suggesting the noted decrease in placental ACE2 protein was due to post-translational influences rather than alteration of mRNA transcripts.

Acute Maternal SARS-CoV-2 Infections in the Third Trimester Are Associated with Increases in Placental ADAM17 Activity and Circulating Maternal Serum ACE2

To next investigate the potential post-translational mechanism behind decreased placental ACE2 expression associated with acute maternal SARS-CoV-2 infections, ADAM17 activity was evaluated in dissected villous placental tissues. Because ADAM17 is a cell-surface enzyme known to cleave surface ACE2,[19] the study examined whether the activity of ADAM17 in the placenta varied in relation to the timing of maternal SARS-CoV-2 infections in pregnancy. For this analysis, a fluorescence-based assay evaluating kinetic ADAM17 cleavage activity over time was used.[33,34] In comparison with the control group, placental tissues from remote SARS-CoV-2 infections in the second

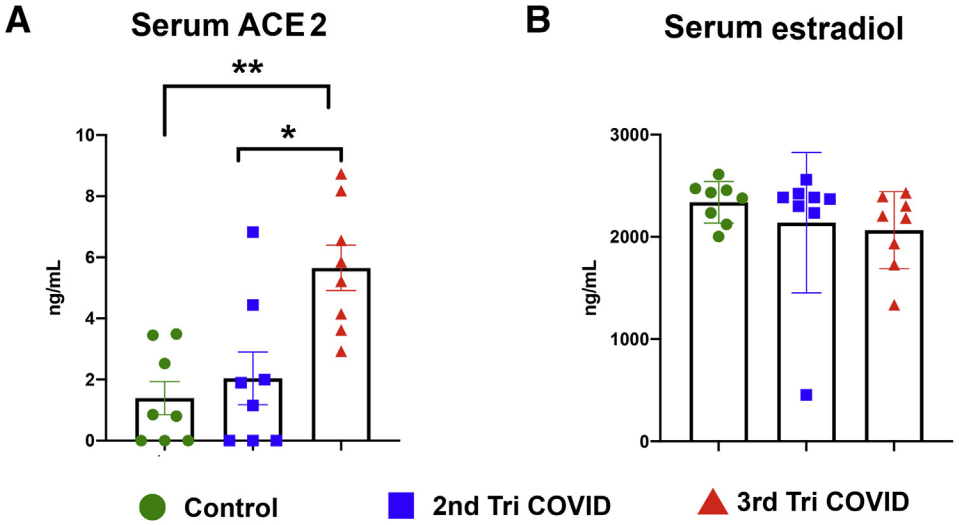

Figure 3 Maternal SARS-CoV-2 infection in the third trimester is associated with increased circulating maternal serum angiotensin-converting enzyme 2 (ACE2). Soluble ACE2 (**A**) and serum estradiol (**B**) in maternal serum collected at delivery from patient groups, as described in Figure 1. Error bars: SEM. *$P < 0.05$, **$P < 0.01$.

trimester of pregnancy showed a modest elevation that did not reach statistical significance in ADAM17 activity (Figure 2A). However, placental tissues from pregnancies with acute third-trimester maternal SARS-CoV-2 infections had a significant increase in ADAM17 activity over time when compared with control villous placental tissues (mean fold increase: 1.5; $P < 0.01$ control versus 3rd Tri COVID) (Figure 2B).

The combined results of decreased villous placental ACE2 protein expression, increased *ACE2* gene expression, and increased ADAM17 activity suggested a cleavage of ACE2 from the villous placental tissue. As villous placental tissue is directly juxtaposed with the maternal blood, the study next examined whether the changes in ACE2 expression and ADAM17 activity also correlated with any alterations in circulating ACE2 levels in maternal serum. To conduct this analysis, ACE2 abundance was evaluated in maternal serum samples that matched with placental samples analyzed for ACE2 expression and ADAM17 activity. In line with the trends noted with ADAM17 activity, serum ACE2 was significantly increased in acute third-trimester SARS-CoV-2 infections compared with control and second-trimester maternal SARS-CoV-2 infections (Figure 3A).

Finally, the study evaluated whether changes in placental and serum ACE2 abundance correlated with alterations in circulating estrogen levels in our cohort. Estrogen regulates ACE2 expression within airway epithelial cells[35] and has been implicated in the sex differences noted between COVID-19 severity among adults.[36] However, the role of estrogen on ACE2 regulation in pregnancy has been less well defined. Among our cohort, there was no significant difference in maternal serum estrogen (assayed as estradiol) levels between our control and COVID groups (Figure 3B).

Discussion

The current study identifies new evidence on the dynamics of placental ACE2 expression in SARS-CoV-2 infections in pregnancy and the influence of the gestational stage of maternal infection relative to delivery. The current findings suggest that the ACE2 protein expression changes are the result of placental ACE2 shedding mediated by ADAM17 in response to maternal SARS-CoV-2.

The dynamic nature of placental ACE2 expression noted in this study could account for the variation of reports identifying the presence or absence of ACE2 within trophoblast epithelial cells.[37] Subsequent studies[24–26] have strongly substantiated prepandemic publications[23] for the presence of ACE2 at the maternal-fetal interface. The 3rd Tri COVID–specific increase in ACE2 mRNA could be due to compensatory up-regulation of ACE2 transcripts in response to the active shedding process in this disease state. Recently, a truncated form of human ACE2 (namely, delta ACE2) was reported to be up-regulated by interferons.[38] The primer and probe set in the current study flanks only the region in the prototype ACE2; therefore, the expression detected is exclusive of delta ACE2. In addition, there were no significant differences in the maternal serum estradiol between our patient groups, further suggesting influences other than hormonal signaling affecting the dynamic expression of placental ACE2 in these pregnancies. Ongoing evaluation of other influences on ACE2 post-translational regulation and ADAM17 activity will be required in future studies to characterize the mechanisms governing this process more clearly. Multiple studies have identified signs of placental inflammation in pregnancies affected by acute maternal SARS-CoV-2, including significant fibrosis,[24,32] increased mononuclear cell infiltrates,[39] and up-regulation of interferon response genes.[30,40,41] Thus, proinflammatory cytokine regulation and immune response signaling pathways will be a key area of focus for future analysis of ACE2 and ADAM17 regulation in these tissues.

Consistent with previous studies,[24–26] the current study found that ACE2 was not expressed in the endothelium of fetal blood vessels in control or COVID placental tissues. These findings highlight an important contrast in the pulmonary and placental responses to SARS-CoV-2. In healthy lung tissue, the pulmonary endothelium has minimal expression of ACE2, but in patients with fatal COVID-19, ACE2 expression is increased on the pulmonary endothelium.[42] Type I interferons α and β induce endothelial ACE2 expression, subsequently facilitating SARS-CoV-2 endothelial invasion *in vitro*.[42] In stark contrast with the lung, the lack of ACE2 on the fetal endothelium in COVID placental tissues further supports the likely central role of ACE2 regulation as a protective countermeasure against perinatal SARS-CoV-2 transmission.

This is also the first study to evaluate serum ACE2 levels in pregnant women affected by COVID-19. Serum ACE2 levels are increased in pregnant relative to non-pregnant women, and further relative increases have been associated with small for gestational age fetal growth.[43] Although serum ACE2 in non-pregnant adult COVID-19 patients has been widely evaluated,[44–46] no studies to date have characterized maternal serum ACE2 content in pregnancies with maternal SARS-CoV-2 infection. Although combined data from the current study suggest that the increase in maternal serum ACE2 in acute 3rd Tri COVID pregnancies could be the result of increased ACE2 placental shedding, the source of circulating maternal serum ACE2 levels in these pregnancies (lung, placenta, or both) requires further investigation.

This study has several limitations. First, the cohort is a small sample size and the gestational evaluation only spanned infections in the second and third (but not first) trimesters of pregnancy. In addition, as stated above, soluble ACE2 in maternal serum cannot be directly identified as placental in origin and could be derived from multiple sources. Ongoing mechanistic studies, including relevant animal models, are needed to evaluate ACE2 expression

relative to maternal COVID-19 infection in all trimesters of pregnancy, to clarify the placental origin of maternal serum ACE2, and to functionally correlate ADAM17 activity with ACE2 placental regulation. Furthermore, the impact of these alterations in placental ACE2 on maternal and infant physiology will require close long-term follow-up.

In conclusion, this work highlights a previously unrecognized 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. Taken together, these data provide support for the growing body of evidence on the importance of ACE2 regulation in the placental response against SARS-CoV-2. The human placenta has many functional and structural parallels with the human lung,[47] and thus continues to be an important primary human tissue for identification of key targets to combat COVID-19 pathogenesis.

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