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SUPPLEMENT ARTICLE

ACE2, TMPRSS2, and L-SIGN Expression in Placentae From HIV-Positive Pregnancies Exposed to Antiretroviral Therapy—Implications for SARS-CoV-2 Placental Infection

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Background. Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) binding receptor ACE2 and the spike protein priming protease TMPRSS2 are coexpressed in human placentae. It is unknown whether their expression is altered in the context of HIV infection and antiretroviral therapy (ART).

Methods. We compared mRNA levels of SARS-CoV-2 cell-entry mediators ACE2, TMPRSS2, and L-SIGN by quantitative polymerase chain reaction in 105 placentae: 45 from pregnant women with HIV (WHIV) on protease inhibitor (PI)-based ART, 17 from WHIV on non-PI–based ART, and 43 from HIV-uninfected women.

Results. ACE2 levels were lower, while L-SIGN levels were higher, in placentae from WHIV on PI-based ART compared to those on non-PI–based ART and to HIV-uninfected women. TMPRSS2 levels were similar between groups. Black race was significantly associated with lower expression of ACE2 and higher expression of L-SIGN. ACE2 levels were significantly higher in placentae of female fetuses.

Conclusions. We identified pregnant women of black race and WHIV on PI-based ART to have relatively lower expression of placental ACE2 than those of white race and HIV-uninfected women. This may potentially contribute to altered susceptibility to COVID-19 in these women, favorably by reduced viral entry or detrimentally by loss of ACE2 protection against hyperinflammation.

Keywords. COVID-19; placenta; renin-angiotensin system; HIV protease inhibitors; race; infant sex; receptor; neonate; AIDS; detrimental.

Infection by severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) causes coronavirus disease 2019 (COVID-19), which has posed a serious threat globally [1, 2]. Data are emerging on the clinical manifestations of COVID-19 in pregnant women. SARS-CoV-2 infection during pregnancy is associated with increased risk of preterm labor, and babies born to infected mothers have a higher risk of admission to the neonatal unit [3–6]. There are sporadic reports of miscarriage, stillbirth, fetal demise, and neonates testing positive for the virus [7–9]. The risk of SARS-CoV-2 placental infection seems to be low [10–13], although reports of electron microscopy observations of virions invading the syncytial layer [14–16] and presence of strong staining of SARS-CoV-2 nucleocapsid/spike glycoprotein in the syncytial layer [16–19] have contributed to growing evidence that SARS-CoV-2 can infect the placenta.

The placenta also seems to be susceptible to the effects of maternal COVID-19 disease, even in the absence of detectable or very low levels of SARS-CoV-2 mRNA or protein in the placenta [8, 20–23]. This is evident from histopathological abnormalities such as villous fibrin deposition, maternal vascular malperfusion, fetal vascular malperfusion, and villitis/intervillositis observed in placentae from women with even mild COVID-19 disease [8, 20–24]. Based on current evidence, the rate of SARS-CoV-2 placental infection is considered low [10–13], although it appears that there is considerable potential for SARS-CoV-2 to affect placental function and fetal development [25].

SARS-CoV-2 invades host cells by binding to the angiotensin-converting enzyme 2 (ACE2) receptor [26–28], a component of the renin-angiotensin system (RAS), which is a critical regulator of blood pressure, electrolyte balance, and fluid homeostasis [29–31]. Many components of RAS, including ACE2, are upregulated in normal pregnancy [32, 33]. Upregulation of ACE2 mediates conversion of angiotensin II, a vasoconstrictor, to angiotensin-(1–7), a vasodilator, and contributes to relatively low blood pressures in pregnancy [34]. Upon binding ACE2, SARS-CoV-2 causes its

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downregulation, enhancing RAS imbalance with increased angiotensin II relative to decreased angiotensin-(1–7), which can cause vasoconstriction, inflammation, and coagulopathy [35, 36].

Much of the literature on how ACE2 levels regulate the pathogenesis of COVID-19 is conflicting. While many authors argue that ACE2 is the underlying reason behind many of the risk factors for severe COVID-19 [37–40], there is a growing body of literature which argues that ACE2 upregulation is a protective factor for SARS-CoV-2 outcomes due to its role in limiting the potent vasoconstrictive effect of angiotensin II [36, 41–46]. Therefore, ACE2 expression may have paradoxical effects, aiding SARS-CoV-2 infection, yet conversely limiting viral pathogenicity. Further studies are needed to elucidate the precise effects that altered ACE2 expression has on the acquisition of SARS-CoV-2 infection and associated severity in COVID-19.

Upon ACE2 binding, SARS-CoV-2 employs the host serine protease TMPRSS2 for spike protein priming, facilitating viral fusion and cellular infection [26]. In humans, *ACE2* and *TMPRSS2* genes are expressed in the placenta throughout the 3 trimesters of pregnancy [47–49], with the highest mRNA expression in the first trimester and decreasing expression with advancing gestation [50, 51]. In 2 recently published reports investigating localization of ACE2 and TMPRSS2 in COVID-19–exposed term placentae, the ACE2 receptor was consistently localized within the outer syncytiotrophoblast layer of chorionic villi, whereas TMPRSS2 was reported to be absent or only present weakly in the villous endothelium and rarely in the syncytiotrophoblast layer [19, 52]. In spite of the relative absence of TMPRSS2, all 15 placentae in a study tested positive for SARS-CoV-2 infection, and there were 5 cases of fetal transmission [19]. This suggests that the SARS-CoV-2 virus may be using alternative cellular entry pathway molecules to enter the placenta. It has been identified previously that SARS-CoV, the closely related coronavirus responsible for the SARS outbreak, uses C-type lectins DC-SIGN (encoded by the gene CD209) and/or L-SIGN (also known as CLEC4M) as independent receptors or as enhancer factors that facilitate ACE2 mediated virus infection [53–56]. A study demonstrated that L-SIGN is endogenously expressed in human endothelial cells and mediates SARS-CoV-2 entry and infection [57, 58]. Recently, a preprint article has established that the N-terminal domain (NTD) of the spike protein mediates SARS-CoV-2 infection by associating with L-SIGN and DC-SIGN. Serum samples from SARS-CoV-2–infected patients were found to contain antibodies against NTD and a patient-derived monoclonal antibody against NTD inhibited SARS-CoV-2 infection of L-SIGN or DC-SIGN–expressing cells [59]. L-SIGN also serves as an attachment receptor for other viruses such as human immunodeficiency virus (HIV) [60].

Emerging data indicate that HIV infection may be associated with increased risk of COVID-19 diagnosis [61] and people with HIV may be at a slightly higher risk of death from COVID-19 [62–66]. The presence of comorbidities, a low CD4 cell count, and lack of an effective antiretroviral therapy contribute to the risk of severe COVID-19 outcomes among people living with HIV [67]. Currently there are no data on the pathogenesis of SARS-CoV-2 in pregnant women with HIV (WHIV), as well as the risk of vertical transmission in this population. Furthermore, it is not known whether the expression of SARS-CoV-2 cell-entry mediators is altered in placentae of WHIV exposed to antiretroviral therapy (ART). We previously reported that pregnant WHIV who received protease inhibitor (PI)-based ART had higher levels of estradiol in the maternal and umbilical cord plasma [68]. As estradiol is known to downregulate the expression of ACE2 [69, 70], we hypothesized that WHIV exposed to PI-based ART have lower placental expression of ACE2. Here, we compared the gene expression pattern of SARS-CoV-2 cell-entry mediators: ACE2, TMPRSS2 and L-SIGN/CLEC4M, in term placentae of WHIV exposed to PI-ART, non-PI-ART, and HIV-uninfected women. Because COVID-19 has disproportionally affected racial and ethnic communities [71], we further explored associations between placental expression of SARS-CoV-2 cell-entry mediators and race. Finally, as the placenta is an organ shared by mother and fetus, we also explored the influence of fetal sex on placental SARS-CoV-2 cell-entry mediator expression.

METHODS

Study Population

Placentae included in this study were collected from women recruited to the Angiogenesis and Adverse Pregnancy Outcomes in Women with HIV (AAPH) cohort (recruited in Toronto, Canada). Details on the AAPH cohort have been published previously [72]. Briefly, participants were aged >18 years, with singleton pregnancy. Exclusion criteria included preexisting hypertension, diabetes, renal, autoimmune, or collagen vascular disease, active opportunistic infection for the WHIV, body mass index (BMI) > 40, and current illicit or recreational drug use. None of the women were current tobacco smokers or had alcohol use disorder. All available placentae (n = 105) were included in this study; 62 from WHIV on ART (45 on PI-based ART, 9 on non-nucleoside reverse transcriptase inhibitor (NNRTI)-based ART, 8 on integrase strand transfer inhibitor (INSTI)-based ART), and 43 women without HIV (control group). Participants were recruited between May 2010 and April 2019.

Ethical Considerations

This study was approved by the Institutional Research Ethics Board at University Health Network (REB No. 20–5526) and was performed in accordance with the Tri-Council Policy Statement on Ethical Conduct for Research Involving Humans. All participants gave written informed consent

for the AAPH study and for inclusion of their samples and data into a biobank program to support studies in HIV and pregnancy.

Sample Collection

Placenta samples were collected immediately after delivery. Placental core sections were sampled from 3 sites on the maternal surface, rinsed in phosphate buffered saline, further dissected into smaller pieces, and immersed in Allprotect tissue reagent (Qiagen). Samples were stored at −80°C until processing.

RNA Isolation and Quantitative Polymerase Chain Reaction

Total RNA was isolated from the placental tissue using the mirVana miRNA Isolation Kit (Thermo Fisher Scientific) per the manufacturer's protocol. RNA quality and concentration were determined using the Nano-Drop1000 Spectrophotometer (Thermo Fisher Scientific). Total RNA, 10 μg, was treated with DNase I, RNase-free (Thermo Fisher Scientific), followed by addition of 5 mM EDTA and reverse transcribed into cDNA using the iScript cDNA Synthesis Kit (Bio-Rad Laboratories). ACE2, TMPRSS2, and L-SIGN mRNA levels were assessed by quantitative polymerase chain reaction (qPCR) using LightCycler 480 SYBR Green I Master reaction mix (Roche) and the LightCycler 480 detection instrument (Roche). The cycling conditions were as follows: initial denaturation at 95°C (5 minutes), followed by 40 cycles of denaturation at 95°C (10 seconds), annealing at 60°C (15 seconds), and extension at 72°C (15 seconds). Gene expression was normalized to *YWHAZ* gene, which presented stable expression among all groups. The primer sequences of all evaluated genes are shown in Supplementary Table 1. Relative expression of target genes was obtained using the 2ΔΔCT method [73].

Statistical Analysis

For demographic and clinical data, medians with interquartile ranges (continuous variables) or frequencies (categorical variables) were calculated and compared using Mann-Whitney *U* test or Fisher exact test, respectively. ACE2, TMPRSS2, and L-SIGN mRNA levels were log-transformed and differences between groups were assessed using Kruskal-Wallis test with Dunn multiple comparison posttest, or Mann-Whitney *U* test, as appropriate. Correlations were assessed using Pearson *r* test. Regression analysis was used to examine relationships between $\log_e$-transformed ACE2, TMPRSS2, or L-SIGN and ART-exposure status (categorized as none, PI-ART, non-PI-ART), race (categorized as black, white, or other), and fetal sex (female or male). Statistical analyses were performed using GraphPad Prism version 5.0 and Stata version 13.0.

RESULTS

Study Populations

We included 105 placentae from the AAPH cohort (recruited in Toronto, Canada), 43 (41%) from women without HIV (control group), and 62 (59%) from WHIV on ART. Of WHIV, 45 (72%) were taking a PI-based regimen, 9 (15%) an NNRTI-based regimen, and 8 (13%) an INSTI-based regimen. For all analyses, placentae exposed to NNRTI or INSTI regimens were grouped together in the non-PI–based ART group. Demographic information is shown in Table 1. Maternal age, maternal prepregnancy BMI, race, mode of delivery, and fetal sex were similar between groups. All placentae from the HIV-uninfected group were delivered at term, while 56 (90%) of the

Table 1. Demographics

Characteristics	HIV− (n = 43)	HIV+ (n = 62)	HIV+ on PI (n = 45)	HIV+ on non-PI (n = 17)
Maternal age, y, median (IQR)	33 (30–36)	33 (30–37)	33 (30–36)	35 (31–38)
Maternal prepregnancy BMI, median (IQR)	24 (21–29)	24 (21–29)	25 (22–30)	24 (20–29)
Race				
Black	26 (61)	45 (73)	35 (78)	10 (59)
White	14 (33)	12 (19)	7 (16)	5 (29)
Other	3 (7)	5 (8)	3 (7)	2 (12)
Delivery mode				
Vaginal	28 (65)	33 (53)	25 (56)	8 (47)
Scheduled cesarean delivery	11 (26)	18 (29)	11 (24)	7 (41)
Emergency cesarean delivery	4 (9)	11 (18)	9 (20)	2 (12)
Term birth	43 (100)	56 (90)	40 (89)	16 (94)
Preterm birth	0 (0)	6 (10)	5 (11)	1 (6)
Fetal sex				
Female	26 (60)	28 (45)	21 (47)	7 (41)
Male	17 (40)	34 (55)	24 (53)	10 (59)

Data are No. (%) except where indicated.

No significant differences were noted for the HIV-positive group (HIV+) compared to HIV-uninfected (HIV−), or between HIV+ on PI-based ART vs HIV+ on non-PI–based ART, using Mann-Whitney *U* test or Fisher exact test as appropriate.

Abbreviations: BMI, body mass index; HIV, human immunodeficiency virus; IQR, interquartile range; PI, protease inhibitor.

HIV-positive group were delivered at term and 6 (10%) were delivered preterm. The median gestational week at birth was 40 for the HIV-uninfected group and 39 for the HIV-positive group. HIV plasma viral load was below detectable limits for 52 (84%) of WHIV and unavailable for 2 (3%) women. Median $CD4^+$ T-cell count at time of recruitment for the WHIV was 565 cells/mm^3. $CD4^+$ T-cell count was below 250 cells/mm^3 for 4 (6.4%) women and unavailable for 1 (2%) woman.

PI-Based ART Exposure and Black Race Are Associated With Lower ACE2 and Higher L-SIGN Placental Expression Levels

We have previously shown that estradiol levels are elevated in pregnancies exposed to PI-based ART but not in those exposed to NNRTIs or INSTIs [68, 74, 75]. Given that ACE2 expression levels have been shown to be influenced by estradiol levels [69, 70], we hypothesized that placentae from women exposed to PI-based ART will have lower levels of ACE2. Compared to the control group, ACE2 mRNA levels were significantly lower in placentae exposed to PI-based ART: median of $\log_e$-transformed values in arbitrary units was −0.56 (interquartile range [IQR], −1.03 to 0.11) for PI-based ART versus 0.12 (IQR, −0.28 to 0.50) for control ($P < .01$; Figure 1). ACE2 mRNA levels were similar between the control group and the group exposed to non-PI–based ART. We next explored if maternal estradiol levels measured between gestational week 33 and 37 correlated with placental ACE2 expression levels. Estradiol levels were only available for 30 WHIV, all of whom were taking a PI-based regimen, and 31 women in the HIV-uninfected group. We observed a significant negative correlation between estradiol levels and placental ACE2 mRNA levels in the HIV-positive group on PI-based ART ($r = −0.43$, $P = .019$; Figure 2). No correlation was observed in the HIV-uninfected group.

In contrast to ACE2, mRNA levels of L-SIGN were significantly higher in placentae exposed to PI-based ART compared to those exposed to non-PI–based ART and compared to controls: median of $\log_e$-transformed values in arbitrary units was 0.78 (IQR, 0.08 to 1.37) for PI-based ART versus −0.30 (IQR, −1.22 to 0.78) for non-PI–based ART ($P < .01$) and versus 0.0 (IQR, −0.68 to 0.78) for control ($P < .01$). The expression of TMPRSS2 was similar between the groups.

We next examined if race influenced expression levels of the SARS-CoV-2 receptors (Figure 3). We found that lower mRNA of ACE2 and higher mRNA expression of L-SIGN in placentae from women who identified as black compared to those who identified as white: median of $\log_e$-transformed values in arbitrary units for ACE2 was −0.26 (IQR, −0.90 to 0.19) for black versus 0.24 (IQR, −0.17 to 0.56) for white race ($P < .05$); and for L-SIGN it was 0.61 (IQR, −0.14 to 1.22) for black versus −0.13 (IQR, −1.24 to 0.50) for white race ($P < .01$). TMPRSS2 mRNA levels did not vary by race. Demographics and clinical information were similar between the different races (Supplementary Table 2) with the exception of prepregnancy BMI, which was significantly lower in white women compared to black women. Differences in ACE2 and L-SIGN mRNA levels between black and white women remained significant when we adjusted for maternal BMI.

We also examined if fetal sex was associated with receptor expression levels (Figure 4). L-SIGN and TMPRSS2 mRNA levels did not differ by sex. However, ACE2 mRNA levels were significantly higher in placentae associated with a female fetus compared to those associated with a male fetus: median of $\log_e$-transformed values in arbitrary units was 0.09 (IQR, −0.45 to 0.57) for female versus −0.41 (IQR, −0.97 to 0.17) for male ($P = .0036$).

We did not observe significant associations between ACE2, L-SIGN, or TMPRSS2 mRNA levels and maternal age or

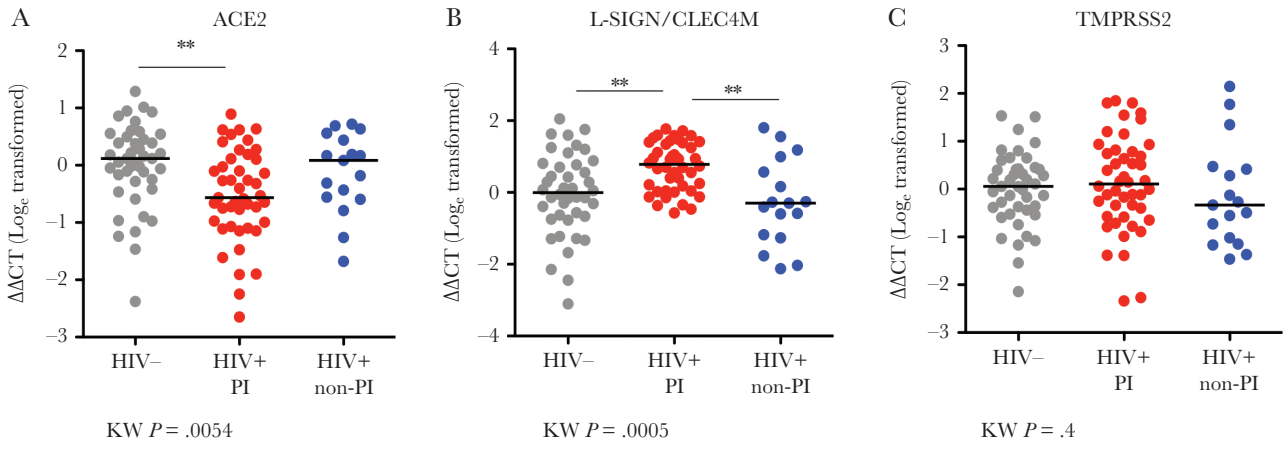

Figure 1. Protease inhibitor exposure in pregnancy is associated with lower ACE2 and higher L-SIGN expression levels in the placenta. $\log_e$ transformed mRNA levels of ACE2 (*A*), L-SIGN/CLEC4M (*B*), and TMPRSS2 (*C*) in placentae of HIV-uninfected women (HIV−, grey), women with HIV (HIV+) on PI-based ART (red), and women with HIV on non-PI–based ART (blue). Statistical comparison using Kruskal-Wallis test with Dunn posttest. *P* values for the Kruskal-Wallis test are shown below each graph. Asterisks indicate *P* values for the Dunn posttest: **, $P < .01$. n = 43 HIV−, n = 45 HIV+ PI-based ART, and n = 17 HIV+ non-PI–based ART. Abbreviations: ART, antiretroviral therapy; HIV, human immunodeficiency virus; PI, protease inhibitor.

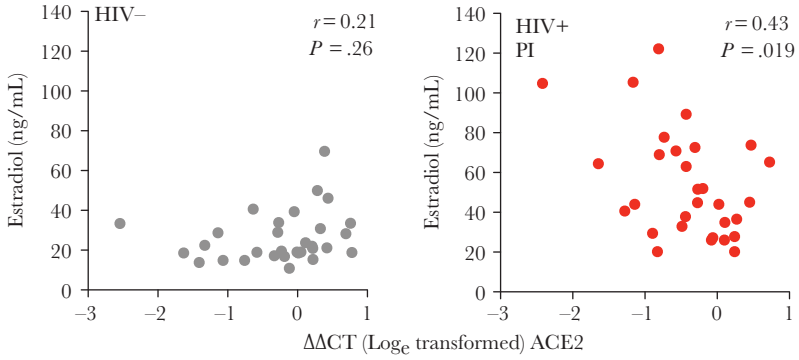

Figure 2. Maternal estradiol levels correlate with placental ACE2 mRNA levels in WHIV on PI-based ART. Maternal peripheral estradiol levels measured between gestational week 33 and 37 were plotted against log$_e$ transformed expression levels of ACE2 for HIV-uninfected women (HIV–, grey) and WHIV on PI-based ART (HIV+ PI, red). Correlations were assessed using Pearson *r* test. A significant correlation was observed in the HIV+ PI group. Abbreviations: HIV, human immunodeficiency virus; PI, protease inhibitor; WHIV, women with HIV.

maternal BMI. In WHIV we did not observe significant associations between ACE2, L-SIGN, or TMPRSS2 mRNA levels and viral load, CD4 count, or preterm birth. In multivariable regression analysis, exposure to PI-based ART, race, and fetal sex, all remained significantly associated with ACE2 mRNA levels (Supplementary Table 2). Similarly, both exposure to PIs and race remained significantly associated with L-SIGN mRNA levels (Supplementary Table 3).

DISCUSSION

Emerging evidence points to HIV as a potential risk-factor for death from COVID-19 [62–66], yet the impact of HIV infection and ART on the clinical presentation, birth outcomes, and placental pathology of pregnancies complicated by COVID-19 remains to be investigated [76, 77]. To better understand the risk of SARS-CoV-2 placental infection in WHIV treated with ART, we performed an integrated analysis of the gene expression of SARS-CoV-2 cell-entry mediators in term placentae of WHIV who received ART compared to the expression in placentae from HIV-uninfected women. We found lower expression of ACE2 and higher expression of L-SIGN/CLEC4M in placentae from WHIV on PI-based ART compared to those from HIV-uninfected women, while ACE2 and L-SIGN mRNA levels were similar in placentae from WHIV on non-PI–based ART compared to HIV-uninfected women. TMPRSS2 mRNA levels were similar across all groups. In agreement with previous reports that ACE2 expression levels are influenced by estradiol levels [69, 70], we observed a negative correlation between late third trimester maternal estradiol levels and placental ACE2 mRNA levels in WHIV on PI-based ART. This correlation was not observed in the HIV-uninfected group. We also observed differential expression profiles for ACE2 and L-SIGN based on race, with black race significantly associated with lower placental mRNA expression of ACE2 and higher expression of L-SIGN compared to white race. TMPRSS2 mRNA levels did not vary by race.

Our data may help stratify the risk of SARS-CoV-2 placental infection in pregnant WHIV taking ART. We identified

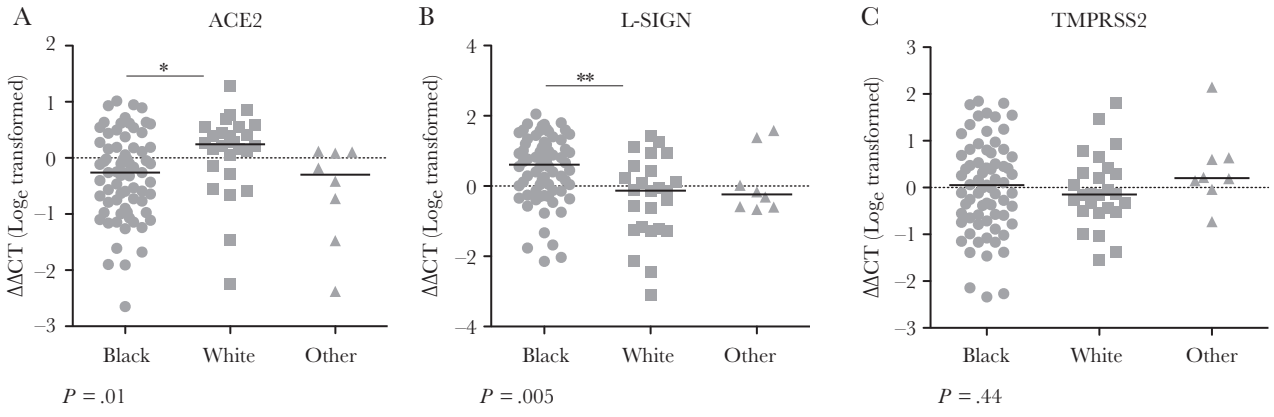

Figure 3. Placental mRNA levels of ACE2 and L-SIGN differ by race. Log$_e$ transformed mRNA expression levels of ACE2 (*A*), L-SIGN/CLEC4M (*B*), and TMPRSS2 (*C*) in placentae by race. Statistical comparison using Kruskal-Wallis test with Dunn posttest. *P* values for the Kruskal-Wallis test are given below graphs. Asterisks indicate *P* value for the Dunn posttest: * $P < .05$, ** $P < .01$. n = 71 black women, n = 26 white women, n = 8 other women (4 Asia, 4 Hispanic).

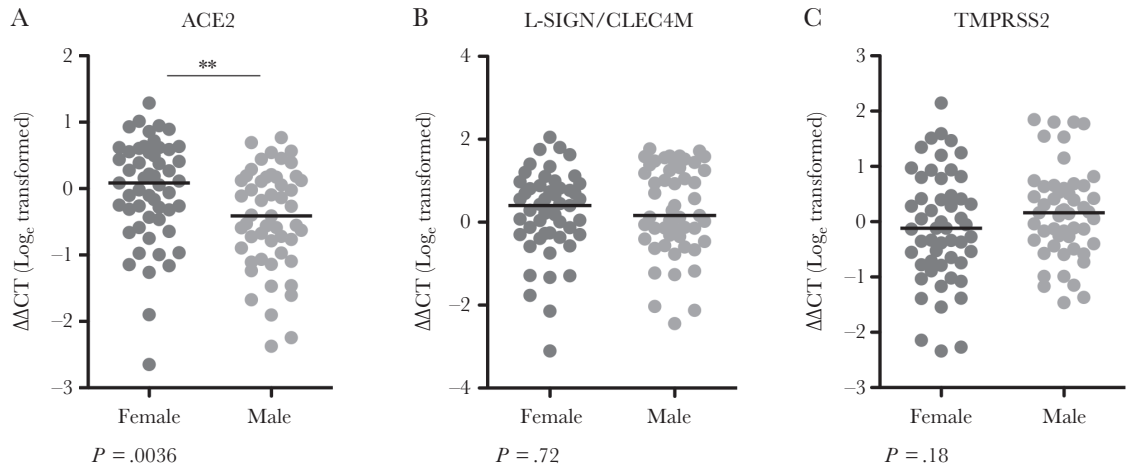

Figure 4. Placental mRNA expression levels of ACE2 differ by infant sex. Log$_e$ transformed mRNA levels of ACE2 (*A*), L-SIGN/CLEC4M (*B*), and TMPRSS2 (*C*) in placentae by fetal sex (dark grey female, light grey male). Statistical comparisons by Mann-Whitney *U* test with *P* values shown below graphs. ** $P < .01$. n = 54 female infants and n = 51 male infants.

pregnant WHIV taking PI-based ART and pregnant women of black race to have lower baseline ACE2 mRNA levels compared to HIV-uninfected women and women of white race, which may reduce their risk of placental infection, although the higher expression of L-SIGN mRNA in the same group of women may mitigate this protection. SIGN receptors are well known for their ability to potentiate viral infection of permissive cell types in *trans*, for example, DC-SIGN–positive dendritic cells incubated with HIV are able to infect T lymphocytes very efficiently, even after very thorough washing [60, 78]. Therefore, potentiation of viral infection in *trans* could be the mechanism by which L-SIGN/DC-SIGN may mediate SARS-CoV-2 infection of ACE2-low cells. Hence, we speculate that due to the increased mRNA levels of L-SIGN in pregnant women of black race and WHIV on PI-based ART, the probability of placental infection might still exist, in spite of the reduced ACE2 levels.

In the event of placental infection due to severe maternal COVID-19, we would speculate that the baseline lower ACE2 levels may emerge as unfavorable for the pregnancy due to further downregulation of ACE2 by SARS-CoV-2 binding and loss of the ACE2 vasodilatory function in the local placental RAS system. This RAS imbalance may result in placental inflammation and vasoconstriction, which over the course of pregnancy may adversely affect the developing fetus, as reported previously, outside the context of COVID-19 [79–83]. However, these mechanistic pathways, and their relationship to outcomes in maternal SARS-CoV-2 infection, remain to be examined.

In healthy term placentae, ACE2 has been detected by immunohistochemistry in syncytiotrophoblasts, cytotrophoblasts, and fetal capillary endothelium [84, 85]. Placental expression of L-SIGN is also localized to the fetal capillary endothelium [60]. Therefore, colocalization of these 2 SARS-CoV-2 receptors in the fetal endothelium may potentiate vertical transmission. It is possible that the significantly higher expression of L-SIGN in pregnant women of black race and WHIV who are on PI-based ART may increase their susceptibility to vertical transmission of SARS-CoV-2. However, it may manifest only in sporadic cases of placental infection in which the syncytial barrier might be breached, perhaps due to placental inflammation. These hypotheses need to be evaluated in research studies.

Mortality from COVID-19 has been particularly high in African American communities [71, 86–89]. Higher mortality among black people could be due to higher prevalence of the known risk factors for COVID-19 complications, such as hypertension, diabetes, obesity, and cardiovascular disease among the black ethnic group, as well as socioeconomic factors [90–93]. Furthermore, a study found that black people with HIV were more likely to die from COVID-19 than other people with HIV [63]. A recent report states that there is a genetic predisposition for lower expression levels of ACE2 in African populations [94], which is consistent with our data reporting lower placental expression of ACE2 in black pregnant women. An ACE2 polymorphism found to be associated with cardiovascular and pulmonary conditions was reported in the African/African American population [95]. Hence, ACE2 and its genomic variants might influence interindividual variability in disease susceptibility and severity of COVID-19.

We also detected significantly higher ACE2 mRNA levels in placentae of female fetuses compared to those of male fetuses. This finding is consistent with a study reporting higher ACE2 expression in different tissues in Asian females as compared to males [96], although the association between sex and ACE2 expression is still debatable.

One strength of our study is the simultaneous measurement of the expression levels of all major SARS-CoV-2 cell-entry

mediators—ACE2, TMPRSS2, and L-SIGN—in a large number of term placentae from WHIV compared to placentae from HIV-uninfected women with similar demographics. Further, the original cohort excluded women with hypertension, diabetes, or obesity and did not include recreational or illicit drug users or current tobacco smokers. While these characteristics limit the influence of potential confounding factors in our findings, they also limit the external validity of our data. A limitation of our study is that we could not assess the placental expression of ACE2, TMPRSS2, and L-SIGN in the first and second trimesters of pregnancy in WHIV treated with ART. Another limitation is that we were not able to evaluate the placental protein levels and localization of these factors, nor definitively separate the effects of HIV from those of ART. In our race analyses we are limited to the comparison of only white and black race, based on participant self-identification. Future studies should evaluate the impact of Hispanic or Asian race. Finally, we only had estradiol levels on 61 of the 105 participants so our correlation analyses between estradiol and ACE2 levels should be viewed with caution.

Overall, our data show that pregnant women of black race and WHIV who are on PI-based ART have lower mRNA levels of ACE2 but higher levels of L-SIGN, which can alter their susceptibility to SARS-CoV-2 placental infection. Once infection is acquired, clinical manifestations might be worse in these women as they may be at a higher risk of placental abnormalities due to RAS dysregulation, leading to pregnancy complications, and possibly transplacental transmission. These data may better inform clinical considerations surrounding risk stratification and prevention approaches for SARS-CoV-2–affected pregnancies exposed to HIV and ART. However, our data should be interpreted cautiously because there may be other undefined pathways regulating SARS-CoV-2 placental infection.

Supplementary Data

Supplementary materials are available at *The Journal of Infectious Diseases* online. Consisting of data provided by the authors to benefit the reader, the posted materials are not copyedited and are the sole responsibility of the authors, so questions or comments should be addressed to the corresponding author.

Notes

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