

Urinary Placental Growth Factor and Risk of Preeclampsia

Richard J. Levine, MD, MPH

Ravi Thadhani, MD, MPH

Cong Qian, MS

Chun Lam, MD

Kee-Hak Lim, MD

Kai F. Yu, PhD

Anastasia L. Blink, MPH

Benjamin P. Sachs, MD, DPH

Franklin H. Epstein, MD

Baha M. Sibai, MD

Vikas P. Sukhatme, MD, PhD

S. Ananth Karumanchi, MD

PREECLAMPSIA IS A COMMON hypertensive disorder of pregnancy characterized by systemic endothelial dysfunction and diagnosed by the appearance of hypertension and proteinuria.^{1,2} For this reason, it is recommended that women undergo blood pressure and urinary protein screening at each prenatal visit throughout gestation.³ Potentially life-threatening complications of preeclampsia include seizures, cerebral hemorrhage, disseminated intravascular coagulation, and renal failure; and the time between the first detection of hypertension and proteinuria and the subsequent development of these complications can be extremely short.^{3,4} The only known cure for preeclampsia is delivery of the placenta. If maternal signs develop before the fetus is mature, the risk of neonatal morbidity and mortality due to premature delivery is markedly increased.

Evidence from our group and others suggests that preeclampsia may be caused by an imbalance of angiogenic factors.⁵⁻¹² Circulating soluble fms-

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 (PlGF), 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 PlGF is readily filtered.

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

Design, Setting, and Patients Nested 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 PlGF concentrations, before and after normalization for urinary creatinine.

Results Among normotensive controls, urinary PlGF 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 PlGF 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 PlGF 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 PlGF concentrations (<118 pg/mL), compared with all other quartiles, was 22.5 (95% confidence interval, 7.4-67.8).

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

JAMA. 2005;293:77-85

www.jama.com

like tyrosine kinase 1 (sFlt1, also referred to as sVEGFR1), an antiangiogenic protein, binds the proangiogenic

proteins, vascular endothelial growth factor (VEGF) and placental growth factor (PlGF), preventing their interac-

Author Affiliations: Division of Epidemiology, Statistics, and Prevention Research, National Institute of Child Health and Human Development, Department of Health and Human Services, Bethesda, Md (Drs Levine and Yu and Ms Blink); Departments of Medicine and Obstetrics, Massachusetts General Hospital, and Harvard Medical School (Dr Thadhani), Renal Division, Department of Medicine, Beth Israel Deaconess Medical Center and Harvard Medical School (Drs Lam, Epstein, Sukhatme, and Karumanchi), and Department of Obstetrics and Gynecology, Beth Israel Deaconess Medical Center, Harvard Medical School, and Harvard School of Public Health (Drs Lim, Sachs, and

Karumanchi), Boston, Mass; Allied Technology Group, Rockville, Md (Mr Qian); and Department of Obstetrics and Gynecology, University of Cincinnati College of Medicine, Cincinnati, Ohio (Dr Sibai).

Financial Disclosures: Drs Sukhatme and Karumanchi are named as coinventors on a pending patent filed by Beth Israel Deaconess Medical Center for the use of angiogenesis-related proteins for diagnosis and treatment of preeclampsia.

Corresponding Author: S. Ananth Karumanchi, MD, Beth Israel Deaconess Medical Center, Renal Division, Dana 517, 330 Brookline Ave, Boston, MA 02215 (sananth@bidmc.harvard.edu).

tion with endothelial cell receptors and thereby inducing endothelial dysfunction.¹³ Administration of sFlt1 to rats results in hypertension, glomerular endotheliosis, and proteinuria, the hallmarks of preeclampsia.⁶ Among participants in the Calcium for Preeclampsia Prevention (CPEP) trial,¹⁴ 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 signs of preeclampsia.⁵

Although longitudinal measures of these angiogenic mediators in serum might be ideal for ascertaining the risk of preeclampsia, obtaining such measurements during routine prenatal care could be challenging. An alternative and less invasive screening method may be to measure these proteins in urine. Although sFlt1 is too large a molecule (≈ 100 kDa) to be filtered into urine in the absence of renal damage, PlGF and VEGF, much smaller proteins (≈ 30 kDa and ≈ 45 kDa, respectively), are readily filtered. Unlike urinary PlGF, which is derived entirely from circulating blood, the major sources of urinary VEGF are cells of the kidney itself (glomerular podocytes and tubular cells^{15,16}); 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.^{14,17} 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.¹⁸ Furthermore, we have demonstrated that alterations in circulating sFlt1 and PlGF antedate clinical preeclampsia in these patients.⁵ Analysis of previous work revealed no significant differences in maternal serum sFlt1 or PlGF concentrations according to infant sex.^{5,6}

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 preeclampsia¹⁴ or on the concentrations of angiogenic factors in serum⁵ 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 $\geq 3+$ [300 mg/dL] on dipstick testing). Gestational hypertension was hypertension as defined herein in the absence of proteinuria. Detailed definitions have been published.^{14,17} 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.¹⁹

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).⁶ 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

Table 1. Baseline Characteristics of CPEP Main Study Cases and Controls and Their Infants*

| Characteristics | Cases (n = 120) | Controls (n = 118) |
|--|-----------------|--------------------|
| Age, mean (SD), y | 20.8 (4.5) | 20.0 (3.4) |
| Body mass index, mean (SD)† | 27.3 (6.8) | 25.0 (6.1) |
| Blood pressure, mean (SD), mm Hg | | |
| Systolic | 109 (9) | 106 (9) |
| Diastolic | 62 (8) | 59 (7) |
| Gestational age at delivery, mean (SD), wk | 38.1 (2.6) | 38.9 (2.5) |
| Current smoker | 9 (7.5) | 13 (11.0) |
| Ever married | 25 (20.8) | 22 (18.6) |
| Race/ethnicity‡ | | |
| White, non-Hispanic | 24 (20.0) | 33 (28.0) |
| White, Hispanic | 21 (17.5) | 14 (11.9) |
| African American | 69 (57.5) | 68 (57.6) |
| Other/unknown | 6 (5.0) | 3 (2.5) |
| Birth weight, mean (SD), g | 3100 (796) | 3247 (596) |
| Delivery at <37 wk | 26 (21.7) | 9 (7.6) |
| Small for gestational age (<10th percentile) | 18 (15.0) | 4 (3.4) |

Abbreviation: CPEP, Calcium for Preeclampsia Prevention trial.

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

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

‡Racial or ethnic group was self-reported.

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 (Metra creatinine assay kit, Quidel Corp, San Diego, Calif).

Statistical Analysis

The χ^2 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

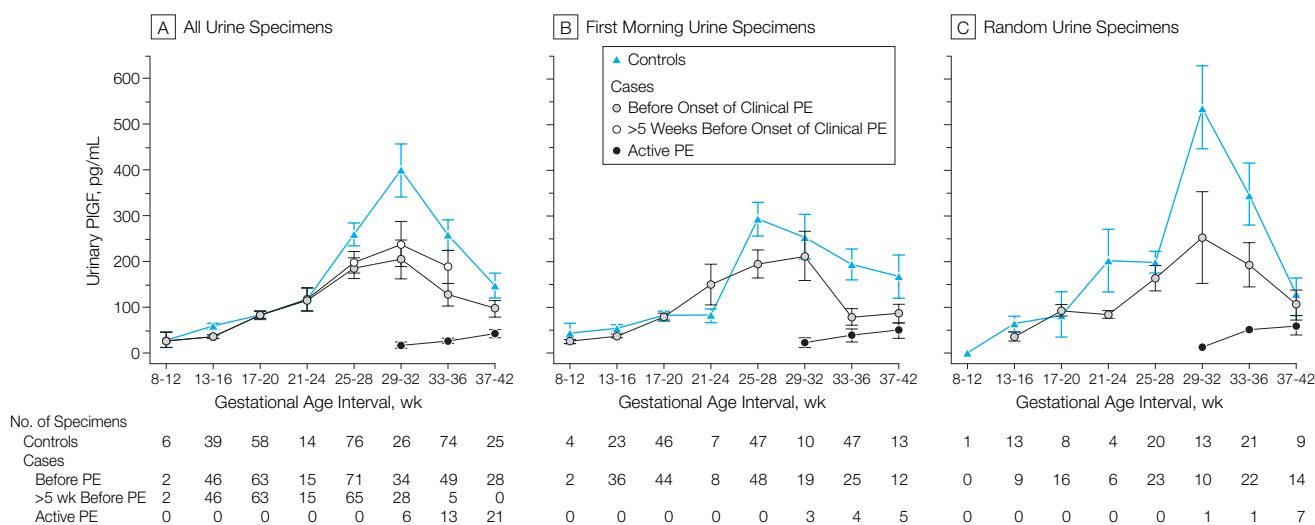
Characteristics of the Women. Of the 120 women with preeclampsia, 80 had mild and 40 had severe disease. Compared with controls, women with preeclampsia had greater body mass index ($P = .007$), higher systolic and diastolic blood pressure at enrollment in the CPEP trial ($P = .001$ and $.006$, respectively), and larger proportions of

their current pregnancies complicated by preterm delivery ($P = .002$) or resulting in SGA infants ($P = .002$).⁵ Patient and infant characteristics have been described previously⁵ and are briefly summarized in TABLE 1.

Differences in Urinary PIGF After Onset of Preeclampsia. We first ascertained that urinary levels of PIGF 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 PIGF than specimens from controls (mean PIGF level, 32 vs 234 pg/mL; $P < .001$ and 50 vs 227 pg/mg of creatinine; $P < .001$).

Gestational Changes in Urinary PIGF. To evaluate gestational patterns, we performed cross-sectional analyses of urine obtained within gestational age intervals of 4 to 5 weeks, with PIGF 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 PIGF 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

Figure 1. Urinary PIGF by Intervals of Gestational Age



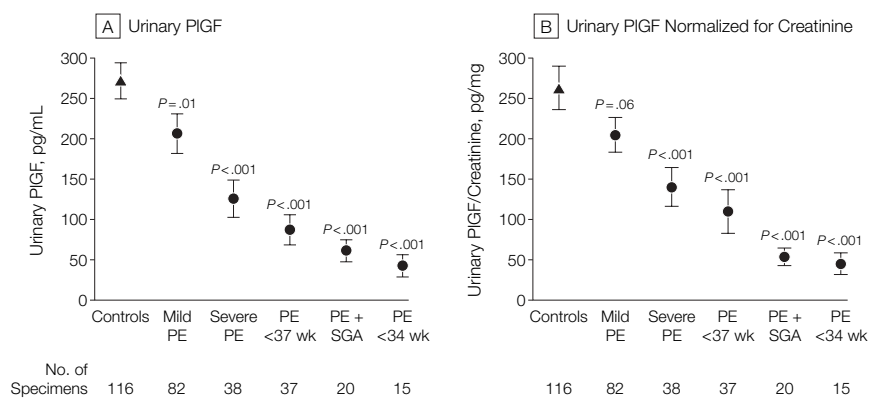
A, Mean urinary placental growth factor (PIGF) concentrations in normotensive women (controls) and in women (cases) before onset and after onset (active preeclampsia [PE]) of clinical PE, according to gestational age. Also shown for women who subsequently developed PE (cases) are the mean urinary concentrations of PIGF 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 ($P < .001$), 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 PIGF 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 PIGF 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 at 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 PIGF 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,

Figure 2. Mean Urinary Concentrations of PlGF at 21 to 32 Weeks of Gestation According to Preeclampsia Status and Severity, Before and After Normalization for Creatinine



Urinary placental growth factor (PlGF) concentrations in picograms per milliliter and in picograms of PlGF per milligram of creatinine are shown at 21 to 32 weeks of gestation in controls and in women who subsequently developed clinical preeclampsia (PE) according to whether they had mild PE, severe PE, PE with an onset at less than 37 weeks of gestation, PE and a small-for-gestational-age (SGA) infant, or PE with an onset at less than 34 weeks of gestation. All specimens from women in whom PE later developed were obtained before the onset of clinical symptoms. The P values given are for comparisons with specimens from controls. Error bars represent SEs.

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 preeclampsia 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 gesta-

tion 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 5 weeks before the onset of clinical disease. However, a number of control specimens also had low urinary PlGF. To distinguish these specimens from specimens obtained within 5 weeks prior to preeclampsia, we examined serum measurements of the ratio of sFlt1 to PlGF. The ratio accounts for both the increased sFlt1 and decreased PlGF observed before onset of preeclampsia. A scatter plot of the ratios of sFlt1 to PlGF concentrations in paired sera is shown in Figure 3B. Ratios are elevated (>5) in all specimens obtained within 5 weeks before the onset of preeclampsia and exceed almost all control values.

Urinary sFlt1 and Urinary VEGF in Preeclampsia. We randomly selected 22 cases and 22 controls for analysis of urinary sFlt1 and VEGF in samples obtained at 21 to 32 weeks of gestation before onset of clinical preeclampsia. In 16 of 22 case specimens (73%) and 19 of 22 control specimens (86%), urinary sFlt1 was undetectable. In contrast, urinary VEGF was detected in all specimens but was not significantly altered in cases before or after the onset of hypertension and proteinuria (before onset, 272 vs 248 pg/mL in the groups of 22 randomly selected cases and controls, respectively; $P = .56$ and after onset, 167 vs 103 pg/mL in 22 gestational age-matched cases and controls, respectively; $P = .61$).

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*

| Quartile | Control Specimens, No. | Preeclampsia <37 wk | | Preeclampsia ≥37 wk | |
|-------------------------------|------------------------|---------------------|------------------|---------------------|----------------|
| | | Specimens, No.† | OR (95% CI)‡ | Specimens, No.† | OR (95% CI)‡ |
| PlGF, pg/mL | | | | | |
| 13-20 wk | | | | | |
| 1: ≤29 | 25 | 6 | 0.6 (0.2-2.4) | 19 | 0.9 (0.3-2.3) |
| 2: 29-59 | 24 | 12 | 1.3 (0.4-4.3) | 25 | 1.4 (0.6-3.3) |
| 3: 59-88 | 24 | 5 | 0.7 (0.2-2.7) | 19 | 1.1 (0.5-2.8) |
| 4: >88 | 24 | 6 | 1.0 | 17 | 1.0 |
| 21-32 wk | | | | | |
| 1: ≤118 | 29 | 30 | 31.3 (5.6-174.7) | 33 | 2.2 (1.0-5.1) |
| 2: 118-230 | 29 | 4 | 2.6 (0.4-16.8) | 21 | 1.3 (0.6-3.0) |
| 3: 230-309 | 29 | 1 | 0.6 (0.1-7.6) | 11 | 0.7 (0.3-1.7) |
| 4: >309 | 29 | 2 | 1.0 | 18 | 1.0 |
| 33-42 wk | | | | | |
| 1: ≤55 | 25 | 2 | NA | 31 | 4.2 (1.4-12.5) |
| 2: 55-113 | 25 | 0 | NA | 21 | 2.5 (0.8-7.7) |
| 3: 113-318 | 25 | 0 | NA | 17 | 2.1 (0.7-6.5) |
| 4: >318 | 24 | 0 | NA | 6 | 1.0 |
| PlGF/creatinine, pg/mg | | | | | |
| 13-20 wk | | | | | |
| 1: ≤26 | 25 | 8 | 0.5 (0.1-2.2) | 21 | 0.9 (0.3-2.5) |
| 2: 26-52 | 24 | 9 | 0.7 (0.2-3.0) | 25 | 1.3 (0.5-3.2) |
| 3: 52-78 | 24 | 5 | 0.4 (0.1-1.8) | 15 | 0.8 (0.3-2.2) |
| 4: >78 | 24 | 7 | 1.0 | 19 | 1.0 |
| 21-32 wk | | | | | |
| 1: ≤120 | 29 | 29 | 15.4 (3.7-64.3) | 33 | 2.6 (1.1-6.3) |
| 2: 120-180 | 29 | 2 | 0.9 (0.1-6.1) | 13 | 1.0 (0.4-2.6) |
| 3: 180-323 | 29 | 3 | 0.9 (0.2-5.1) | 22 | 1.7 (0.7-4.0) |
| 4: >323 | 29 | 3 | 1.0 | 15 | 1.0 |
| 33-42 wk | | | | | |
| 1: ≤69 | 24 | 2 | NA | 34 | 2.6 (1.0-6.6) |
| 2: 69-153 | 25 | 0 | NA | 23 | 1.7 (0.6-4.5) |
| 3: 153-268 | 25 | 0 | NA | 8 | 0.6 (0.2-1.8) |
| 4: >268 | 24 | 0 | NA | 10 | 1.0 |

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 + Q4 are given in the "Results" section of the text.

and women who remained normotensive during pregnancy but delivered an SGA infant with normotensive women whose infant was not SGA (controls) and with women with preeclampsia before 37 weeks. The clinical characteristics of the women in this study and of their infants are summarized in TABLE 3. The characteristics of women with preeclampsia and their infants were similar to those reported for such women in the main study. Compared with normotensive women 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 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 during pregnancy but delivered an SGA infant did not differ from those of normotensive

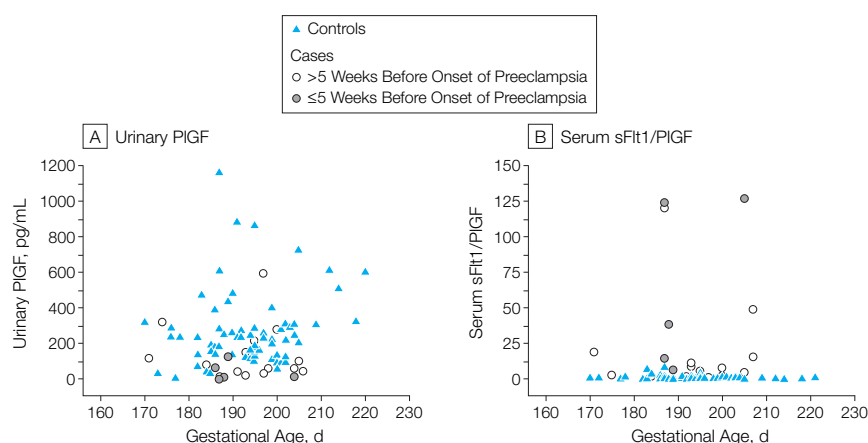
controls whose infant was not born SGA. Similarly, levels in patients with gestational hypertension did not differ from those of normotensive controls. However, levels of urinary PlGF in patients who developed preeclampsia before 37 weeks of gestation (collected on average 42 days prior to clinical disease onset) were much lower than controls (77 vs 206 pg/mL; $P < .001$). Within each group, PlGF concentra-

tions among women who delivered male or female infants did not differ significantly.

COMMENT

In this study of 120 women with preeclampsia and 118 normotensive controls, urinary concentrations of PlGF were significantly lower beginning at 25 to 28 weeks of gestation among the women who subsequently developed

Figure 3. 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



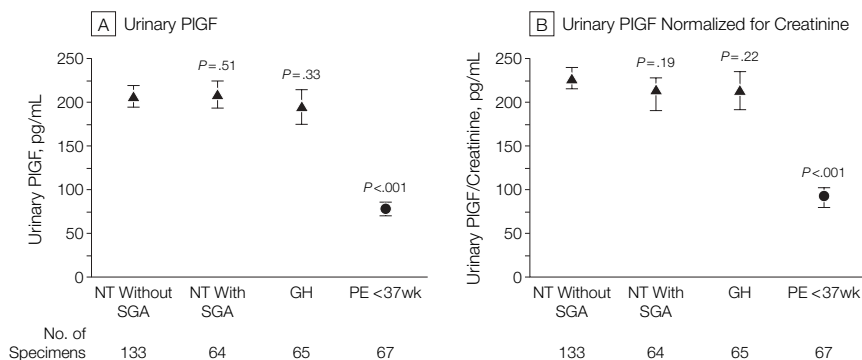
Measurements were obtained from paired urine and serum specimens obtained from 20 women before development of preeclampsia at less than 37 weeks of gestation and from 69 normotensive controls.

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

| Characteristics | Normal Blood Pressure, Not SGA (n = 120) | Normal Blood Pressure and SGA (n = 60) | P Value† | Gestational Hypertension (n = 60) | P Value† | Preeclampsia <37 wk (n = 59) | P Value† |
|--|--|--|----------|-----------------------------------|----------|------------------------------|----------|
| Age, mean (SD), y | 21.8 (4.6) | 21.3 (4.9) | .49 | 22.2 (5.3) | .61 | 21.1 (4.7) | .30 |
| Body mass index, mean (SD)‡ | 25.8 (6.1) | 22.8 (3.6) | <.001 | 28.3 (7.4) | .02 | 27.6 (6.9) | .08 |
| Blood pressure, mean (SD), mm Hg | | | | | | | |
| Systolic | 106 (9) | 106 (8) | .84 | 108 (9) | .24 | 111 (8) | .001 |
| Diastolic | 60 (7) | 60 (8) | .84 | 62 (9) | .16 | 65 (7) | <.001 |
| Gestational age at delivery, mean (SD), wk | 39.0 (1.8) | 38.7 (1.4) | .18 | 39.6 (1.7) | .06 | 34.6 (2.3) | <.001 |
| Current smoker | 15 (12.5) | 13 (21.7) | .11 | 3 (5.0) | .11 | 4 (6.8) | .24 |
| Ever married | 34 (28.3) | 16 (26.7) | .81 | 15 (25.4) | .68 | 16 (27.1) | .86 |
| Race/ethnicity§ | | | | | | | |
| White, non-Hispanic | 46 (38.3) | 20 (33.3) | .11 | 20 (33.3) | .69 | 16 (27.1) | .43 |
| White, Hispanic | 16 (13.3) | 16 (26.7) | | 6 (10.0) | | | |
| African American | 55 (45.8) | 24 (40.0) | | 33 (55.0) | | | |
| Other/unknown | 3 (2.5) | 0 | | 1 (1.7) | | | |
| Birth weight, mean (SD), g | 3273 (456) | 2538 (278) | <.001 | 3437 (559) | .04 | 2193 (726) | <.001 |
| Delivery <37 wk | 13 (10.8) | 6 (10.0) | .86 | 3 (5.0) | .19 | 50 (84.8) | <.001 |
| SGA (<10th percentile) | 0 | 60 (100) | <.001 | 2 (3.3) | .11 | 18 (30.5) | <.001 |

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.

Figure 4. Mean Urinary Concentrations of PlGF 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 (PlGF) concentrations in picograms per milliliter and in picograms of PlGF 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.

preeclampsia. Differences between the 2 groups became more pronounced at 29 to 36 weeks. In our previous study of serum concentrations of angiogenic proteins, serum free PlGF was lower in cases than in controls beginning at 13 to 16 weeks of gestation, becoming even lower after 25 weeks of gestation.⁵ As predicted from serum measurements, in the current study, urinary PlGF at 21 to 32 weeks of gestation was especially decreased in women who developed preeclampsia before 37 weeks or during a pregnancy complicated by an SGA infant and in those within 5 weeks of the onset of clinical signs. Furthermore, among women in the lowest quartile of urinary PlGF concentrations (<118 pg/mL) at 21 to 32 weeks of gestation, the risk of developing preeclampsia before 37 weeks of gestation or during a pregnancy complicated by an SGA infant was markedly elevated. Risk was high irrespective of adjustment for urinary creatinine concentrations and evident even in random urine specimens—although the association was stronger with first morning specimens, which are likely to be more concentrated. Thus, urinary PlGF was especially useful for identifying patients who would ben-

efit most from early diagnosis. We have also demonstrated that a strategy of following urine measurement of PlGF with serum measurements of sFlt1 and PlGF in selected patients may minimize false-positive results from urine testing.

Urinary PlGF was much lower at 21 to 32 weeks of gestation in women who developed preeclampsia before 37 weeks than in women who developed gestational hypertension or delivered an SGA infant, 2 obstetrical conditions with similarities to preeclampsia. Thus, it appears that a low urinary PlGF concentration at this stage of pregnancy may distinguish preeclampsia from gestational hypertension and intrauterine growth retardation.

Urinary VEGF concentrations were reported recently to be modestly elevated in 37 women with severe preeclampsia compared with 32 with uncomplicated pregnancy.²⁰ We found nonsignificant elevations of urinary VEGF before and after the onset of preeclampsia, consistent with the hypothesis that urinary VEGF reflects primarily local renal VEGF production. Since urinary VEGF originates almost entirely from renal podocyte and tubular cells,^{15,16} it has not been exposed to circulating sFlt1, which is too large a molecule to filter freely through

an intact glomerulus. Therefore, while reduced urinary PlGF in women with preeclampsia likely reflects reduced circulating free PlGF (the result of binding to excess circulating sFlt1), urinary VEGF does not reflect the angiogenic imbalance in the blood.

Limitations of this study must be acknowledged. We used specimens obtained almost 10 years ago, and although we did find differences between cases and controls, specimen deterioration may have affected reported values. Moreover, since there was an average of only 3 urine specimens per woman throughout pregnancy, we could not follow up suspicious results with repeated measurements of urine and serum to search for trends, as could be done in clinical practice. Finally, we did not determine whether there are alterations in urinary PlGF throughout gestation in obstetrical 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 PlGF are dramatic in women who will develop early-onset preeclampsia, normotensive women who subsequently develop gestational hypertension or deliver an SGA infant have none.

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.⁶ 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 PlGF concentrations. Among those with low levels, serial serum measurements of sFlt1 and PlGF could then be used to identify more precisely individuals at high risk. Prospective longitudinal studies with measurements throughout pregnancy are needed to assess the validity of these observations.

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: Levine, Qian, Blink.

Study supervision: Levine, Karumanchi.

Calcium for Preeclampsia Prevention Study Group:

University of Alabama at Birmingham: J. C. Hauth, R. Goldenberg, B. S. Stofan; University of New Mexico at Albuquerque: L. B. Curet, G. M. Joffe, V. Dorato; University of Tennessee at Memphis: B. M. Sibai, S. A. Friedman, B. M. Mercer, T. Carr; Case Western Reserve University at MetroHealth Medical Center, Cleveland, Ohio: P. M. Catalano, A. S. Petrusis, L. Barabach; Oregon Health Sciences University, Portland: C. Morris, S. L. Jacobson, K. McCracken; EMMES Corp, Rockville, Md: J. R. Esterlitz, M. G. Ewell, D. M. Brown; National Institute of Child Health and Human Development (NICHD): R. J. Levine, R. DerSimonian, J. D. Clemens, M. A. Klebanoff, E. G. Raymond, J. G. Rigau-Perez, H. Shifrin; National Heart, Lung, and Blood Institute (NHLBI): J. A. Cutler, D. E. Bild. Data Safety and Monitoring Board: M. Lindheimer, C. Begg, T. Chalmers, M. Druzin, R. Sokol.

Funding/Support: This study was funded by the American Society of Nephrology Carl W. Gottschalk Research Scholar Award, Beth Israel Deaconess Medical Center Department of Medicine, Obstetrics &

Gynecology seed funds to Dr Karumanchi, and by intramural funding of Dr Levine from the NICHD. Salary support for Dr Karumanchi was provided through a National Institutes of Health physician-scientist award (KO8-DK-002825). The CPEP trial was supported by the NICHD under contracts N01-HD-1-3121 through 3126, N01-HD-3154, and N01-HD-5-3246, with co-funding from the NHLBI. Dr Thadhani is funded by grant R01-HD-3-9223 from the NICHD.

Role of the Sponsors: The funding organizations had no role in the design and conduct of the study; collection, management, analysis, and interpretation of the data; or the preparation and review of the manuscript for submission. The manuscript was approved for submission by the Division of Epidemiology, Statistics, and Prevention Research of the NICHD.

Acknowledgment: We are indebted to the CPEP Study Group, who assembled the database and specimen repository used here, and to the patients who took part in the study. We thank Patricia Moyer for assistance with the figures.

REFERENCES

- Walker JJ. Pre-eclampsia. *Lancet*. 2000;356:1260-1265.
- Roberts JM. Endothelial dysfunction in preeclampsia. *Semin Reprod Endocrinol*. 1998;16:5-15.
- Sibai BM. Diagnosis and management of gestational hypertension and preeclampsia. *Obstet Gynecol*. 2003;102:181-192.
- Sibai BM, Caritis S, Hauth J. What we have learned about preeclampsia. *Semin Perinatol*. 2003;27:239-246.
- Levine RJ, Maynard SE, Qian C, et al. Circulating angiogenic factors and the risk of preeclampsia. *N Engl J Med*. 2004;350:672-683.
- Maynard SE, Min JY, Merchan J, et al. Excess placental soluble fms-like tyrosine kinase 1 (sFlt1) may contribute to endothelial dysfunction, hypertension, and proteinuria in preeclampsia. *J Clin Invest*. 2003;111:649-658.
- Tsatsaris V, Goffin F, Munaut C, et al. Overexpression of the soluble vascular endothelial growth factor receptor in preeclamptic patients: pathophysiological consequences. *J Clin Endocrinol Metab*. 2003;88:5555-5563.
- Koga K, Osuga Y, Yoshino O, et al. Elevated serum soluble vascular endothelial growth factor receptor 1 (sVEGFR-1) levels in women with preeclampsia. *J Clin Endocrinol Metab*. 2003;88:2348-2351.
- Taylor RN, Grimwood J, Taylor RS, McMaster MT, Fisher SJ, North RA. Longitudinal serum concentrations of placental growth factor: evidence for abnormal placental angiogenesis in pathologic pregnancies. *Am J Obstet Gynecol*. 2003;188:177-182.
- Zhou Y, Damsky CH, Fisher SJ. Preeclampsia is associated with failure of human cytotrophoblasts to mimic a vascular adhesion phenotype: one cause of defective endovascular invasion in this syndrome? *J Clin Invest*. 1997;99:2152-2164.
- Torry DS, Wang HS, Wang TH, Caudle MR, Torry RJ. Preeclampsia is associated with reduced serum levels of placenta growth factor. *Am J Obstet Gynecol*. 1998;179:1539-1544.
- Thadhani R, Mutter WP, Wolf M, et al. First trimester placental growth factor and soluble fms-like tyrosine kinase 1 and risk for preeclampsia. *J Clin Endocrinol Metab*. 2004;89:770-775.
- Kendall RL, Thomas KA. Inhibition of vascular endothelial cell growth factor activity by an endogenously encoded soluble receptor. *Proc Natl Acad Sci U S A*. 1993;90:10705-10709.
- Levine RJ, Hauth JC, Curet LB, et al. Trial of calcium to prevent preeclampsia. *N Engl J Med*. 1997;337:69-76.
- Brown LF, Berse B, Tognazzi K, et al. Vascular permeability factor mRNA and protein expression in human kidney. *Kidney Int*. 1992;42:1457-1461.
- Simon M, Grone HJ, Jöhren O, et al. Expression of vascular endothelial growth factor and its receptors in human renal ontogenesis and in adult kidney. *Am J Physiol*. 1995;268:F240-F250.
- Levine RJ, Esterlitz JR, Raymond EG, et al. Trial of Calcium for Preeclampsia Prevention (CPEP): rationale, design, and methods. *Control Clin Trials*. 1996;17:442-469.
- Levine RJ, Qian C, LeShane ES, et al. Two-stage elevation of cell-free fetal DNA in maternal sera before onset of preeclampsia. *Am J Obstet Gynecol*. 2004;190:707-713.
- Zhang J, Bowes WA Jr. Birth-weight-for-gestational-age patterns by race, sex, and parity in the United States population. *Obstet Gynecol*. 1995;86:200-208.
- Roes EM, Steegers EA, Thomas CM, et al. High levels of urinary vascular endothelial growth factor in women with severe preeclampsia. *Int J Biol Markers*. 2004;19:72-75.