Cellular Fetal Microchimerism in Preeclampsia

Hilary S. Gammill, Tessa M. Aydelotte, Katherine A. Guthrie, Evangelyn C. Nkwopara, J. Lee Nelson

Abstract—Previous studies have shown elevated concentrations of free fetal DNA and erythroblasts in maternal circulation in women with preeclampsia compared with those with normal pregnancy. Pluripotent and immunocompetent fetal cells also transfer to the maternal circulation during pregnancy, but whether concentrations of fetal mononuclear cells also differed in preeclampsia was unknown. We sought to quantify cellular fetal microchimerism in maternal circulation in women with preeclampsia and healthy controls. We studied women with preeclampsia and compared them with women with healthy pregnancies at similar gestational age. To identify a targetable polymorphism unique to the fetus to quantify fetal microchimerism, participants and family members were genotyped for the human leukocyte antigen loci DRB1, DQA1, and DQB1, as well as several other polymorphisms. A panel of polymorphism-specific quantitative polymerase chain reaction assays was used to identify and quantify fetal microchimerism in maternal peripheral blood mononuclear cells. Of 53 preeclampsia samples tested for cellular fetal microchimerism, 17 (32%) were positive when compared with 6 of 57 (6%) control samples (unadjusted odds ratio for detection, 4.0; 95% confidence interval, 1.5–11.1; P=0.007). The concentration of cellular fetal microchimerism (expressed as genome equivalents of fetal microchimerism per 100,000 maternal genome equivalents) was also higher among women with preeclampsia: median 0.0, mean 5.7, range 0 to 153.7, compared with those with controls: median 0.0, mean 0.3, range 0 to 9.1, P=0.002. We conclude that women with preeclampsia harbor cellular fetal microchimerism more commonly and at higher concentrations compared with women with uncomplicated pregnancy. The functional capacity and phenotype of these fetal cells are not yet known. (Hypertension. 2013;62:00-00.)

Key Words: chimerism ■ hypertension ■ maternal-fetal exchange ■ preeclampsia ■ pregnancy complications

A mother and fetus exchange cells and DNA during pregnancy. Fetal cells can persist as microchimerism long-term within maternal circulation and tissues.1 Fetal cellular microchimerism includes pluripotent and immune competent cells that can durably persist, and its presence has been associated with later-life disease risk.2,3 For example, increased fetal microchimerism is associated with some autoimmune diseases,4 and decreased fetal microchimerism is found in some malignancies.7,8 Such associations may reflect functional significance of cellular microchimerism, such as in an alloautoimmune or allosurveillance capacity. Whether fetal cellular microchimerism primarily derives from the transfer of intact cells from the fetal circulation or from exported trophoblast and syncytiotrophoblast microparticles, fetal erythrocytes, and erythroblasts, as well as cell-free fetal DNA (cffDNA),5 also results in circulating concentrations of syncytiotrophoblast microparticles, fetal erythrocytes, and erythroblasts, and cffDNA, and fetal erythroblasts are rapidly and definitively cleared from the maternal system postpartum.9,10

Preeclampsia is associated with increased fetal–maternal trafficking. In 1893, Schmorl reported the first detection of trophoblast cells in the maternal lung in eclampsia.11 Since then, studies have shown that in overt preeclampsia, women harbor higher circulating concentrations of syncytiotrophoblast microparticles, fetal erythrocytes, and erythroblasts, as well as cffDNA and total cell-free DNA. Precociously evident preeclampsia, cffDNA is elevated and primarily derives from apoptotic or aponecrotic shedding of trophoblast material.12 Fetal microchimerism within the PBMC compartment, which is characterized by incomplete clearance, immune competence, and pluripotency, may contribute to the maternal immune dysfunction seen in preeclampsia and may lead to long-term persistence after preeclamptic pregnancy.

We conducted a case–control study to test the hypothesis that fetal cellular microchimerism is higher in preeclampsia compared with that in healthy pregnancies.

Methods

This study was approved by the Institutional Review Committees of the University of Washington and the Fred Hutchinson Cancer Research Center. All individual participants gave written informed consent before participation in this study. All study procedures were in accordance with institutional guidelines and adhered to the Declaration of Helsinki and Title 45, United States Code of Federal Regulations, Part 46, Protection of Human Subjects.

Women with preeclampsia were recruited at the time of clinical diagnosis at the University of Washington Medical Center between 2007 and 2010. Preeclampsia was defined as hypertension (systolic blood pressure, >140 or diastolic blood pressure, >90) persistent...
Because an HLA polymorphism unique to the fetus may not always be used to identify fetal microchimerism. Six aliquots of DNA from PBMC were tested from each blood draw, with total reaction volumes of 50 μL. The maximum amount of DNA tested per aliquot was 25,000 genome equivalents (gEq) because higher concentrations of DNA may inhibit the PCR. A calibration curve was created on each plate to quantify the total number of gEq of DNA tested in each reaction. DNA quantities were reported as the DNA gEq number of fetal cells per 100,000 maternal cell equivalents using a conversion factor of 6.6 pg of DNA per cell. Total DNA tested per sample was considered acceptable if ≥30,000 gEq.

Multiple safeguards were taken to avoid the risk of contamination. The optical detection system of the 7000 Sequence Detector obviates the need to reopen reaction tubes after amplification. DNA extractions and quantitative PCR preparations were performed under a UV light equipped safety hood, with UV run for 30 minutes between experiments. Filtered tips were used during pipetting. Each experiment included multiple control wells to ensure the absence of contamination.

Statistical Considerations

The primary outcome for analysis was pregnancy status (preeclampsia case versus uncomplicated pregnancy control) and the principal predictor of interest was the presence of fetal microchimerism. Logistic regression models were used to estimate the association between the presence of fetal microchimerism and case status while adjusting for confounding factors.

Fetal microchimerism concentrations were also analyzed by case status. By definition, microchimeric cells occur at low concentrations; therefore, the data distribution is skewed to the right and approximates a Poisson distribution. For this reason, we analyzed the concentrations as the outcome in log-linear regression models, estimating the rate of fetal microchimerism detection as the number of fetal gEq as a proportion of the number of maternal gEq tested. Negative-binomial models were fit to account for the higher level of variability in the data than expected in a Poisson model; interpretation of the resulting estimates is identical to those of a Poisson model.

Approximately 20% of samples were analyzed with 2 different assay types, 1 based on an HLA polymorphism and the other on a non-HLA polymorphism. Of these, 60% were consistently negative, 7% were consistently positive, and 33% had variable results. To account for this variability, an indicator of assay type (HLA or non-HLA) was included in all models. Robust SEs were calculated using generalized estimating equations to account for correlation between 2 results from the same subject.

Factors examined as potential confounders included maternal age at sample collection, quantitative PCR assay type (HLA/non-HLA), parity (no/yes), fetal sex, race, smoking status, body mass index, gestational age at sample collection, and number of cell equivalents tested. A factor was defined as a confounder if there was a discrepancy of ≥10% in the estimated coefficient of interest between the multivariable model, including the factor and the model without it. Differences in subject characteristics between groups were assessed using the χ2 test for categorical variables and Fisher exact test for continuous variables. P values from regression models were derived from the Wald test, and no adjustments were made for multiple comparisons. Analyses were performed on SAS software version 9 (SAS Institute, Inc. Cary, NC).

Results

Study participants included 46 women with preeclampsia (53 samples) and 47 women with uncomplicated pregnancies.
(57 samples). No participant (control or with preeclampsia) had a prior history of blood transfusion, and none was the recipient of an organ or hematopoietic cell transplant. Samples from 7 of 46 (15%) of preeclampsia cases and 10 of 47 (21%) of healthy controls were assayed twice ($P=0.45$).

Many characteristics of the women with preeclampsia varied significantly from those with healthy pregnancies. Participants with preeclampsia were younger and more likely to be nonwhite. Participants with preeclampsia were also associated with earlier gestational age at delivery and lower birthweight compared with the participants with normal pregnancies (Table 1). Despite being derived from the same gestational age range, the gestational age at which samples were drawn was slightly earlier in the participants with preeclampsia than those with controls.

The mean totals of maternal gEq tested among the 53 preeclampsia samples and the 57 control samples were 105±137 (SD, 30±95) and 107±749 (SD, 28±93), respectively ($P=0.64$).

Of the 53 samples from women with preeclampsia tested for fetal microchimerism, 17 (32%) were positive. In comparison, fetal microchimerism was detected in 6 of 57 (6%) samples from women with normal pregnancies. In an unadjusted model, the estimated odds ratio was 4.0 with 95% confidence interval (CI) 1.5 to 11.1; $P=0.007$. For overall positivity in the logistic regression model, adjustment for assay type and maternal age at sample collection diminished the association, giving an odds ratio of 3.5 (95% CI, 0.7–17.4; $P=0.13$).

Fetal cellular microchimerism concentrations are shown in the Figure. The median fetal microchimerism concentration for both groups was 0 gEq/100000 maternal cell equivalents, as expected because of the anticipated skewed distribution. The fetal microchimerism value range for the women with preeclampsia was 0 to 153.7 gEq per 100000 maternal cell equivalents, with a mean concentration of 5.7 gEq per 100000 maternal cell equivalents. For the healthy control group, the range was 0 to 9.1 gEq per 100000 maternal cell equivalents, with a mean concentration of 0.3 gEq per 100000 maternal cell equivalents (Table 2). The concentration of fetal microchimerism was significantly higher in women with preeclampsia compared with those with normal pregnancies in an unadjusted model (detection rate ratio, 17.4; 95% CI, 2.7–110.4; $P=0.002$). This association remained significant in the quantitative model after adjusting for assay type, maternal age and gestational age at sample collection; the estimated detection rate ratio was 15.8 (95% CI, 3.2–77.8; $P<0.001$). Two preeclampsia values reflected particularly high concentrations 98.1 and 153.7 gEq of fetal microchimerism per 100000 maternal cell equivalents; the next highest value was 11.0 gEq per 100000 maternal cell equivalents. When these values were excluded from the model, the estimated detection rate ratio was attenuated but remained statistically significant: 4.6 (95% CI, 1.6–13.2; $P=0.004$ after adjustment).

A preplanned analysis of the subset of nulliparous participants, also excluding cases with chronic hypertension, showed qualitatively similar results. These analyses included 30 women (34 samples) with preeclampsia and 30 women (37 samples) with normal pregnancies. In a model adjusted for assay type and maternal age at sample collection, the

![Table 1. Clinical and Demographic Characteristics for Study Participants](image)

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Normal Pregnancy (n=47)</th>
<th>Preeclampsia (n=46)</th>
<th>P Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Maternal age, y, mean±SD</td>
<td>32.5±3.7</td>
<td>27.3±5.3</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Nulliparity, n (%)</td>
<td>30 (64)</td>
<td>35 (76)</td>
<td>0.20</td>
</tr>
<tr>
<td>Gestational age at delivery, wk, mean±SD</td>
<td>39.8±1.2</td>
<td>32.4±3.9</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Gestational age at sample collection, wk, mean±SD</td>
<td>34.2±3.9</td>
<td>32.1±4.0</td>
<td>0.01</td>
</tr>
<tr>
<td>Birthweight, g</td>
<td>3458±386</td>
<td>1731±909</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Unknown</td>
<td>10</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>White, n (%)</td>
<td>42/47 (89)</td>
<td>27/42 (64)</td>
<td>0.005</td>
</tr>
<tr>
<td>Cesarean delivery, n (%)</td>
<td>10/41 (24)</td>
<td>10/46 (22)</td>
<td>0.77</td>
</tr>
<tr>
<td>Male fetal sex, n (%)</td>
<td>19/45 (42)</td>
<td>24/46 (52)</td>
<td>0.34</td>
</tr>
<tr>
<td>Smoking, n (%)</td>
<td>0/31 (0)</td>
<td>8/46 (17)</td>
<td>0.01</td>
</tr>
<tr>
<td>BMI, mean±SD</td>
<td>23.7±3.7</td>
<td>32.9±8.9</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Autoimmune disease, n (%)</td>
<td>0/47 (0)</td>
<td>0/46 (0)</td>
<td>…</td>
</tr>
<tr>
<td>Pregestational diabetes mellitus, n (%)</td>
<td>0/47 (0)</td>
<td>4/46 (9)</td>
<td>0.04</td>
</tr>
<tr>
<td>Gestational diabetes mellitus, n (%)</td>
<td>2/38 (5)</td>
<td>4/46 (9)</td>
<td>0.5</td>
</tr>
<tr>
<td>Systolic BP, mm Hg, mean±SD</td>
<td>n/a</td>
<td>164±19</td>
<td>…</td>
</tr>
<tr>
<td>Diastolic BP (mm Hg), mean±SD</td>
<td>n/a</td>
<td>99±10</td>
<td>…</td>
</tr>
<tr>
<td>Renal dysfunction (Cr≥1.0 mg/dL), n (%)</td>
<td>n/a</td>
<td>10 (22)</td>
<td>…</td>
</tr>
<tr>
<td>Hepatic dysfunction (AST≥40 U/L), n (%)</td>
<td>n/a</td>
<td>13 (28)</td>
<td>…</td>
</tr>
<tr>
<td>Thrombocytopenia (platelet count,&lt;100000 cells/mm³), n (%)</td>
<td>n/a</td>
<td>5 (11)</td>
<td>…</td>
</tr>
<tr>
<td>Overall severe preeclampsia, n (%)</td>
<td>n/a</td>
<td>37 (80)</td>
<td>−</td>
</tr>
</tbody>
</table>

AST indicates aspartate transaminase; BMI, body mass index; BP, blood pressure; and Cr, creatinine.
Discussion

We have shown that women with preeclampsia harbor fetal cellular microchimerism more often and at higher concentrations than women with uncomplicated pregnancies. Strengths of our investigation include that it was conducted in a group of women with carefully defined preeclampsia in comparison with a thoroughly characterized group of women with healthy pregnancies and normal pregnancy outcomes. Maternal age, gestational age, and parity were accounted for in the analysis because these are important confounders. Our methodology, based on family genotyping and quantitative PCR–targeting of nonshared polymorphisms, is well established and allows for specific identification of fetoplacental cellular material and quantification of microchimerism sequestered in bone marrow or other tissues. In this study, samples were limited to peripheral blood; thereby, we were not able to detect microchimerism sequestered in bone marrow or other tissues.

Our investigation of cellular fetal microchimerism adds to a substantial literature on the transfer of fetal material and deportation of trophoblast to the maternal circulation in pregnancy. Schmorl first demonstrated transfer of fetoplacental material to the maternal lung in eclamptic pregnancies in 1893. Since then, the ubiquity of trophoblastic emboli to maternal lung has been demonstrated, and isolation of SNA from maternal peripheral blood has been accomplished using specific density preparations and antibody-based methods. Although efforts have focused on harnessing fetal–maternal transfer for the rapidly advancing field of noninvasive prenatal testing, it has been consistently clear that abnormalities in transplacental transfer are associated with adverse pregnancy outcomes, especially preeclampsia. Prior studies have shown that there is increased apoptosis within anchoring villi and among invading cytотrophoblast in preeclampsia. This is thought to result from cyclic hypoxia/reperfusion of the preeclampsia placenta that is caused by insufficient spiral artery remodeling, leading to persistent vasoreactivity. Maternal circulating concentrations of trophoblast-derived material, fetal erythroblasts, and cffDNA are higher in preeclampsia both during and preceding clinical disease. Detection and concentration of cffDNA correlate with disease severity, with higher concentrations in more clinically severe disease possibly reflecting the degree of underlying placental dysfunction.

In this study, we focused on fetoplacental cellular transfer to the maternal circulation by specifically investigating the PBMC compartment. It is likely that the PBMC compartment contains primarily mononuclear cells, especially leukocytes, as well as SNA or other trophoblast-derived material. The presence of SNA is of particular interest given recent work demonstrating that SNA in women with preeclampsia are transcriptionally active both in a placental explant model and in maternal lung. In addition, although the potential for trophoblast-derived material to persist in the maternal system is unknown, it is clear that the placenta may be an important

Table 2. Concentration of Cellular Fetal Microchimerism Among Subjects With Preeclampsia and Subjects With Normal Pregnancy

<table>
<thead>
<tr>
<th>Group</th>
<th>Concentration (gEq Per 100,000 Maternal gEq)</th>
<th>Detection Rate Ratio (95% CI; <em>P</em> Value)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Median Range Mean</td>
<td>Unadjusted</td>
</tr>
<tr>
<td>Normal pregnancy, n=47 subjects (57 samples)</td>
<td>0.0 0–9.1 0.3</td>
<td>...</td>
</tr>
<tr>
<td>Preeclampsia, n=46 subjects (53 samples)</td>
<td>0.0 0–153.7 5.7</td>
<td>17.4 (2.7–110.4; <em>P</em>=0.002)</td>
</tr>
</tbody>
</table>

CI indicates confidence interval; and gEq, genome equivalent.

*Adjusted for assay type, maternal age, and gestational age at sample collection.
source of hematopoietic stem cells. The other fetal component of the PBMC compartment, intact fetal mononuclear cells, is transferred to the maternal circulation late in gestation in normal pregnancy. In contrast to cffDNA and erythroblasts, which are rapidly cleared, intact fetal cells can be detected decades later and have been associated with later-life disease risk, including higher risk of some autoimmune disease and protection from some malignancy. The pluripotent potential and immunocompetence of transferred fetal cells may contribute to the heightened maternal inflammation and immune dysfunction seen in preeclampsia. The significance of cellular fetal microchimerism is underscored by the possibility that durable persistence of pluripotent fetal cells may contribute to long-term maternal health. Enduring allogeneic fetal cells could contribute to later-life maternal disease risks associated with preeclampsia, such as the development of cardiovascular and renal disease, or to protection from cancer. Perspectives

In summary, fetal–maternal cellular transfer is increased in preeclampsia compared with that in healthy pregnancies. From a clinical perspective, delineation of the mechanisms underlying fetoplacental cell transfer to the mother in complicated pregnancies may identify novel targets for intervention, both for benefit in immediate obstetric outcome and potentially for later-life health. Many questions remain, including whether cellular fetal microchimerism durably persists after preeclampsia, in the months and years postpartum. In addition, there may be phenotypic differences in the cells acquired in preeclampsia compared with those in uncomplicated pregnancies, both fetal and placental in origin. Delineation of the specific cell types involved in transplacental exchange in human pregnancy and longitudinal follow-up of women with preeclampsia will advance our understanding and direct future studies.

Acknowledgments

We acknowledge the women and their families who agreed to participate in this research.

Sources of Funding

This research was supported by National Institutes of Health grants HD01264, HD067221, AI45659, and AI41721 and the Preeclampsia Foundation.

Disclosures

None.

References


**Novelty and Significance**

**What Is New?**

- Previous studies have shown increased fetoplacental transfer to the mother in pregnancies complicated by preeclampsia compared with that in uncomplicated pregnancies. We have demonstrated higher overall concentrations of fetal cellular material in the maternal circulation applying a widely applicable and quantitative approach.

- Fetal–maternal cellular transfer follows a different pattern in healthy pregnancies than fetal DNA, has the capacity to generate durably persistent microchimerism, and has been associated with later-life disease risk.

**What Is Relevant?**

- The study examines fetal–maternal cell transfer in pregnancies complicated by preeclampsia compared with that in healthy pregnancies.

**Summary**

Transfer of fetal cellular material occurs more often and at higher concentrations in women with preeclampsia compared with women with uncomplicated pregnancies.
Cellular Fetal Microchimerism in Preeclampsia

Hilary S. Gammill, Tessa M. Aydelotte, Katherine A. Guthrie, Evangelyn C. Nkwopara and J. Lee Nelson

_Hypertension_, published online October 7, 2013;

_Hypertension_ is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
Copyright © 2013 American Heart Association, Inc. All rights reserved.
Print ISSN: 0194-911X. Online ISSN: 1524-4563

The online version of this article, along with updated information and services, is located on the World Wide Web at:

http://hyper.ahajournals.org/content/early/2013/10/07/HYPERTENSIONAHA.113.01486