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;62:1062-1067.)

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.2–5 For example, increased fetal microchimerism is associated with some autoimmune disease;6 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 deported trophoblast and syncytial nuclear aggregates (SNAs) is unknown. The maternal peripheral blood mononuclear cell (PBMC) compartment likely contains both. Microparticles and cell-free fetal DNA (cffDNA) also transfer to the maternal circulation during pregnancy.9 In contrast to fetal cellular microchimerism within the PBMC compartment, cffDNA and fetal erythroblasts are rapidly and definitively cleared from the maternal system postpartum.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. Preceding clinically evident preeclampsia, cffDNA is elevated and primarily derives from apoptotic or aponecrotic shedding of trophoblast material12. 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...
for ≥6 hours with proteinuria (defined as a timed urine collection with ≥300 mg of protein in 24 hours, a random urine sample with a protein/creatinine ratio of ≥0.5, or a urine dipstick assessment of ≥3). Severe preeclampsia was defined by the presence of any of the following: severe hypertension (systolic blood pressure, >160 or diastolic blood pressure, >110) persistent for 24 hours, seizures (eclampsia), hemolysis, elevated liver enzymes, thrombocytopenia, pulmonary edema, renal dysfunction, or fetal growth restriction. Subjects with chronic hypertension were included if a timed urine collection from early in pregnancy was available, and a 2-fold elevation in proteinuria was demonstrated in conjunction with worsening hypertension. All samples were drawn before the onset of labor.

Participants with uncomplicated pregnancy outcomes were derived from a population of healthy women with a singleton pregnancy followed longitudinally between November 1995 and December 2008 in Seattle, WA. After delivery, medical records and a self-reported questionnaire were collected and reviewed for clinical and demographic information. Information obtained included age, ethnicity, medical history, reproductive history, dating of the pregnancy, pregnancy complications, and maternal and fetal outcomes. History of transfusion was also queried. To ensure uncomplicated obstetric outcomes among the control population, previously established exclusionary criteria were applied after delivery. Specifically, among the controls, we excluded subjects with the following obstetric complications: preeclampsia, gestational hypertension, preterm birth (spontaneous or indicated), pregestational diabetes mellitus, placenta previa, placental abruption/antenatal bleeding, trauma, small for gestational age fetal size, and other complications. The absence of each of these complications was determined by the subject’s obstetrician. For all participants, gestational age was determined by the last menstrual period corroborated by a first or second trimester ultrasound or by the earliest ultrasound available. Longitudinal data on microchimerism (both fetal and maternal) in healthy pregnancy were previously reported in a subset of control participants in the current study.15,16

For participants with preeclampsia, maternal peripheral blood was drawn at the time of diagnosis before the onset of labor. Control participants underwent peripheral blood draws each trimester, and samples drawn during the latter half of pregnancy (20–42-week gestation, the same gestational age range at which women could be diagnosed with preeclampsia) and before the onset of labor, were included in this analysis. For both preeclampsia subjects and controls, fetal cord blood or a segment of cord tissue for genotyping was obtained immediately after delivery. All maternal and cord blood samples were drawn into acid citrate dextrose solution A-vacutainer tubes. PBMC were isolated from whole blood by Ficoll Histopaque (Pharmacia Biotech, Uppsala, Sweden) gradient centrifugation at a density of 1.077 g/mL, cryopreserved in dimethylsulfoxide, and stored in liquid nitrogen. Samples from controls and women with preeclampsia were processed and stored in an identical manner. Genomic DNA was extracted from PBMC or whole blood using Wizard Genomic DNA Purification Kits (Promega, Madison, WI) according to manufacturer’s instructions. DNA was extracted from cord tissue when cord blood was not available using QIAmp DNA Mini Kit (Qiagen, Hilden, Germany).

Because of the extensive polymorphism in human leukocyte antigen (HLA) genes, HLA genotyping of women and their fetuses usually results in identification of a polymorphism that is unique to the fetus and can then be targeted to identify and quantify fetal microchimerism. HLA genotyping was conducted using Dynal line strips (before January 2008) or Luminex-based (One Lambda, beginning January 2008) polymerase chain reaction (PCR)-sequence-specific oligonucleotide probe techniques. All maternal and fetal samples were HLA genotyped for the class II loci DRB1, DQA1, and DQB1. HLA relationships were then examined to identify nonshared HLA polymorphisms that could be used to identify fetal microchimerism. Because an HLA polymorphism unique to the fetus may not always be available for all families, genotyping for several other polymorphisms was also performed. Genotyping for these non-HLA loci used a conventional PCR system described previously.17 To assess similarity of results across the HLA-based and non–HLA-based assays, some samples were tested with each type of assay.

After identifying a polymorphism that was unique to the fetus, we used the appropriate assay from a panel of polymorphism-specific quantitative PCR assays that we developed for this purpose to test DNA extracted from maternal PBMC for 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 for the polymorphism-specific assay was included to quantify the amount of fetal microchimerism and to validate the assay for each experiment. Every sample was also tested for a nonpolymorphic gene, betaglobin. A betaglobin calibration curve (obtained from commercially prepared human genomic DNA [Promega]) was concurrently evaluated 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.19 Total DNA tested per sample was considered acceptable if ≥2×10^3 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 a 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 via t test for continuous factors. 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...
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 non-white. 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, 30.095) and 107.749 (SD, 28.093), 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/100,000 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 100,000 maternal cell equivalents, with a mean concentration of 5.7 gEq per 100,000 maternal cell equivalents. For the healthy control group, the range was 0 to 9.1 gEq per 100,000 maternal cell equivalents, with a mean concentration of 0.3 gEq per 100,000 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, 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

<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>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>Gestational 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;100,000 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.
Preeclampsia: Cellular Fetal Microchimerism

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 from the current pregnancy. Our study limitations include practical issues limiting the number of participants investigated and the number of cells tested for each participant. It is likely that our results represent an underestimation of the true prevalence of microchimerism. Microchimerism, by definition, occurs at very low concentrations. For feasibility reasons, we were able to test an aliquot of \( \approx 100,000 \) maternal PBMC. This stochastic sampling of a rare occurrence implies that microchimerism prevalence would be greater if larger amounts of maternal cell equivalents could be tested. In addition, some individuals may harbor microchimerism at concentrations below the lower limit (threshold) of detection of the assay, again leading to generalized underestimation. There is no reason to expect that this underestimation would vary by gestational age or across the groups of normal pregnancy and preeclampsia thus rendering comparisons appropriate. In addition, microchimerism may occur in any tissue. 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; ( P ) Value)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Median</td>
<td>Range</td>
</tr>
<tr>
<td>Normal pregnancy, ( n=47 ) subjects (57 samples)</td>
<td>0.0</td>
<td>0–9.1</td>
</tr>
<tr>
<td>Preeclampsia, ( n=46 ) subjects (53 samples)</td>
<td>0.0</td>
<td>0–153.7</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 cfDNA 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

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Disclosures

None.

References


**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?**

- Further understanding of fetal–maternal cell transfer in complicated pregnancies may enable identification of potential novel therapeutic targets to modulate later-life disease risks after preeclampsia, particularly cardiovascular disease.

**Summary**

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

**Novelty and Significance**

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