SARS-CoV-2 ACE2 and TMPRSS2 Receptor Protein Expression Patterns Throughout Gestation

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This new information has not been published or presented previously. Figure 1 is from data previously published (Reference 14) and is so referenced in the legend. The figure is new and has not been previously published.

**Abstract**

We previously demonstrated that the late gestation placental expression pattern of ACE2 (the primary SARS-CoV-2 receptor) is localized to the villous syncytiotrophoblast (ST), usually in a polarized membranous pattern at the ST base sparing the apical surface (that directly exposed to maternal blood). We found that the late gestation placental expression pattern of TMPRSS2 (the spike proteinase required for SARS-CoV-2 cellular infection), is usually absent in the trophoblast but rarely, weakly expressed in the placental endothelium. We now show the developmental protein expression patterns of ACE2 and TMPRSS2 by immunohistochemistry throughout gestation, from first through third trimester. We found TMPRSS2 expression was rarely detectable in villous endothelium and very rarely detectable in the ST across gestation. We found ACE2 expression varied during gestation with circumferential ST expression more common in early gestations and polarized expression more common in later gestation. Although this study is small, these preliminary results suggest that earlier gestation pregnancies may be more vulnerable to infection than later gestation pregnancies.

Key words: ACE2, TMPRSS2, Placental expression, SARS-CoV-2

**Background**

Most viruses that infect the placenta do so via hematogenous spread leading to infection of the villi [1, 2]. Infection of the placenta, and subsequently, the fetus, typically occurs by direct villous trophoblastic infection or a paracellular route via damage to the villous trophoblastic barrier. The virus must be transported across the villous stroma and through the vascular endothelium to enter the fetal bloodstream and cause congenital (vertical) infection [3]. Alternatively, the virus can enter the placental/fetal blood stream more directly via villous vasculosyncytial membrane infection, where the villous syncytiotrophoblast (ST) and the villous capillary endothelium are apposed, essentially functioning as a single unit [4]).

Despite a large number of women with COVID-19 during pregnancy, SARS-CoV-2 rarely infects the placenta [5, 6]. Furthermore, vertical infection of the fetus by SARS-COV-2 is quite rare and has been documented in only a few case reports usually in term gestations [7-10]. Transmission and infection in early pregnancy has not been well studied. Lack of maternal-fetal transmission is likely due in large part to the apparent low prevalence of SARS-CoV-2 viremia in pregnancy[6], while reported rates of viremia in non-pregnant populations have ranged widely, from 1 – 27 % [11, 12]. No viremia was detected in 62 SARS-CoV-2-infected pregnant women we studied and reported previously [6].

To enter and infect cells, SARS-CoV-2 requires a receptor, most commonly ACE2, and a serine protease, usually TMPRSS2 [13]. Receptor and protease must be co-expressed in cells for SARS-CoV-2 infection to occur. In our previous work we showed that ACE2 is expressed in the ST, extravillous trophoblast (EVT), and cytotrophoblast (CT) and that TMPRSS2 was usually not detected by immunohistochemistry in any later-gestation placental cells [6, 14]. The function of ACE2 in the placenta is thought to activate the renin-angiotensin system in chorionic villi and extravillous

trophoblast. ACE2 may play a role in trophoblast invasion and changes in vascular flow [15-18]. When present, TMPRSS2 was rarely and very weakly detected in the placental (fetal) endothelium and ST (in a uniform pattern) [6, 14]. The role of TMPRSS2 expression in the placenta is unknown. Based on our prior findings, we hypothesized that one barrier to vertical SARS-CoV-2 transmission is that ACE2 protein expression pattern is polarized towards the stromal (basal) side of the villous ST and rarely expressed on the apical side where it would be directly exposed to maternal blood and SARS-CoV-2 viremia (Figure 1) [6, 14]. Polarized ACE2 expression, away from maternal blood, would likely limit viral adhesion to the ST, and widespread absence of TMPRSS2 in the ST would likely inhibit viral entry into the villous trophoblast.

While this protein expression pattern may play a protective role against placental and vertical SARS-CoV-2 infection in later gestations, the receptor expression pattern in early-gestation placentas has not been described. Importantly, if the polarized expression pattern develops over gestation and is not present in first or second trimester placentas, early gestational SARS-CoV-2 infections may cross the placenta more readily and lead to vertical SARS-CoV-2 transmission, possibly with damaging fetal effects (analogous to the more damaging effects seen with early gestation Zika virus [ZikV] infection [19]). To better understand the risk of early gestational vertical SARS-CoV-2 transmission, we examined placental receptor expression patterns from early first trimester through to mid third trimester.

**Methods**

We ascertained and included pre-pandemic women or women testing negative for SARS-CoV-2 infection who underwent a therapeutic pregnancy termination or preterm delivery between 1/1/2019 and 12/1/2020 by searching the Massachusetts General Hospital pathology database. We reviewed placental or villous tissue hematoxylin and eosin slides and pathology reports and

retrieved appropriate formalin fixed paraffin embedded (FFPE) blocks for immunohistochemical (IHC) studies. Initial diagnoses were rendered by perinatal pathologists, either by DJR or by pathologists she trained, using the Amsterdam Consensus diagnostic nosology [20]. We performed IHC on 5 micron sections from FFPE blocks including villous tissue using an automated stainer (Bond-III; Leica Microsystems Bannockburn, IL) with ACE2 monoclonal antibody (clone CL4035 [1:15,000], Thermo Fisher Scientific, Waltham, MA) and TMPRSS2 antibody (clone PA5-83286 [1:1,000] Thermo-Invitrogen, Carlsbad, CA) in accordance with the manufacturer's recommendations. The slides were stained in a single batch with appropriate controls. A perinatal pathologist (DJR) reviewed IHC slides and qualitatively scored each for location and intensity of the signal. The study was approved by the Mass General Brigham Institutional Review Board (2020P001116) and was exempt of the requirement for informed consent.

**Results**

We examined 12 cases ranging in gestational age from 5 weeks 3 days to near term at 36.0 weeks (Table 1). Both IHC stains produced signals in control tissue (Figures 1 and 2). ACE2 expression was uniformly strong in the villous ST, CT (Figure 2) and EVT (data not shown) in a membranous pattern. We again observed a common distinct ACE2 expression polarity in the ST favoring basal over apical expression (10/12 cases), but occasionally observed a primarily circumferential membranous expression (3/12 cases, one overlap with the basal expression pattern) (Figure 2A). Among three cases with predominantly circumferential staining, two cases (cases 1 and 6) had an earlier gestational age, whereas one case (case 11) has a later gestational age (Table 1). TMPRSS2 expression was usually not detected in villous tissues but occasionally weakly detected in the ST (cases 1 and 6) or placental endothelium (cases 5 and 10) and was not detected in the EVT (data not shown). The TMPRSS2 expression pattern was cytoplasmic to faintly membranous (Figure 2, B and C).

**Discussion**

Here, we demonstrate that the ACE2 receptor needed for cellular SARS-CoV-2 placental infection, is expressed in the placental ST, EVT, and CT across all three pregnancy trimesters. Similar to our previous findings in third trimester pregnancies exposed to SARS-CoV-2 [6, 14], we find rare, weak expression of the serine protease TMPRSS2 in the placental endothelium and very rarely in the ST (Figures 1B, 2B, and 2C). Overall, we conclude that TMPRSS2 is not detectable or weakly expressed in the placental villous tissues throughout pregnancy. We also again show that ACE2 expression is polarized, such that the apical ST surface directly exposed to maternal blood usually does not express the protein, but rather, ACE2 expression is biased to the ST stromal (basal) side (Figures 1A and 2A). We suggest that this expression pattern offers some barrier to placental cellular SARS-CoV-2 infection when spread hematogenously. However, in early gestation ACE2 is expressed more frequently circumferentially in the ST (Figure 2A). Relatively higher ACE2 expression on the apical ST surface in early gestation suggests a weakness of the ST barrier function which could result in higher placental infection rates and vertical SARS-CoV-2 transmission in the setting of early pregnancy exposures, although this has not been reported to date. As more pregnant women recover from first trimester SARS-CoV-2 infection, it is important to remain vigilant to possible placental infection and vertical transmission. Experience with other TORCH or TORCH-like infections suggests that infections in early gestation may be more morbid to the fetus than those in later gestation [21, 22].

Vertical transmission of some RNA viruses, including HIV and ZikV, is also dependent on receptor and cofactor cellular expression. The placental expression pattern of these factors has not been fully described, but in the case of HIV, receptors are expressed weakly on the ST and expression appears to be circumferential [23]. In contrast, ZikV appears to infect the placenta via paracellular pathways, where its receptors and cofactors are not expressed on the ST but are instead present in CT, EVT, and Hofbauer cells [24]. Unfortunately, placental expression patterns of other RNA viruses have not

been well described [14]. Understanding which cell types express viral receptors and cofactors, and their expression patterns, are critical to understanding the mechanisms of placental infection, vertical transmission, and developing targeted prevention and treatment.

The main limitation of this study is the small sample size. We also did not include SARS-CoV-2 infected placentas, as we chose to examine the native expression pattern of these factors. In addition, SARS-CoV-2 infected placentas are rare and to date we have none from the first or second trimester. In our previous work [14] and unpublished subsequent cases, we did not find evidence of altered expression in the presence of SARS-CoV-2 infection. We cannot exclude possible effects of fetal anomalies on these expression patterns but feel such effects are unlikely based on our prior clinical and research experience.

In summary, these findings suggest vertical SARS-CoV-2 infection is possible in early gestation and may be facilitated based more prevalent ACE2 receptor expression on the apical side of the ST. The prevalence of vertical infection in early pregnancy and effects on the fetus and newborn remain unknown and are critical gaps in knowledge needed to appropriately advise women and their medical providers.



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**Table 1 Case Data**

| Case number | Gestational age (weeks) | Clinical history | ACE2 expression pattern in ST | TMPRSS2 expression |
|---|---|---|---|---|
| 1 | 5 + 3d | TAB | ~80 % circumferential,~20% polarized | Very weakly positive in ST and endometrial glands |
| 2 | 7 | TAB | Polarized | Negative |
| 3 | 8 | TAB | ~75% polarized, ~25% circumferential | Negative |
| 4 | 8 + 5d | TAB | Polarized | Negative |
| 5 | 8 + 6d | TAB | ~80% polarized, ~20% circumferential | Faint, villous endothelium |
| 6 | 12 | TAB, fetal anencephaly | Circumferential | Negative in ST, weakly positive in endometrial glands |
| 7 | 18 | PPROM | ~80% polarized, ~20% circumferential | Negative |
| 8 | 21 | TAB, AV Canal defect | Polarized | Negative |
| 9 | 23 | TAB, fetal forebrain anomalies | ~50% polarized, ~50% circumferential | Negative |
| 10 | 28 | PET with severe features | Polarized | Faint, villous endothelium |
| 11 | 33 | Chorio, NRFHT | ~20% polarized, ~80% circumferential | Negative |
| 12 | 36 | IUGR, Breech, severe oligo | ~80% polarized, ~20% circumferential | Negative |

AV Canal – atrioventricular canal heart defect, Chorio – acute chorioamnionitis, d – days, IUGR – intrauterine growth restriction, NRFHT – non-reassuring fetal testing, oligo – oligohydramnios, PET – pre-eclampsia, PPROM – preterm premature rupture of membranes, ST – syncytiotrophoblast, TAB – therapeutic abortion

**Figure Legends**

**Figure 1. ACE2 and TMPRSS2 expression in the placenta of a woman testing negative for SARS-CoV-2 at 33 weeks 4 days (new figure from a case published in [14])**

A. ACE2 expression showing polarized expression of ACE2 in the ST basally (towards the stromal side of the ST) cells (arrow) with absent apical expression. 20X.
B. TMPRSS2 expression showing very weak expression in the villous endothelium (arrowheads). 20X.

ST - syncytiotrophoblast

**Figure 2. ACE2 and TMPRSS2 expression in early gestation**

A. ACE2 expression in case 5 showing both polarized expression (arrowhead) and circumferential expression (arrow) in the same villous ST. 20X.
B. TMPRSS2 expression in case 10 showing weak staining in the villous endothelium (arrow). 40X.
C. TMPRSS2 expression in case 1 showing very weak expression in circumferentially in the villous ST (arrows). 20X.
D. TMPRSS2 control expression in prostatic epithelium. Note that weaker expression in some of the glandular epithelium is similar to that in B and C. 10X.
E. ACE2 control expression in small intestinal epithelium. 20X.

ST – syncytiotrophoblast

## References


1. Kaplan C. The placenta and viral infections. Semin Diagn Pathol **1993**; 10:232-50.
2. Koi H, Zhang J, Parry S. The mechanisms of placental viral infection. Ann N Y Acad Sci **2001**; 943:148-56.
3. Mahyuddin AP, Kanneganti A, Wong J, et al. Mechanisms and evidence of vertical transmission of infections in pregnancy including SARS-CoV-2. Prenat Diagn **2020**.
4. Benirschke K, Burton GJ, Baergen RN. Pathology of the Human Placenta. 6 ed. Berlin: Springer-Verlag, **2012**.
5. Schwartz DA, Dhaliwal A. INFECTIONS IN PREGNANCY WITH COVID-19 AND OTHER RESPIRATORY RNA VIRUS DISEASES ARE RARELY, IF EVER, TRANSMITTED TO THE FETUS: EXPERIENCES WITH CORONAVIRUSES, HPIV, hMPV RSV, AND INFLUENZA. Arch Pathol Lab Med **2020**.
6. Edlow AG, Li JZ, Collier AY, et al. Assessment of Maternal and Neonatal SARS-CoV-2 Viral Load, Transplacental Antibody Transfer, and Placental Pathology in Pregnancies During the COVID-19 Pandemic. JAMA Netw Open **2020**; 3:e2030455.
7. Schwartz DA, Morotti D. Placental Pathology of COVID-19 with and without Fetal and Neonatal Infection: Trophoblast Necrosis and Chronic Histiocytic Intervillositis as Risk Factors for Transplacental Transmission of SARS-CoV-2. Viruses **2020**; 12.
8. Patane L, Morotti D, Giunta MR, et al. Vertical transmission of COVID-19: SARS-CoV-2 RNA on the fetal side of the placenta in pregnancies with COVID-19 positive mothers and neonates at birth. Am J Obstet Gynecol MFM **2020**:100145.
9. Kirtsman M, Diambomba Y, Poutanen SM, et al. Probable congenital SARS-CoV-2 infection in a neonate born to a woman with active SARS-CoV-2 infection. CMAJ **2020**; 192:E647-E50.
10. Alamar I, Abu-Arja MH, Heyman T, et al. A Possible Case of Vertical Transmission of Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2) in a Newborn With Positive Placental In Situ Hybridization of SARS-CoV-2 RNA. J Pediatric Infect Dis Soc **2020**; 9:636-9.
11. Wang W, Xu Y, Gao R, et al. Detection of SARS-CoV-2 in Different Types of Clinical Specimens. JAMA **2020**; 323:1843-4.
12. Fajnzylber J, Regan J, Coxen K, et al. SARS-CoV-2 viral load is associated with increased disease severity and mortality. Nat Commun **2020**; 11:5493.
13. Hoffmann M, Kleine-Weber H, Schroeder S, et al. SARS-CoV-2 Cell Entry Depends on ACE2 and TMPRSS2 and Is Blocked by a Clinically Proven Protease Inhibitor. Cell **2020**; 181:271-80 e8.
14. Hecht JL, Quade B, Deshpande V, et al. SARS-CoV-2 can infect the placenta and is not associated with specific placental histopathology: a series of 19 placentas from COVID-19-positive mothers. Mod Pathol **2020**; 33:2092-103.
15. Anton L, Merrill DC, Neves LA, et al. Activation of local chorionic villi angiotensin II levels but not angiotensin (1-7) in preeclampsia. Hypertension **2008**; 51:1066-72.
16. Pique-Regi R, Romero R, Tarca AL, et al. Does the human placenta express the canonical cell entry mediators for SARS-CoV-2? Elife **2020**; 9.
17. Valdes G, Corthorn J, Bharadwaj MS, Joyner J, Schneider D, Brosnihan KB. Utero-placental expression of angiotensin-(1-7) and ACE2 in the pregnant guinea-pig. Reprod Biol Endocrinol **2013**; 11:5.
18. Valdes G, Neves LA, Anton L, et al. Distribution of angiotensin-(1-7) and ACE2 in human placentas of normal and pathological pregnancies. Placenta **2006**; 27:200-7.

19. Noronha L, Zanluca C, Azevedo ML, Luz KG, Santos CN. Zika virus damages the human placental barrier and presents marked fetal neurotropism. Mem Inst Oswaldo Cruz **2016**; 111:287-93.
20. Khong TY, Mooney EE, Ariel I, et al. Sampling and Definitions of Placental Lesions: Amsterdam Placental Workshop Group Consensus Statement. Arch Pathol Lab Med **2016**; 140:698-713.
21. Pass RF, Fowler KB, Boppana SB, Britt WJ, Stagno S. Congenital cytomegalovirus infection following first trimester maternal infection: symptoms at birth and outcome. J Clin Virol **2006**; 35:216-20.
22. Mlakar J, Korva M, Tul N, et al. Zika Virus Associated with Microcephaly. N Engl J Med **2016**; 374:951-8.
23. David FJ, Autran B, Tran HC, et al. Human trophoblast cells express CD4 and are permissive for productive infection with HIV-1. Clin Exp Immunol **1992**; 88:10-6.
24. Miranda J, Martin-Tapia D, Valdespino-Vazquez Y, et al. Syncytiotrophoblast of Placentae from Women with Zika Virus Infection Has Altered Tight Junction Protein Expression and Increased Paracellular Permeability. Cells **2019**; 8.

Figure 1

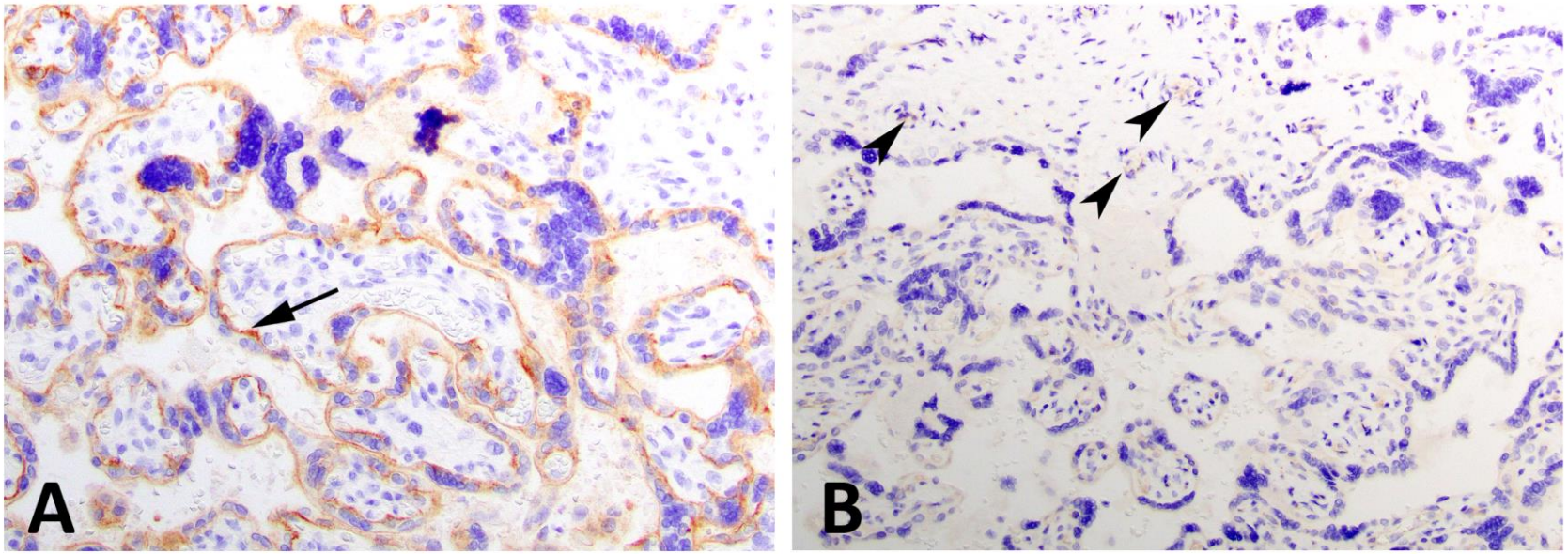

Figure 2