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Racial-ethnic differences in midtrimester maternal serum levels of angiogenic and antiangiogenic factors

Juan YANG, PhD¹, Michelle PEARL, PhD², Gerald N DELORENZE, PhD³, Roberto ROMERO, MD⁴, Zhong DONG, PhD⁴, Laura JELLIFFE-PAWLOWSKI, PhD⁵, Robert CURRIER, PhD¹, Monica FLESSEL, PhD¹, and Martin KHARRAZI, PhD⁶

¹Genetic Disease Screening Program, California Department of Public Health (Drs. Yang, Currier, and Flessel), Richmond, CA

²Sequoia Foundation (Dr. Pearl), Richmond CA

³Division of Research, Kaiser Permanente Northern California (Dr. DeLorenze), Oakland, CA

⁴Perinatology Research Branch, *Eunice Kennedy Shriver* National Institute of Child Health and Human Development (Drs. Romero and Dong), Detroit, MI

⁵Department of Epidemiology and Biostatistics, University of California San Francisco (Dr. Jelliffe-Pawlowski), San Francisco, CA

⁶Environmental Health Investigations Branch, California Department of Public Health (Dr. Kharrazi), Richmond CA

Abstract

BACKGROUND—Little is known about racial-ethnic differences in the distribution of maternal serum levels of angiogenic and antiangiogenic factors and their associations with early-onset preeclampsia.

OBJECTIVE—To investigate the distribution of mid-trimester maternal serum levels of placental growth factor, soluble endoglin, and soluble vascular endothelial growth factor receptor 1 and their associations with early-onset preeclampsia in Whites, Hispanics, and Blacks.

STUDY DESIGN—A population-based nested case-control design was used to identify cases and controls of White, Hispanic, and Black origin from a 2000 to 2007 live-birth cohort in five southern California counties. Cases included 197 women (90 Whites, 67 Hispanics, and 40 Blacks) with early-onset preeclampsia defined as hypertension and proteinuria with onset prior to 32 weeks according to hospital records. Controls included a random sample of 2,363 women without early-onset preeclampsia. Maternal serum specimens collected at 15–20 weeks of gestation as part of routine prenatal screening were tested for placental growth factor, soluble endoglin, and soluble vascular endothelial growth factor receptor 1. Serum levels of the three factors were log-normally distributed. Adjusted natural logarithmic means were compared

CORRESPONDING AUTHOR: Juan Yang, Genetic Disease Screening Program, California Department of Public Health, 850 Marina Bay Parkway, F175, Mail Stop 8200 Richmond, CA 94804, Telephone: 510-412-1520; Fax: 510-412-1511; juan.yang@cdph.ca.gov.

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between cases and controls and between racial-ethnic groups. Odds ratios and 95% confidence intervals derived from logistic regression models were calculated to measure the magnitude of the associations.

RESULTS—Cases showed lower adjusted logarithmic means of placental growth factor but higher adjusted logarithmic means of soluble endoglin than controls across all three groups ($P < 0.05$). Cases also had higher adjusted means of soluble vascular endothelial growth factor receptor 1 than controls in Whites (7.75 log pg/mL versus 7.52 log pg/mL, $P < 0.05$) and Hispanics (7.73 log pg/mL versus 7.40 log pg/mL, $P < 0.05$) but not in Blacks (7.85 log pg/mL versus 7.69 log pg/mL, $P = 0.47$). Blacks were found to have higher levels of placental growth factor in both cases and controls when compared to Whites and Hispanics (adjusted means: 4.69 log pg/mL and 5.20 log pg/mL in Blacks, 4.08 log pg/mL and 4.78 log pg/mL in Whites, and 3.89 log pg/mL and 4.70 log pg/mL in Hispanics, respectively, $P < 0.05$). Hispanic cases had the highest adjusted mean of soluble endoglin compared to White and Black cases (9.24 log pg/mL, 9.05 log pg/mL, and 8.93 log pg/mL, respectively, $P < 0.05$). The weakest association of early-onset preeclampsia with placental growth factor and soluble endoglin was observed in Blacks. The adjusted odds ratio per log pg/mL increase of the two analytes were 0.219 (95% confidence interval, 0.124-0.385) and 5.02 (95% confidence interval, 2.56-9.86) in Blacks in comparison to 0.048 (95% confidence interval, 0.026-0.088) and 36.87 (95% confidence interval, 17.00-79.96) in Whites ($P < .05$) and 0.028 (95% confidence interval, 0.013-0.060) and 86.68 (95% confidence interval, 31.46-238.81) in Hispanics ($P < .05$), respectively. As for soluble vascular endothelial growth factor receptor 1, the association was not significantly different among the racial-ethnic groups.

CONCLUSIONS—Racial-ethnic differences were observed in the distribution of midtrimester maternal levels of placental growth factor and soluble endoglin and in the associations with early-onset preeclampsia. These differences should be considered in future studies to improve etiologic and prognostic understanding of early-onset preeclampsia.

Keywords

early-onset preeclampsia; placental growth factor; soluble endoglin; soluble vascular endothelial growth factor receptor-1; racial-ethnic differences

INTRODUCTION

Preeclampsia is one of the most dangerous pregnancy-associated disorders. Affecting 3-5% of pregnancies in the United States, preeclampsia causes 25% of maternal deaths and 25,000-40,000 perinatal deaths each year.^{1,2} Preeclampsia is heterogeneous in onset and severity of clinical manifestations.³ Preeclampsia occurring at <32 weeks of pregnancy, also known as early-onset preeclampsia with an incidence range of 0.2%-1.4%,⁴ carries a disproportionately higher risk of maternal and infant mortality and therefore is considered a more severe form of the disease than preeclampsia occurring later in pregnancy.^{1,3} Angiogenesis is the process of new blood vessel formation and the growth of new blood vessels from existing blood vessels.^{1,5} These processes are critical to the establishment and functioning of the placenta.^{1,6} The placenta produces high levels of angiogenic factors, such as placental growth factor (PlGF) in order to maintain an angiogenic balance for normal fetal development.⁶ Dramatically reduced levels of PlGF and increased levels of antiangiogenic

factors, such as soluble endoglin (sEng) and soluble vascular endothelial growth factor receptor 1 (sVEGFR-1) in maternal circulation have been repeatedly reported as important predictors for preeclampsia with more pronounced effects for early-onset compared to late-onset preeclampsia.^{1,7-15}

Black women have been shown to be at higher risk for preeclampsia than White women (4.0% versus 2.8%).¹⁶ Hispanics have been found to have lower risk than non-Hispanics (1.3% versus 5.3%)¹⁷ although results are not consistent.^{18,19} While many studies have been published on angiogenic and antiangiogenic factors as screening markers for preeclampsia,^{1,3} few have examined racial-ethnic differences in the distribution of these factors and in their associations with preeclampsia. To date, no population-based study that we are aware of has concurrently compared the pattern of mid-pregnancy angiogenic and antiangiogenic factors in White, Hispanic, and Black women. A comprehensive study of racial-ethnic differences in maternal angiogenic imbalance is needed to better understand the pathogenesis of preeclampsia within and across racial-ethnic groups.

Utilizing a nested case-control study design, this study aimed to better understand the relationship among maternal serum concentrations of PlGF, sEng, and sVEGFR-1 at 15-20 weeks of gestation in White, Hispanic, and Black women with and without early-onset preeclampsia.

MATERIALS AND METHODS

Case Identification

All early-onset preeclampsia cases (n=197) evaluated in this study were identified by the California Very Preterm Birth Study²⁰ from prenatal screening records and validated via chart review at the hospital of delivery. Detailed study design and methods have been published elsewhere.²⁰ Briefly, very preterm births of White, Hispanic, or Black origin were identified from a population-based cohort created through record linkage of 346,456 mother-baby pairs with stored maternal serum specimens linked to certificates of live births in California from January 2000 to April 2007. White and Hispanic births were defined as both parents being non-Hispanic White and Mexican Hispanic White, respectively, based on birth records. Mexican Hispanic White referred to self-reported White with Hispanic ethnicity origin of Mexican, Mexican-American, or Chicano. Black births were defined as Black mothers of any ethnicity, regardless of father's race-ethnicity. All of these women participated in routine prenatal screening for aneuploidies and neural tube defects between 15 and 20 weeks through the Genetic Disease Screening Program within the California Department of Public Health and had serum banked after screening.^{20,21} Study samples were limited to singleton live births delivered in five Southern California counties (Los Angeles, Orange, Riverside, San Bernardino, and San Diego). Within this large cohort, the medical records of 1,117 chronologically identified very preterm births of <32 weeks of gestation (429 Whites, 440 Hispanics, and 248 Blacks) were abstracted to distinguish very preterm births with known causes, including preeclampsia, which formed the case group for this study. Cases with a birth weight > 2,500 g were excluded based on suspected misdating. Pregnancies with structural abnormalities reported on delivery records or identified by the California Birth Defects Monitoring Program registry were also excluded.

Preeclampsia was defined as hypertension accompanied by proteinuria with onset after 20 weeks of gestation in the California Very Preterm Birth Study. Hypertension was defined as blood pressure ≥ 140 mm Hg systolic or ≥ 90 mm Hg diastolic on two occasions 6 hours apart. Proteinuria was defined as ≥ 0.3 grams of protein in a 24-hour urine collection or $\geq 1+$ dipstick on a urine test on two occasions 6 hours apart. Early-onset preeclampsia was defined as preeclampsia with delivery before 32 weeks of gestation. Gestational age estimates for cases were mostly based on ultrasound (61.5%) and date of last menstrual period (29.5%) from the California Prenatal Screening Program with a small part (9.0%) based on maternal hospital chart review.

Hospital chart review identified 207 cases of early-onset preeclampsia with a maternal mid-trimester specimen available for laboratory testing. Ten of the 207 cases were excluded due to maternal specimens collected beyond the gestational window of 15-20 weeks. The final sample included 90 White, 67 Hispanic, and 40 Black cases. Details of the sample selection are listed in the Figure.

Control Identification

Controls were non-cases selected at an original sample size of 10 times the number of cases before specimen pulling using simple random sampling within the same race-ethnicity specific singleton live-birth cohort as cases. Births with adverse outcomes such as late-onset preeclampsia, preterm birth, and low birth weight were also eligible controls. Controls of White, Hispanic, and Black origin were drawn separately for each racial-ethnic group. For women with multiple pregnancies during the study period, only the first pregnancy was chosen. Control births without banked serum specimens or with structural malformations according to the California Birth Defect Monitoring Program registry were excluded. The final sample included 916 White, 709 Hispanic, and 738 Black controls (Figure). Gestational age estimates for controls were based on ultrasound (55.8%), date of last menstrual period (34.9%), and physical exam (9.3%) from the California Prenatal Screening Program.

Biological Specimens

Banked maternal blood specimens were retrieved from the California Biobank Program.²² As part of the State Prenatal Screening Program, obstetrical care service providers at doctors' offices or drawing stations collected venous blood from pregnant women who voluntarily enrolled at 15-20 weeks of gestation. The blood, which was collected in serum separator tubes, was sent to one of the seven Newborn and Prenatal Screening Laboratories by mail under ambient conditions. Specimens arrived for triple-marker prenatal testing within seven days of collection (median time = 3 days).^{20,21} After routine testing, leftover specimens were refrigerated and then processed for long-term storage at -20°C . Women consented to the routine prenatal screening test in writing and were notified that the blood becomes the property of the State, which allows for a variety of approved research uses of the leftover blood under the highest level of security and confidentiality standards. While women have the option to request the specimen not be used for research purposes, very few assert this right. Specimen vials from cases and controls were intermingled, with vial positions randomly assigned during shipping and laboratory testing. Laboratory personnel were unaware of subjects' case-control status.

Laboratory methods

Maternal serum levels of PIGF, sEng, and sVEGFR-1 were determined by immunoassays (R&D Systems Inc., Minneapolis, MN) utilizing the quantitative sandwich enzyme immunoassay technique. Laboratory testing was conducted at the research core laboratory of the Perinatology Research Branch, *Eunice Kennedy Shriver* National Institute of Child Health and Human Development, National Institutes of Health, Department of Health and Human Services. Standard serum specimens were used in each laboratory run and analyte value was determined by interpolation from standard curves. The assays utilized 120 μ L, 25 μ L, and 25 μ L of serum for the PIGF, sEng, and VEGFR-1 assays, respectively. The inter- and intra-assay coefficients of variation obtained in the laboratory were 4.6% and 3.6% for PIGF, 5.3% and 4.3% for sEng, and 2.8% and 2.7% for sVEGFR-1, respectively. The sensitivity of the assays was 5.7 pg/mL for PIGF, 40.0 pg/mL for sEng, and 12.9 pg/mL for sVEGFR-1.

Data analysis

The laboratory levels of PIGF, sEng, and sVEGFR-1 were linked to birth records, prenatal screening program data, and maternal hospital chart data (cases only) for analysis. Maternal characteristics were compared between cases and controls. Gestational weeks at delivery and at preeclampsia diagnosis for cases were compared among the three racial-ethnic groups. The Chi-square test and Student *t* test were used to estimate the statistical significance of the comparisons for categorical and continuous variables, respectively. Natural logarithmic transformed circulating levels of PIGF, sEng, and sVEGFR-1 were normally distributed in cases and controls. Means (log pg/mL) and standard errors were calculated for cases and controls of each racial-ethnic group and were adjusted by maternal age, education, parity, specimen storage year, gestational age at specimen collection, and maternal weight at or near specimen collection. The adjusted logarithmic means were compared using the Tukey honestly significant difference²³ test between cases and controls in each racial-ethnic group and between racial-ethnic groups among cases and controls, respectively. Odds ratios (OR) and 95% confidence intervals (CI) derived from binomial logistic regressions were used to measure effect magnitude of analyte levels in association with case-control status. The logarithmic transformed levels (log pg/mL) of the three analytes were used in the logistic regression to reduce both variance and outlier influence. Race-ethnicity is a significant effect modifier for PIGF and sEng in association with early-onset preeclampsia; therefore, association results were presented separately for each race-ethnicity group. Maternal age, education, parity, specimen storage year, gestational weeks at specimen collection, and maternal weight at specimen collection were included as potential confounders. Standard Z test was conducted to compare ORs between racial-ethnic groups. Given the above-mentioned final sample sizes, this study had adequate power of 80% to detect association of moderate or higher effect estimates (OR 2.6) between cases and controls in each racial-ethnic group under a logistic model with a two-sided test.

All of the statistical analyses were conducted using software (SAS, Version 9.2 for Windows; SAS Institute, Cary, NC). The study protocol was approved by the California Health and Human Services Agency Committee for the Protection of Human Subjects (Project number 10-10-52).

RESULTS

The prevalence of early-onset preeclampsia (<32 weeks of gestation) was 0.13% for Whites (92 of 69,800), 0.14% for Hispanics (72 of 51,614), and 0.41% for Blacks (43 of 10,582) based on the linked birth cohort for this study (Figure), within the lower range previously reported.⁴ Compared to controls, White cases were more likely to be nulliparous (81.11% versus 50.11%, $P < 0.05$), and Hispanic cases were older than controls (28.61 years on average versus 26.22 years, $P < 0.05$), as presented in Table 1. Cases of White and Hispanic origin were heavier at or near the time of specimen collection than controls (76.20 kg on average versus 70.02 kg in Whites and 76.33 kg versus 67.22 kg in Hispanics, $P < 0.05$, respectively). Maternal education and gestational age at specimen collection were not associated with case-control status in any racial-ethnic group. Gestational age at delivery and at preeclampsia diagnosis among cases did not differ across racial-ethnic groups.

Cases had lower adjusted logarithmic mean levels of PIGF but higher adjusted logarithmic mean levels of sEng than controls for each of the three racial-ethnic groups ($P < 0.05$; Table 2). Cases also had higher adjusted mean levels of sVEGFR-1 than controls in Whites (7.78 log pg/mL versus 7.52 log pg/mL, $P < 0.05$) and Hispanics (7.73 log pg/mL versus 7.40 log pg/mL, $P < 0.05$) but not in Blacks (7.85 log pg/mL versus 7.69 log pg/mL, $P = 0.47$). Blacks had larger adjusted logarithmic means of PIGF in both cases and controls and higher adjusted logarithmic means of sVEGFR-1 in controls when compared to Whites and Hispanics ($P < 0.05$ for all comparisons). Blacks also had larger adjusted means of sEng in controls than Whites (8.58 log pg/mL versus 8.53 log pg/mL, $P < 0.05$) but smaller adjusted means of sEng in cases than Hispanics (8.93 log pg/mL versus 9.24 log pg/mL, $P < 0.05$). Analyte levels were similar between Whites and Hispanics, except that Hispanic cases had higher adjusted means of sEng (9.24 log pg/mL versus 9.05 log pg/mL, $P < 0.05$) and White controls had higher adjusted means of sVEGFR-1 (7.52 log pg/mL versus 7.40 log pg/mL, $P < 0.05$). The adjusted logarithmic mean levels of sVEGFR1 in cases were similar across all three racial-ethnic groups.

Logistic regression results with adjustment for covariates (Table 3) revealed strong associations of midtrimester PIGF, sVEGFR-1, and sEng levels with early-onset preeclampsia. Women with higher PIGF level were at lower risk for early-onset preeclampsia in all three racial-ethnic groups, with the weakest associations observed among Blacks (adjusted OR [AOR], 0.219; 95% CI, 0.124-0.385 per log pg/mL increase [$P < 0.05$] in comparison to AOR, 0.048; 95% CI, 0.026-0.088 in Whites, and AOR, 0.028; 95% CI, 0.013-0.060 in Hispanics). Elevated levels of sVEGFR-1 and sEng were positively associated with early-onset preeclampsia. Women with a single logarithmic unit increase of sEng levels had a more than five-fold increase in risk for early-onset preeclampsia in all three racial-ethnic groups with the weakest association observed in Blacks (AOR, 5.02; 95% CI, 2.56-9.86 [$P < 0.05$] in comparison to AOR, 36.87; 95% CI, 17.00-79.96 in Whites, and AOR, 86.68; 95% CI, 31.46-238.81 in Hispanics). There was no significant difference of PIGF and sEng in association with early-onset preeclampsia between Whites and Hispanics. Elevated odds of early-onset preeclampsia associated with higher sVEGFR-1 levels were observed in Whites (AOR, 3.24; 95% CI, 2.04-5.14) and Hispanics (AOR, 3.68; 95% CI, 2.16-6.29) but not in Blacks (AOR, 1.2; 95% CI, 0.7-2.1).

COMMENT

In this investigation, we observed a number of relationships suggesting that midtrimester angiogenic and antiangiogenic factors are associated with early-onset preeclampsia. We also observed patterns that suggested the strength of these relationships may differ by racial-ethnic group. While low levels of PIGF and high levels of sEng were strongly associated with early-onset preeclampsia across racial-ethnic groups, the associations were stronger in Whites and Hispanics than Blacks (AOR in Whites and Hispanics were more than 4.4 times the AOR in Blacks). Higher sVEGFR-1 levels were only associated with an increased risk of early-onset preeclampsia in women who were Hispanic or White. To our knowledge, this is the first study that describes the racial-ethnic differences of midtrimester angiogenic and antiangiogenic factors in association with early-onset preeclampsia.

The relationships between midtrimester PIGF, sEng, and sVEGFR-1 levels and early-onset preeclampsia have been widely documented.^{1,3} Previous studies have shown reduced PIGF levels²⁴ in both the first²⁵ and second²⁶ trimesters and elevated sEng^{24,25,27} and sVEGFR-1^{24,27,28} levels in the second trimester of preeclamptic pregnancies compared to pregnancies without preeclampsia. Results from this study confirmed previous findings and found very strong associations of PIGF and sEng levels and moderately strong associations of sVEGFR-1 levels with early-onset preeclampsia. Differences in the effect magnitude between the three analytes and early-onset preeclampsia have rarely been reported.

Racial-ethnic differences have been well reported in maternal circulation of many proteins such as alpha-fetoprotein²⁹ and tumor necrosis factor alpha (TNF- α)³⁰ but rarely mentioned for angiogenic and antiangiogenic factors. Most previous studies on angiogenic and antiangiogenic factors were conducted in White participants and there is a paucity of information on other ethnic groups.^{31,32} Black women were found to have slightly higher levels of PIGF, sEng, and sVEGFR-1 than White women and women of other races in one study³³ but in another study no difference in PIGF and sVEGFR-1 was found compared to White women.³⁴ A study of sVEGFR-1 levels by parity³⁵ also did not find significant differences in PIGF and sVEGFR-1 levels within the first or second pregnancies of Hispanic women compared to White women. Our study agreed with that of Mijal et al³³ indicating higher levels of PIGF and sVEGFR-1 in Black controls than White and Hispanic controls, and with that of Faupel-Badger et al³⁴ and Wolf et al³⁵ showing no PIGF differences between White and Hispanic controls. Our study also found lower levels of sVEGFR-1 in Hispanic than White controls, which is different from the Wolf et al³⁵ study. This study also detected higher sEng levels in Black compared to White controls.

Recent studies have advanced our understanding of etiology of preeclampsia although it remains largely unknown.^{1,3,36} Preeclampsia is hypothesized as the result of an exaggerated inflammatory response due to maternal constitutional factors, genetic predisposition, and/or immunological maladaptation.³ In the last decade a two-stage pathogenic model has gained acceptance as the underlying pathophysiology of early-onset preeclampsia.^{31,37-40} In the first stage, inadequate placentation due to shallow cytotrophoblastic invasion restricts blood supply to the placenta and creates a hypoxic placental environment in the first trimester. Oxidative stress then induces ischemic lesions to the placenta, which may promote the

release of a variety of molecules including but not limited to PIGF, sEng, and sVEGFR-1 into maternal circulation.^{1,41-44} In the second stage, the released molecules initiate a cascade of cellular and molecular changes via multiple pathophysiological mechanisms—angiogenesis, exaggerated inflammatory response, endothelial and vascular dysfunction, thrombosis, renin-angiotensin system activation, metabolic and dietary effects, and others such as neuroendocrine system effects¹⁶⁻²⁰—leading to maternal syndromes in the second trimester and later gestation.²¹ In support of angiogenesis as one of the most important mechanisms^{44,45} contributing to early-onset preeclampsia, this study detected very strong associations of lower PIGF levels and higher sEng levels with early-onset preeclampsia.

Our novel finding of racial-ethnic differences of PIGF and sEng levels in association with early-onset preeclampsia, if confirmed by other studies to not be due to other factors or mechanisms not studied within the current framework, suggests that there might be different regulations and/or processes of the angiogenic mechanism within racial-ethnic groups. It could also suggest that each racial-ethnic group has its own key pathophysiological mechanisms in the incidence for early-onset preeclampsia, if the racial-ethnic differences can be replicated in future research investigating other pathophysiological mechanisms for early-onset preeclampsia.

Strengths of the present study include its use of a nested case-control design to concurrently compare the distribution of midtrimester PIGF, sEng, and sVEGFR-1 levels in Whites, Hispanics, and Blacks and their associations with early-onset preeclampsia. Racial-ethnic groups were well defined, the outcome was restricted to early-onset preeclampsia, and the population-based sample size was large. Despite these strengths, there were a number of study limitations. First, the study used maternal serum specimens stored at -20°C rather than -80°C for more than five years by the California Biobank Program²² without immediate refrigeration after routine specimen collection. Although platelets, eosinophils, monocytes, and leukocytes in serum could also release angiogenic factors (i.e., sVEGFR-1) during clotting,⁴⁶ both serum and plasma have been widely used in previous studies with similar concentration ranges detected from the two specimen types.⁴⁶ Previous studies have documented the stability of angiogenic factors in specimens stored for more than 12 years²⁷ and have shown that serum levels of angiogenic factors were not affected by storage duration and temperature.^{3,47} Comparing the adjusted logarithmic means of PIGF, sEng, and sVEGFR-1 levels by storage years, we found no change in PIGF and sVEGFR-1 levels and a slight decrease in sEng levels with >12 storage years among Hispanic controls (Supplementary Table 1). Biomarker stability may be stronger at storage temperature of -80°C than at -20°C .⁴⁸ The impact of storage temperature on analyte values in this study is unknown; however, both case and control specimens were handled in a similar way, therefore, differential effect (bias) across case-control and racial-ethnic groups is unlikely. Second, this study tested PIGF, sEng, and sVEGFR-1 concentrations in maternal blood specimens routinely collected from participants in the Prenatal Screening Program during 15-20 weeks. Screening program participants, approximately 70% of women delivering live births in California,²⁰ broadly represent the California birth population in terms of race-ethnicity and education.⁴⁹ However, women under 20 or over 35 years of age, with late or no prenatal care, or public insurance, are less likely to participate.⁴⁹ It is not clear how these selection factors may influence the distribution of PIGF, sEng, and sVEGFR-1 and their

associations with early-onset preeclampsia. Future studies will need to have more complete coverage of the pregnant population. In addition, the angiogenic and antiangiogenic imbalance in preeclamptic pregnancy is considered to start at the end of first trimester and peak at mid-trimester to term.⁵⁰ While ready access and availability of banked specimens provided a unique opportunity to investigate the study questions, the one-time-only blood collection limited our ability to fully understand the concentration change over the course of pregnancy and to study PlGF, sEng, and sVEGFR-1 levels in early pregnancy in association with early-onset preeclampsia. Such an investigation represents an important next step.

In summary, this study confirmed the relationship between early-onset preeclampsia and angiogenic and antiangiogenic factors and found that racial-ethnic differences may exist. Our study results point to the need for future research in homogenous population groups stratified by race-ethnicity to potentially improve etiologic and prognostic value of these analytes.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Condensation

- Racial-ethnic differences were observed in angiogenic and antiangiogenic factors associated with early-onset preeclampsia.

FIGURE. Identification flowchart of early-onset preeclampsia cases and controls
Step-by-step sample selection. Exclusion rates in each step vary between race-ethnicity groups due to different population distributions and exclusion details by race-ethnicity groups.

Table 1

Selected maternal characteristics of early-onset preeclampsia cases and controls by racial-ethnic groups, California births, 2000-2007.

Characteristics	White		Hispanic		Black	
	Controls (N=916)	Cases (N=90)	Controls (N=709)	Cases (N=67)	Controls (N=738)	Cases (N=40)
Maternal age (year, mean $\pm$ SD)	30.00 $\pm$ 4.67	30.22 $\pm$ 5.26	26.22 $\pm$ 5.72	28.61 $\pm$ 6.49 ^a	26.58 $\pm$ 5.81	27.20 $\pm$ 5.60
High school graduate (%)	23.25	18.89	85.05	82.09	53.79	50.00
Nulliparity (%)	50.11	81.11 ^b	35.26	41.79	36.18	45.00
Maternal weight (kilograms) at or near specimen collection (mean $\pm$ SD)	70.02 $\pm$ 14.98	76.20 $\pm$ 17.85 ^a	67.22 $\pm$ 14.40	76.33 $\pm$ 18.98 ^a	78.03 $\pm$ 20.87	80.42 $\pm$ 21.18
Gestational age at specimen collection 18 weeks of gestation ^a (%)	15.61	14.44	25.81	29.85	27.51	22.50
Delivery < 26 weeks of gestation ^a (%)	NA	10.00	NA	13.43	NA	12.50
Preeclampsia diagnosis < 26 weeks of gestation ^c (%)	NA	14.44	NA	16.42	NA	15.00

All data are from birth certificates with the exception of gestational age.

SD, standard deviation; NA, not applicable.

^aStudent *t* test $P < 0.05$ comparing cases to controls

^bChi-square test $P < 0.05$ comparing cases to controls

^cGestational age based on prenatal screening and hospital charts for cases and prenatal screening only for controls

Table 2

Adjusted^f natural logarithmic mean and standard error of PlGF, sEng, and sVEGFR-1 concentrations (log pg/mL) among cases and controls by racial-ethnic groups, California births, 2000–2007

Percentiles	White		Hispanics		Black	
	Controls (N=916)	Cases (N=90)	Controls (N=709)	Cases (N=67)	Controls (N=738)	Cases (N=40)
PlGF						
Mean	4.78 ^{a b}	4.08 ^{a c}	4.70 ^{a d}	3.89 ^{a e}	5.20 ^{a b d}	4.69 ^{a c e}
Standard error	0.04	0.06	0.04	0.07	0.03	0.09
sEng						
Mean	8.53 ^{a b}	9.05 ^{a f}	8.54 ^a	9.24 ^{a e f}	8.58 ^{a b}	8.93 ^{a e}
Standard error	0.03	0.04	0.03	0.05	0.02	0.06
sVEGFR-1						
Mean	7.52 ^{a b g}	7.78 ^a	7.40 ^{a d g}	7.73 ^a	7.69 ^{b d}	7.85
Standard error	0.04	0.07	0.04	0.07	0.04	0.09

^f Adjusted by maternal age, education, parity, specimen storage year, maternal weight at or near specimen collection, and gestational week at specimen collection.

PlGF, placental growth factor; sEng, soluble endoglin; sVEGFR-1, soluble vascular endothelial growth factor receptor 1.

^a $P < 0.05$ between adjusted means of cases and controls of the same race-ethnicity.

^b $P < 0.05$ between adjusted means of White controls and Black controls

^c $P < 0.05$ between adjusted means of White cases and Black cases

^d $P < 0.05$ between adjusted means of Hispanic controls and Black controls

^e $P < 0.05$ between adjusted means of Hispanic cases and Black cases

^f $P < 0.05$ between adjusted means of White cases and Hispanic cases

^g $P < 0.05$ between adjusted means of White controls and Hispanic controls

Table 3

Odds ratios and 95% confidence intervals of PlGF, sEng, and sVEGFR-1 concentrations (per log pg/mL increase) in association with early-onset preeclampsia, by racial-ethnic groups, California births, 2000-2007

Analytes	White			Hispanics			Black		
	OR 95% CI			OR 95% CI			OR 95% CI		
	Unadjusted	Adjusted ^a	Unadjusted	Adjusted ^a	Unadjusted	Adjusted ^a	Unadjusted	Adjusted ^a	Adjusted ^a
PlGF	0.052 ^b 0.030-0.089	0.048 ^b 0.026-0.088	0.044 ^c 0.023-0.084	0.028 ^c 0.013-0.060	0.244 ^{b c} 0.143-0.417	0.219 ^{b c} 0.124-0.385			
sEng	26.10 ^b 13.33-51.08	36.87 ^b 17.00-79.96	57.91 ^c 23.72-141.40	86.68 ^c 31.46-238.81	4.18 ^{b c} 2.29-7.64	5.02 ^{b c} 2.56-9.86			
sVEGFR-1	2.87 1.88-4.39	3.24 2.04-5.14	2.66 1.64-4.32	3.68 2.16-6.29	1.62 0.91-2.89	1.61 0.88-2.95			

^a Adjusted by maternal age, education, parity, specimen storage year, maternal weight at or near specimen collection, and gestational week at specimen collection.

^b $p < 0.05$ between OR of Whites and Blacks

^c $p < 0.05$ between OR of Hispanics and Blacks

OR, odds ratio; CI, confidence interval