Urinary Placental Growth Factor and Risk of Preeclampsia

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Context Preeclampsia may be caused by an imbalance of angiogenic factors. We previously demonstrated that high serum levels of soluble fms-like tyrosine kinase 1 (sFlt1), an antiangiogenic protein, and low levels of placental growth factor (PIGF), a proangiogenic protein, predict subsequent development of preeclampsia. In the absence of glomerular disease leading to proteinuria, sFlt1 is too large a molecule to be filtered into the urine, while PIGF is readily filtered.

Objective To test the hypothesis that urinary PIGF is reduced prior to onset of hypertension and proteinuria and that this reduction predicts preeclampsia.

Design, Setting, and Patients Nestled case-control study within the Calcium for Preeclampsia Prevention trial of healthy nulliparous women enrolled at 5 US university medical centers during 1992-1995. Each woman with preeclampsia was matched to 1 normotensive control by enrollment site, gestational age at collection of the first serum specimen, and sample storage time at −70°C. One hundred twenty pairs of women were randomly chosen for analysis of serum and urine specimens obtained before labor.

Main Outcome Measure Cross-sectional urinary PIGF concentrations, before and after normalization for urinary creatinine.

Results Among normotensive controls, urinary PIGF increased during the first 2 trimesters, peaked at 29 to 32 weeks, and decreased thereafter. Among cases, before onset of preeclampsia the pattern of urinary PIGF was similar, but levels were significantly reduced beginning at 25 to 28 weeks. There were particularly large differences between controls and cases of preeclampsia with subsequent early onset of the disease or small-for-gestational-age infants. After onset of clinical disease, mean urinary PIGF in women with preeclampsia was 32 pg/mL, compared with 234 pg/mL in controls with fetuses of similar gestational age ($P < .001$). The adjusted odds ratio for the risk of preeclampsia to begin before 37 weeks of gestation for specimens obtained at 21 to 32 weeks, which were in the lowest quartile of control PIGF concentrations ($< 118$ pg/mL), compared with all other quartiles, was 22.5 (95% confidence interval, 7.4-67.8).

Conclusion Decreased urinary PIGF at mid gestation is strongly associated with subsequent early development of preeclampsia.

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tion with endothelial cell receptors and thereby inducing endothelial dysfunction. Administration of sFlt1 to rats results in hypertension, glomerular endotheliosis, and proteinuria, the hallmarks of preeclampsia. Among participants in the Calcium for Preeclampsia Prevention (CPEP) trial, we recently demonstrated that elevated serum concentrations of sFlt1 are evident approximately 5 weeks before the onset of clinical preeclampsia. Low serum concentrations of free PlGF, beginning at 13 to 16 weeks of gestation, and reduced free VEGF also antedated the clinical onset of gestational hypertension, glomerular endotheliosis, and proteinuria, the hallmarks of preeclampsia. We have demonstrated that alterations in circulating angiogenic factors in serum or urine, which is derived entirely from circulating blood, the major sources of urinary VEGF are cells of the kidney itself (glomerular podocytes and tubular cells); thus, urinary VEGF is unlikely to reflect the circulating angiogenic state. Therefore, we used archived urine samples to test the hypothesis that urinary PlGF is reduced well before the onset of hypertension and proteinuria and might predict preeclampsia.

**METHODS Participants and Specimen Collection**

The CPEP trial was a randomized, double-blind clinical trial conducted in 1992-1995 to evaluate the effects of daily supplementation with calcium or placebo on the incidence and severity of preeclampsia. A total of 4589 healthy nulliparous women with singleton pregnancies were enrolled between 13 and 21 weeks of gestation at 5 participating US medical centers and were followed up until 24 hours after delivery. Written informed consent was obtained from all participants. Subsequently, 326 women developed preeclampsia.

Serum and urine specimens were requested from participants before enrollment in the trial, at 26 to 29 weeks of gestation, at 36 weeks if they were still pregnant, and when hypertension or proteinuria was noted. Both first morning and 24-hour urine specimens were requested; if neither was available, a random or “spot” urine specimen was collected. Twenty-four-hour urine specimens were requested from patients in whom preeclampsia was suspected. Because the studies reported here used data and specimens that could not be linked to identifiable women, the Office of Human Subjects Research of the National Institutes of Health granted them exemptions from the requirement for review and approval by the institutional review board.

**Main Study.** For the present study, we selected women with complete outcome information, serum samples obtained at less than 22 weeks of gestation, and a live-born male infant. This group had previously been selected for a study of fetal DNA and preeclampsia, in which fetal and maternal DNA were differentiated through the amplification of a gene on the Y chromosome. Furthermore, we have demonstrated that alterations in circulating sFlt1 and PlGF antedate clinical preeclampsia in these patients. Analysis of previous work revealed no significant differences in maternal serum sFlt1 or PlGF concentrations according to infant sex.

Of the 4589 women enrolled in the CPEP trial, we excluded 253 who were lost to follow-up, 21 whose pregnancy ended before 20 weeks, 13 who had missing data on maternal or perinatal outcomes, 4 who had no data on smoking history, 9 in whom the presence of hypertension had not been verified by the team that reviewed each chart, and 32 others who had a stillbirth, leaving 4257 women. Of these women, 2156 had a male infant. After exclusion of 1 woman whose infant had a chromosomal abnormality, 381 women with gestational hypertension, and 43 without a baseline serum specimen, 1731 women remained. Preeclampsia developed in 175 of these women, whereas 1556 remained normotensive during pregnancy.

Calcium supplementation did not affect urinary levels of PlGF. Specimens collected at 8 to 20 weeks of gestation were considered the baseline specimens and were obtained before the administration of calcium or placebo. At 21 to 32 weeks, mean concentrations of PlGF were 223 vs 228 pg/mL (P = .63) in women receiving placebo vs calcium, respectively; at 33 to 42 weeks, these concentrations were 187 vs 166 pg/mL (P = .53). Similarly, at 21 to 32 weeks, mean levels of PlGF per milligram of creatinine were 226 vs 219 pg/mg (P = .66) and at 33 to 42 weeks were 222 vs 178 pg/mg (P = .62).

Since calcium supplementation had no effect on the risk or severity of preeclampsia or on the concentrations of angiogenic factors in serum or urine, women were chosen without regard to whether they had received calcium supplementation or placebo. For each woman with preeclampsia, 1 normotensive control was selected, matched according to enrollment site, gestational age at the collection of the first serum specimen (within 1 week), and storage time of the samples at –70°C (within 12 months). A total of 120 of 159 matched pairs were randomly chosen for analysis of all serum and urine specimens obtained before labor or delivery. If a woman had more than 1 urine specimen obtained on the same day, we selected 1 specimen, preferring first morning to random and random to 24-hour specimens. We identified 348 urine specimens from 120 preeclampsia cases and 318 urine specimens from 118 normotensive controls. Two normotensive controls from the serum study had no eligible urine specimens and were excluded from further analyses. Of the 238 women in the urine
specimen study, 26 (10.9%) contributed 1 urine specimen, 55 (23.1%) contributed 2 specimens, 111 (46.6%) contributed 3, 35 (14.7%) contributed 4, 10 (4.2%) contributed 5, and 1 (0.4%) contributed 7.

For all controls and cases with onset of preeclampsia before term (<37 weeks), we examined separately samples of urine obtained at 21 to 32 weeks of gestation for which a serum specimen from the same woman had been collected within 3 days of the urine specimen (mean difference, 0.5 days). Among the 90 resulting pairs of urine-serum specimens, 2 were from the same woman; for this woman, we included only the pair closest to the mid point of the gestational age interval. A total of 89 urine-serum specimen pairs remained from 20 cases of preterm preeclampsia and 69 normotensive controls.

Ancillary Study. We performed an ancillary study to ascertain whether urinary PlGF at 21 to 32 weeks of gestation might differ between women with male or female infants and to determine if concentrations of urinary PlGF might be lower than normal in women with gestational hypertension and in women who remained normotensive during pregnancy but who delivered a small-for-gestational-age (SGA) infant. Among the 4256 women in the CPEP trial with adequate data who delivered a live-born infant not known to have a chromosomal abnormality, we excluded 239 with term preeclampsia (≥37 weeks). Of the 4017 women remaining, 3303 had at least 1 urine specimen obtained at 21 to 32 weeks of gestation before onset of labor or delivery and before onset of preeclampsia or gestational hypertension. Among these women, we randomly selected 120 whose pregnancy was normotensive and whose infant was not SGA, 60 with normotensive pregnancy who delivered an SGA infant, 60 with gestational hypertension, and 59 with preterm (<37 weeks) preeclampsia. In each group, we chose half the women who delivered male infants and half who delivered female infants, except for the group with preterm preeclampsia.

In this group, we selected 30 with male infants but could find only 29 with female infants. Placental growth factor was analyzed in all urine specimens obtained at 21 to 32 weeks of gestation.

### Preeclampsia, Gestational Hypertension, and SGA Infants

Preeclampsia was defined as a newly elevated diastolic blood pressure of at least 90 mm Hg and proteinuria of at least 1+ (30 mg/dL) on dipstick testing, each on 2 occasions 4 to 168 hours apart. Severe preeclampsia was defined as the HELLP syndrome (hemolysis, elevated liver enzyme levels, and a low platelet count), eclampsia, or preeclampsia with either severe hypertension (diastolic blood pressure ≥110 mm Hg) or severe proteinuria (urinary protein excretion ≥3.5 g per 24 hours or findings of ≥3+ [300 mg/dL] on dipstick testing). Gestational hypertension was hypertension as defined herein in the absence of proteinuria. Detailed definitions have been published.14,15 The time of onset of preeclampsia was defined as the time of the first elevated blood pressure or urine protein measurement leading to diagnosis of preeclampsia. Similarly, onset of gestational hypertension was the time of the first elevated blood pressure measurement that led to diagnosis. An SGA infant was defined as an infant whose birth weight was below the 10th percentile according to US tables of birth weight for gestational age that accounted for race, parity, and infant sex.10

### Procedures

Assays were performed by personnel who were unaware of pregnancy outcomes. Specimens were randomly ordered for analysis. Enzyme-linked immunosorbent assays for sFlt1, free PlGF, and free VEGF were performed in duplicate, as previously described, with the use of commercial kits (R&D Systems, Minneapolis, Minn.). The minimum detectable doses in the assays for sFlt1, PlGF, and VEGF were 5, 7, and 5 pg/mL, respectively, with interassay and intra-assay coefficients of variation of 7.6% and 3.3%, respectively, for sFlt1; 10.9% and 5.6% for PlGF; and 7.3% and 5.4% for VEGF. The enzyme-linked immunosorbent assay kits for sFlt1, VEGF, and PlGF were validated for use in urine specimens with 96%, 98%, and 99% recovery from spiked urine samples, respectively. Urinary creatinine was measured using a commercially available picric acid colorimetric assay (Metrac creatinine assay kit, Quidel Corp, San Diego, Calif).

### Statistical Analysis

The χ² test was used for comparison of categorical variables and the t test for comparison of continuous variables. Although arithmetic mean concentrations are reported in the text and figures, statistical testing was conducted within each time interval individually after logarithmic transformation, using the generalized estimating equations method (SAS/PROC GENMOD procedure; SAS, version 8.0, SAS Institute Inc, Cary, NC) in crude and adjusted analyses to account for patients with varying numbers of specimens. Odds ratios (ORs) were adjusted with the use of logistic regression analysis. Since matching was complete only for
analyses of the earliest serum specimens in the entire study population, matching was not accounted for in the statistical analyses. For all analyses, \( P < .05 \) was considered statistically significant.

**RESULTS**

**Main Study**

Characteristics of the Women. Of the 120 women with preeclampsia, 80 had mild and 40 had severe disease. Compared with controls, women with preeclampsia had greater body mass index (\( P = .007 \)), higher systolic and diastolic blood pressure at enrollment in the CPEP trial (\( P = .001 \) and .006, respectively), and larger proportions of their current pregnancies complicated by preterm delivery (\( P = .002 \)) or resulting in SGA infants (\( P = .002 \)). Patient and infant characteristics have been described previously and are briefly summarized in Table 1.

**Differences in Urinary PlGF After Onset of Preeclampsia.** We first ascertained that urinary levels of PlGF were altered in women after development of clinical preeclampsia. Among 22 pairs of women with preeclampsia and gestational age–matched controls, specimens of urine obtained after onset of clinical disease had lower levels of PlGF than specimens from controls (mean PlGF level, 32 vs 234 pg/mL; \( P < .001 \) and 50 vs 227 pg/mg of creatinine; \( P < .001 \)).

**Gestational Changes in Urinary PlGF.** To evaluate gestational patterns, we performed cross-sectional analyses of urine obtained within gestational age intervals of 4 to 5 weeks, with PlGF levels expressed as concentrations (Figure 1A) or as picograms per milligram of creatinine. Patterns expressed as concentrations and picograms per milligram of creatinine were similar because mean urinary creatinine concentrations within gestational age intervals did not differ significantly between cases and controls. The PlGF levels in controls increased during the first 2 trimesters, with a more rapid increase after 21 to 24 weeks, reaching a peak at 29 to 32 weeks and

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**Figure 1. Urinary PlGF by Intervals of Gestational Age**

A, Mean urinary placental growth factor (PlGF) concentrations in normotensive women (controls) and in women (cases) before onset and after onset (active preeclampsia [PE]) of clinical PE, according to gestational age. Also shown for women who subsequently developed PE (cases) are the mean urinary concentrations of PlGF after excluding specimens obtained within 5 weeks before onset of PE (open circles). Error bars represent SEs. \( P \) values for the comparisons between specimens from cases before the onset of PE and specimens from controls obtained during the same gestational age interval, after logarithmic transformation and accounting for patients with varying numbers of specimens, were significant at 25 to 28 weeks (\( P < .001 \)), 29 to 32 weeks (\( P = .002 \)), and 33 to 36 weeks (\( P = .005 \)). Comparisons between controls and cases more than 5 weeks before onset of PE were also significant at 25 to 28 weeks (\( P < .005 \)) and 29 to 32 weeks (\( P = .02 \)). The comparisons between specimens obtained from women with active PE and from controls were significant at 29 to 32 weeks (\( P < .005 \)), 33 to 36 weeks (\( P < .001 \)), and 37 to 42 weeks (\( P < .001 \)). The comparisons between specimens obtained from women with active PE and from women in whom PE later developed were also significant at 29 to 32 weeks (\( P < .001 \)), 33 to 36 weeks (\( P < .001 \)), and 37 to 42 weeks (\( P = .003 \)). Note that PlGF concentrations before onset of PE do not include specimens obtained after appearance of hypertension or proteinuria (active PE). Mean urinary creatinine concentrations between cases and controls were not significantly different for the various gestational windows (171 vs 147 mg/dL at 8-12 weeks, 136 vs 139 mg/dL at 13-16 weeks, 118 vs 112 mg/dL at 17-20 weeks, 109 vs 102 mg/dL at 21-24 weeks, 104 vs 117 mg/dL at 25-28 weeks, 110 vs 129 mg/dL at 29-32 weeks, 95 vs 98 mg/dL at 33-36 weeks, and 108 vs 109 mg/dL at 37-42 weeks). B, Mean urinary PlGF concentrations in normotensive women (controls) and in women (cases) before onset and after onset (active PE) of clinical PE according to gestational age, using only first morning urine specimens. Error bars represent SEs. \( P \) values for the comparisons between specimens from cases before the onset of PE and specimens from controls obtained during the same gestational age interval were significant at 25 to 28 weeks (\( P = .002 \)) and at 33 to 36 weeks (\( P = .02 \)). The comparisons between specimens obtained from women with active PE and specimens obtained from controls were significant at 29 to 32 weeks (\( P < .001 \)), and 33 to 36 weeks (\( P = .006 \)). The comparisons between specimens obtained from women with active PE and from those in whom PE later developed were also significant at 29 to 32 weeks (\( P = .003 \)). C, Mean PlGF concentrations before and after onset of clinical PE, using only random urine specimens. Error bars represent SEs. \( P \) values for the comparisons between specimens from cases before the onset of PE and specimens from controls obtained during the same gestational age interval were significant at 29 to 32 weeks (\( P = .01 \)) and at 33 to 36 weeks (\( P = .02 \)). The comparisons between specimens obtained from women with active PE and specimens obtained from controls were significant at 29 to 32 weeks (\( P < .001 \)), 33 to 36 weeks (\( P < .001 \)), and 37 to 42 weeks (\( P = .05 \)). The comparisons between specimens obtained from women with active PE and specimens obtained from women in whom PE later developed were also significant at 29 to 32 weeks (\( P < .001 \)) and at 33 to 36 weeks (\( P < .002 \)).
decreasing thereafter. The levels in women who subsequently developed preeclampsia followed a similar pattern but were significantly lower at 25 to 28, 29 to 32, and 33 to 36 weeks. When specimens obtained within 5 weeks before the onset of preeclampsia were excluded, the differences in the preceding gestational age intervals between controls and women who later had preeclampsia were less pronounced. Among women with specimens obtained in the same gestational age interval, those who already had clinical preeclampsia had significantly lower concentrations at 29 to 32, 33 to 36, and 37 to 42 weeks than those who developed preeclampsia later. Similar gestational age patterns among controls and cases before and after onset of clinical preeclampsia were observed when restricting the analysis of specimens to either first morning (Figure 1B) or random (Figure 1C) urine specimens.

**Relationship of Urinary PlGF and Severity of Preeclampsia.** Before the onset of preeclampsia, there were particularly large differences between the levels of urinary PlGF in controls and those in women who later had preeclampsia with onset before 37 weeks or who had preeclampsia and an SGA infant. Figure 2 shows PlGF concentrations and PlGF expressed as picograms per milligram of creatinine in urine obtained at 21 to 32 weeks of gestation.

Alterations in urinary PlGF levels were also more pronounced in women who subsequently developed preeclampsia before term (<37 weeks of gestation) than in women who had an onset of preeclampsia at term (>37 weeks) (at 21-32 weeks, 87 pg/mL in women with preeclampsia before term vs 223 pg/mL in women with preeclampsia at term; P < .001; at 33-42 weeks, 22 pg/mL in women with preeclampsia before term vs 118 pg/mL in women with preeclampsia at term; P < .001). Results were similar when using PlGF expressed as picograms per milligram of creatinine or after adjusting PlGF concentrations for creatinine, gestational age at specimen collection, storage time, body mass index, and maternal age. Furthermore, PlGF levels in specimens of urine obtained before onset of preeclampsia from women who later had preeclampsia and an SGA infant were lower than in women who later had preeclampsia but whose infants were not SGA (at 21-32 weeks, 62 vs 205 pg/mL; P = .002; at 33-42 weeks, 42 vs 123 pg/mL; P = .06).

**Odds Ratios for Preeclampsia Associated With Urinary PlGF.** To determine the risk of preeclampsia according to urinary PlGF in specimens obtained before the onset of clinical signs, we divided PlGF values into quartiles based on the distribution in controls and calculated adjusted ORs for preeclampsia in each quartile, compared with the highest quartile (Table 2) or with all other quartiles. Among specimens obtained at 21 to 32 weeks of gestation, the lowest quartile of PlGF was associated with a greatly increased risk of preterm preeclampsia and a small increased risk of preeclampsia at term. For preterm preeclampsia, after adjustment for gestational age at specimen collection, storage time, body mass index, and age, using PlGF concentration, the OR for the lowest quartile vs all others was 22.5 (95% confidence interval [CI], 7.4-67.8); using picograms of PlGF per milligram of creatinine, the OR was 16.4 (95% CI, 5.9-45.5). After restricting specimens to first morning urine, adjusted ORs were 39.5 (95% CI, 6.5-240.8) and 20.4 (95% CI, 4.5-92.3) for PlGF concentration and picograms of PlGF per milligram of creatinine, respectively. Using random urine specimens, adjusted ORs were 13.5 (95% CI, 2.3-79.8) and 11.1 (95% CI, 2.0-61.3), respectively. On average, urine specimens obtained at 21 to 32 weeks of gestation from women who developed preeclampsia before 37 weeks were collected 46 days prior to the onset of clinical disease.

For term preeclampsia, after adjustment for the factors noted herein and using all urine specimens obtained at 21 to 32 weeks, ORs were 2.2 (95% CI, 1.2-4.3) and 2.1 (95% CI, 1.1-4.1), respectively, for the lowest quartile vs all other quartiles. The lowest quartile of PlGF was also associated with an increased risk of term preeclampsia vs all other quartiles in specimens obtained at 33 to 42 weeks of gestation (adjusted OR, 2.3; 95% CI, 1.2-4.5 for picograms of PlGF per milligram of creatinine).

When we performed the same analyses in specimens obtained at 21 to 32 weeks of gestation for women who developed preeclampsia during a pregnancy complicated by an SGA infant,
we found that the estimates were unstable (adjusted OR, 405; 95% CI, 27-5983 for picograms of PlGF per milligram of creatinine). This was because there were only 20 such women, all of whom were in the lowest (n = 19) or next lowest (n = 1) quartiles of urinary PlGF. Nevertheless, the data indicate that low urinary PlGF is associated with a substantial increase in risk for pre-eclampsia with an SGA infant.

**Relationship of Urinary PlGF to Proximity to Preeclampsia**. Urinary concentrations of PlGF in specimens obtained at 21 to 32 weeks of gestation and within 5 weeks before the onset of preeclampsia were lower (43 pg/mL) than in specimens obtained more than 5 weeks before clinical disease (196 pg/mL; P < .001). In specimens obtained at 33 to 42 weeks of gestation, concentrations were 110 pg/mL vs 187 pg/mL, respectively (P = .05). There was little difference when PlGF was normalized for creatinine. Figure 3A is a scatter plot of urinary PlGF concentrations at 21 to 32 weeks from all 69 controls and all 20 cases who subsequently developed preeclampsia before term (<37 weeks) and who had a serum specimen obtained within 3 days of the urine specimen (mean difference, 0.5 days). Women who developed preeclampsia before term had lower urinary PlGF concentrations than normotensive controls. Concentrations were lowest (all <150 pg/mL) in specimens obtained within 3 weeks before the onset of clinical disease. However, a number of control specimens also had low urinary PlGF.

**Ancillary Study**

To further test the hypothesis that decreased urinary PlGF is specific for early-onset preeclampsia, we performed a second study in which we analyzed urine specimens obtained at 21 to 32 weeks from women with other obstetrical conditions that may share similarities of pathogenesis. We compared women with gestational hypertension

**Table 2. Odds Ratios for Preeclampsia at Less Than 37 Weeks of Gestation and at 37 Weeks or More of Gestation According to Quartile of Urinary PlGF**

<table>
<thead>
<tr>
<th>Quartile</th>
<th>Control Specimens, No.</th>
<th>Preeclampsia &lt; 37 wk</th>
<th>Preeclampsia ≥ 37 wk</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>PIGF, pg/mL</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>13-20 wk</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1: ≤ 29</td>
<td>25</td>
<td>6</td>
<td>0.6 (0.2-2.4)</td>
</tr>
<tr>
<td>2: 29-59</td>
<td>24</td>
<td>12</td>
<td>1.3 (0.4-4.3)</td>
</tr>
<tr>
<td>3: 59-88</td>
<td>24</td>
<td>5</td>
<td>0.7 (0.2-2.7)</td>
</tr>
<tr>
<td>4: &gt; 88</td>
<td>24</td>
<td>6</td>
<td>1.0</td>
</tr>
<tr>
<td>21-32 wk</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1: ≤ 118</td>
<td>29</td>
<td>30</td>
<td>3.1 (5.6-17.4)</td>
</tr>
<tr>
<td>2: 118-230</td>
<td>29</td>
<td>4</td>
<td>2.6 (0.4-16.8)</td>
</tr>
<tr>
<td>3: 230-309</td>
<td>29</td>
<td>1</td>
<td>0.6 (0.1-7.5)</td>
</tr>
<tr>
<td>4: &gt; 309</td>
<td>29</td>
<td>2</td>
<td>1.0</td>
</tr>
<tr>
<td>33-42 wk</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1: ≤ 55</td>
<td>25</td>
<td>2</td>
<td>NA</td>
</tr>
<tr>
<td>2: 55-113</td>
<td>25</td>
<td>0</td>
<td>NA</td>
</tr>
<tr>
<td>3: 113-318</td>
<td>25</td>
<td>0</td>
<td>NA</td>
</tr>
<tr>
<td>4: &gt; 318</td>
<td>24</td>
<td>0</td>
<td>NA</td>
</tr>
<tr>
<td><strong>PIGF/creatinine, pg/mg</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>13-20 wk</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1: ≤ 26</td>
<td>25</td>
<td>8</td>
<td>0.5 (0.1-2.2)</td>
</tr>
<tr>
<td>2: 26-52</td>
<td>24</td>
<td>9</td>
<td>0.7 (0.2-3.0)</td>
</tr>
<tr>
<td>3: 52-78</td>
<td>24</td>
<td>5</td>
<td>0.4 (0.1-1.8)</td>
</tr>
<tr>
<td>4: &gt; 78</td>
<td>24</td>
<td>7</td>
<td>1.0</td>
</tr>
<tr>
<td>21-32 wk</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1: ≤ 120</td>
<td>29</td>
<td>29</td>
<td>15.4 (3.7-64.3)</td>
</tr>
<tr>
<td>2: 120-180</td>
<td>29</td>
<td>2</td>
<td>0.9 (0.1-6.1)</td>
</tr>
<tr>
<td>3: 180-323</td>
<td>29</td>
<td>3</td>
<td>0.9 (0.2-5.1)</td>
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<tr>
<td>4: &gt; 323</td>
<td>29</td>
<td>3</td>
<td>1.0</td>
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<tr>
<td>33-42 wk</td>
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<td></td>
</tr>
<tr>
<td>1: ≤ 69</td>
<td>24</td>
<td>2</td>
<td>NA</td>
</tr>
<tr>
<td>2: 69-153</td>
<td>25</td>
<td>0</td>
<td>NA</td>
</tr>
<tr>
<td>3: 153-268</td>
<td>25</td>
<td>0</td>
<td>NA</td>
</tr>
<tr>
<td>4: &gt; 268</td>
<td>24</td>
<td>0</td>
<td>NA</td>
</tr>
</tbody>
</table>

Abbreviations: CI, confidence interval; NA, not applicable; OR, odds ratio; PlGF, placental growth factor.

*Quartiles were determined on the basis of control specimens.
†Specimens from cases were all obtained before onset of clinical signs of preeclampsia.
‡Odds ratios were adjusted for gestational age at specimen collection, specimen storage time, maternal age, and body mass index. The reference category was the highest quartile, Q4. Adjusted odds ratios and 95% CIs for comparisons of Q1 vs Q2, Q3, or Q4 are given in the “Results” section of the text.
and women who remained normotensive during pregnancy but delivered an SGA infant were least likely to have smoked during pregnancy. 

Figure 4 depicts urinary PlGF at 21 to 32 weeks of gestation, expressed as concentrations and as picograms per milligram of creatinine. Placental growth factor levels in women who remained normotensive whose infants were not SGA, women with gestational hypertension had greater body mass index and infants of greater birth weight and normotensive women with an SGA infant had lower body mass index and infants of lower birth weight. Normotensive women with SGA infants were most likely and women with hypertensive disorders of pregnancy were least likely to have smoked during pregnancy.

Figure 4 shows the scatter plots of urinary placental growth factor (PlGF) concentrations and ratios of soluble fms-like tyrosine kinase-1 (sFlt1) to PlGF in serum at 21 to 32 weeks by days of gestation. Measurements were obtained from paired urine and serum specimens obtained from 20 women before development of preeclampsia at less than 37 weeks of gestation and from 69 normotensive controls.

### Table 3. Baseline Characteristics of CPEP Ancillary Study Women and Their Infants

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>Normal Blood Pressure, Not SGA (n = 120)</th>
<th>Normal Blood Pressure and SGA (n = 59)</th>
<th>P Value†</th>
<th>Gestational Hypertension (n = 60)</th>
<th>P Value†</th>
<th>Preeclampsia ≤37 wk (n = 59)</th>
<th>P Value†</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age, mean (SD), y</td>
<td>21.8 (4.6)</td>
<td>21.3 (4.9)</td>
<td>.49</td>
<td>22.2 (5.3)</td>
<td>.61</td>
<td>21.1 (4.7)</td>
<td>.30</td>
</tr>
<tr>
<td>Body mass index, mean (SD)‡</td>
<td>25.8 (6.1)</td>
<td>22.8 (3.6)</td>
<td>&lt;.001</td>
<td>28.3 (7.4)</td>
<td>.02</td>
<td>27.6 (6.9)</td>
<td>.08</td>
</tr>
<tr>
<td>Blood pressure, mean (SD), mm Hg</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Systolic</td>
<td>106 (9)</td>
<td>106 (8)</td>
<td>.84</td>
<td>108 (9)</td>
<td>.24</td>
<td>111 (8)</td>
<td>.001</td>
</tr>
<tr>
<td>Diastolic</td>
<td>60 (7)</td>
<td>60 (8)</td>
<td>.84</td>
<td>62 (9)</td>
<td>.16</td>
<td>65 (7)</td>
<td>&lt;.001</td>
</tr>
<tr>
<td>Gestational age at delivery, mean (SD), wk</td>
<td>39.0 (1.8)</td>
<td>38.7 (1.4)</td>
<td>.18</td>
<td>39.6 (1.7)</td>
<td>.06</td>
<td>34.6 (2.3)</td>
<td>&lt;.001</td>
</tr>
<tr>
<td>Current smoker</td>
<td>15 (12.5)</td>
<td>13 (21.7)</td>
<td>.11</td>
<td>3 (5.0)</td>
<td>.11</td>
<td>4 (6.8)</td>
<td>.24</td>
</tr>
<tr>
<td>Ever married</td>
<td>34 (28.3)</td>
<td>16 (26.7)</td>
<td>.81</td>
<td>15 (26.4)</td>
<td>.68</td>
<td>16 (27.1)</td>
<td>.86</td>
</tr>
<tr>
<td>Race/ethnicity§</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>White, non-Hispanic</td>
<td>46 (38.3)</td>
<td>20 (33.3)</td>
<td></td>
<td>20 (33.3)</td>
<td></td>
<td>16 (27.1)</td>
<td></td>
</tr>
<tr>
<td>White, Hispanic</td>
<td>16 (13.3)</td>
<td>16 (26.7)</td>
<td></td>
<td>6 (10.0)</td>
<td></td>
<td>10 (17.0)</td>
<td></td>
</tr>
<tr>
<td>African American</td>
<td>55 (45.8)</td>
<td>24 (40.0)</td>
<td></td>
<td>33 (55.0)</td>
<td></td>
<td>30 (50.9)</td>
<td></td>
</tr>
<tr>
<td>Other/unknown</td>
<td>3 (2.5)</td>
<td>0</td>
<td></td>
<td>1 (1.7)</td>
<td></td>
<td>3 (5.1)</td>
<td>.43</td>
</tr>
<tr>
<td>Birth weight, mean (SD), g</td>
<td>3273 (466)</td>
<td>2538 (278)</td>
<td>&lt;.001</td>
<td>3437 (559)</td>
<td>.04</td>
<td>2193 (726)</td>
<td>&lt;.001</td>
</tr>
<tr>
<td>Delivery &lt;37 wk</td>
<td>13 (10.8)</td>
<td>6 (10.0)</td>
<td>.86</td>
<td>3 (5.0)</td>
<td>.19</td>
<td>50 (84.8)</td>
<td>&lt;.001</td>
</tr>
<tr>
<td>SGA (&lt;10th percentile)</td>
<td>0</td>
<td>60 (100)</td>
<td>&lt;.001</td>
<td>2 (3.3)</td>
<td>.11</td>
<td>18 (30.5)</td>
<td>&lt;.001</td>
</tr>
</tbody>
</table>

**Abbreviations:** CPEP, Calcium for Preeclampsia Prevention trial; SGA, small for gestational age.

*Data are expressed as No. (%) unless otherwise noted.

†P values for difference vs normal blood pressure, not SGA.

‡Body mass index was calculated as weight in kilograms divided by the square of height in meters.

§Racial or ethnic group was self-reported.

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Urinary placental growth factor (PIGF) concentrations in picograms per milliliter and in picograms of PIGF per milligram of creatinine are shown at 21 to 32 weeks of gestation in normotensive (NT) women whose infants were not born small for gestational age (SGA). NT women with SGA infants, women who subsequently developed gestational hypertension (GH), and women who subsequently developed preeclampsia (PE) before 37 weeks of gestation. Specimens from women in whom GH or PE developed were obtained before onset of clinical disease. The mean gestational age at specimen collection was similar in all groups. The P values given are for comparisons with specimens from controls (NT without SGA). Error bars represent SEs.

Figure 4. Mean Urinary Concentrations of PIGF in Normotensive Women With Infants Not Born SGA, Normotensive Women With SGA Infants, Women in Whom GH Developed, and Women in Whom PE Developed Before 37 Weeks of Gestation

Urinary placental growth factor (PIGF) concentrations in picograms per milliliter and in picograms of PIGF per milligram of creatinine are shown at 21 to 32 weeks of gestation in normotensive (NT) women whose infants were not born small for gestational age (SGA). NT women with SGA infants, women who subsequently developed gestational hypertension (GH), and women who subsequently developed preeclampsia (PE) before 37 weeks of gestation. Specimens from women in whom GH or PE developed were obtained before onset of clinical disease. The mean gestational age at specimen collection was similar in all groups. The P values given are for comparisons with specimens from controls (NT without SGA). Error bars represent SEs.

The identification of angiogenic proteins that appear to mediate the maternal syndrome of preeclampsia may present specific targets for therapeutic intervention to restore angiogenic balance. Prevention and treatment are especially needed for women with preeclampsia of early onset or complicated by an SGA infant. However, such women must first be identified before the onset of clinical disease. If a reliable and valid urinary dipstick assay can be developed, one scenario might be to screen all women for low urinary PIGF and serum to search for trends, as could be done in clinical practice. Finally, we did not determine whether there are alterations in urinary PIGF throughout gestation in obstetric conditions with similarities to preeclampsia, such as gestational hypertension or pregnancy complicated by an SGA infant. Nevertheless, our data suggest that at a time when alterations in urinary PIGF are dramatic in women who will develop early-onset preeclampsia, normotensive women who subsequently develop gestational hypertension or deliver an SGA infant have none.

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Author Contributions: Drs Levine and Karumanchi had full access to all of the data in the study and take responsibility for the integrity of the data and the accuracy of the data analysis.

Study concept and design: Levine, Thadhani, Karumanchi.

Acquisition of data: Levine, Lam, Sibai, Karumanchi.

Analysis and interpretation of data: Levine, Thadhani, Qian, Lam, Lim, Yu, Blink, Sachs, Epstein, Sukhatme, Karumanchi.

Drafting of the manuscript: Levine, Thadhani, Epstein, Karumanchi.

Critical revision of the manuscript for important intellectual content: Levine, Thadhani, Qian, Lam, Lim, Yu, Blink, Sachs, Epstein, Sibai, Sukhatme, Karumanchi.

Statistical analysis: Qian, Yu.

Obtained funding: Levine, Karumanchi.

Administrative, technical, or material support: Yu, Blink, Sachs, Epstein, Sibai, Sukhatme, Karumanchi.

Study concept and design: Levine, Thadhani, Epstein, Sibai, Sukhatme, Karumanchi.

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REFERENCES