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Fetal—Not Maternal—*APOL1* Genotype Associated with Risk for Preeclampsia in Those with African Ancestry

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Black Americans are at increased risk for preeclampsia. Genetic variants in apolipoprotein L1 (*APOL1*) account for much of the increased risk for kidney disease in blacks. *APOL1* is expressed in human placenta and transgenic mice expressing *APOL1* develop preeclampsia. We evaluated the role of *APOL1* variants in human preeclampsia. We determined maternal and fetal *APOL1* genotypes in black women with preeclampsia in two populations. At Einstein Montefiore Center (EMC) Affiliated Hospitals, we studied 121 pregnancies in black women with preeclampsia. At University of Tennessee Health Science Center (UTHSC), we studied 93 pregnancies in black women with preeclampsia and 793 pregnancies without preeclampsia. We measured serum markers of preeclampsia soluble fms-like tyrosine kinase 1 (sFlt-1), placental growth factor (PlGF), and soluble endoglin (sEng). Fetal *APOL1* high-risk (HR) genotype was associated with preeclampsia, with odds ratios at EMC and UTHSC of 1.84 (95% CI 1.11, 2.93) and 1.92 (95% CI 1.05, 3.49), respectively. Maternal *APOL1* HR genotype was not associated with preeclampsia. Mothers with the fetal *APOL1* HR genotype had more cerebral or visual disturbances (63% versus 37%, $p = 0.04$). In addition, fetal *APOL1* HR genotype was associated with a higher sFLT-1/PlGF ratio at birth ($p = 0.04$). Fetal *APOL1* high-risk genotype increases the risk for preeclampsia, likely by adversely affecting placental function. Further research is needed to assess whether *APOL1* genetic testing can predict preeclampsia and improve pregnancy outcomes.

Introduction

Preeclampsia (PEE1 [MIM: 189800]) is characterized by systemic hypertension and maternal systemic endothelial dysfunction in pregnancy.[1,2] It results from placentation defects with an imbalance in angiogenic factors including soluble fms-like tyrosine kinase 1 (sFlt-1).[3,4] It accounts for 16% of maternal deaths in developed countries[5] and up to 900,000 infant deaths per year globally.[6] Preeclampsia is more prevalent and more severe in women with African ancestry, particularly those from sub-Saharan Africa.[7–9] While more than 50 candidate genes have been evaluated as possible genetic risk factors for preeclampsia, relatively few studies have focused on subjects with African ancestry.[6,10–15]

Recent discoveries have shown that common coding variants in the apolipoprotein L-1 gene (*APOL1* [MIM: 603743]) that are found only on African chromosomes, termed G1 and G2, are potent risk factors for a spectrum of kidney diseases in black Americans. The *APOL1* G1 allele comprises of two missense variants: rs73885319 (c.1024A>G [p.Ser342Gly], termed G1g) and rs60910145 (c.1152T>G [p.Ile384Met], termed G1m). The *APOL1* G2 allele consists of a 6 bp in-frame deletion, rs71785313 (c.1164_1169delTTATAA [p.Asn388_Tyr389del]). The odds ratios among homozygotes or compound heterozygotes (G1/G1, G2/G2, or G1/G2), termed the high-risk genotypes, vary from ~5 in hypertension-attributed kidney disease to ~29 and ~89 in HIV-associated nephropathy in American blacks and South Africans, respectively.[16–18] *APOL1* arose late in primate evolution but has been retained as a functional gene by only a few primates, including humans. It encodes apolipoprotein L1 and provides innate immunity against most African trypanosomes.[19] APOL1 G1 and G2 gene variants extend protection against trypanosomes causing human African

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trypanosomiasis, which are resistant to the ancestral form of *APOL1*.[20] The "high-risk" genotypes are found in approximately 12% of American blacks.[21]

Several observations support a role for *APOL1* in the development of preeclampsia. Among tissues, placenta has one of the highest levels of both APOL1 mRNA and protein expression.[22–26] Transgenic mice expressing either G0 or G2 *APOL1* in the placenta develop a preeclampsia/eclampsia phenotype with pregnancy-induced hypertension, proteinuria, and seizures, with a more severe phenotype among *APOL1* G2 transgenic mice.[27] Circulating autoantibodies against APOL1 can be found in the blood of women with preeclampsia, and the overall level of APOL1 circulating in the blood of women with preeclampsia is higher than that in women without preeclampsia.[28,29]

We hypothesized that *APOL1* variants play a role in the excess risk for preeclampsia among blacks. To address the relationship of *APOL1* variants with preeclampsia in blacks, we analyzed a case-only study of 121 infants born to mothers with preeclampsia from Einstein Montefiore Center and a case-control study of mothers and infants enrolled in the University of Tennessee Health Science Center CANDLE study, which included 93 pregnancies complicated by preeclampsia and 793 pregnancies without preeclampsia.

Subjects and Methods

Study Design and Oversight

We genotyped the *APOL1* G1 and G2 renal risk variants in mothers and infants in two study locations. The first was a case-only study, at Einstein Montefiore (EMC)-affiliated hospitals in New York, NY, which included 121 pregnancies complicated by preeclampsia. The second was a case-control study at the University of Tennessee Health Sciences Center (UTHSC), Memphis, TN, which included 93 pregnancies with preeclampsia and 793 control pregnancies without preeclampsia. The participants at UTHSC were recruited as part of the Conditions Affecting Neurocognitive Development and Learning in Early Childhood (CANDLE) study.[30] At each study center, institutional review boards approved the study protocol in advance. In the EMC study, informed consent was not required. In the UTHSC study, subjects or their guardians provided written informed consent.

EMC Case-Only Study

Study Population

Births were selected from the 769 births at Einstein Montefiore Center (EMC)-affiliated hospitals between 1997 and 2016 whose mothers (1) were identified as "Black or African American" in the electronic medical record and (2) had "preeclampsia" mentioned in their placental pathology report. For all births with complications such as preeclampsia, placentas were routinely submitted to pathology. From the 769 births, the most recent 186 births with placental pathology samples available for genotyping were obtained for study. If a mother had more than one birth that met inclusion criteria within this time frame, only the first birth was included. Multiple gestations and births without contemporaneous physician diagnosis were excluded. Fetal DNA was successfully genotyped for 121 births and maternal DNA was successfully genotyped in 24 births. Maternal specimen genotyping was limited because of the labor-intensive collection process and low DNA yield; 35 maternal samples were sent for genotyping, 24 (69%) were successfully genotyped.

The prevalence of the *APOL1* genotypes in the preeclampsia cohort was compared to a healthy control population comprised of two separate populations of healthy volunteers: (1) 176 healthy black blood donors without a history or evidence of kidney disease, recruited in Bethesda, MD[31] and (2) 923 black subjects from the southeastern United States (North Carolina, South Carolina, Georgia, Virginia, and Tennessee) without a history of kidney disease or diabetes or first-degree relatives with these diseases.[32]

Design and Data Collection

Physician diagnosis of preeclampsia in the maternal medical record was confirmed, and clinical data required to meet the American College of Obstetrics and Gynecology diagnostic criteria for preeclampsia were collected from the medical record. Data collected included: the presence of new-onset hypertension, proteinuria ($\geq$300 mg of protein per 24 hr, a protein/creatinine ratio of >0.3 g/g, and/or a urine dipstick for protein of $\geq$1+), and evidence of other end-organ dysfunction such as thrombocytopenia (platelet count less than 100,000/μL), impaired liver function (liver enzyme values $\geq$ twice the normal upper limit or severe persistent right upper quadrant pain not responsive to medication or accounted for by another diagnosis), new-onset renal insufficiency (serum creatinine $\geq$ 1.1 mg/dL or a doubling of the serum creatinine not accounted for by another diagnosis), pulmonary edema, or cerebral dysfunction.[1] For women with preexisting hypertension, we required evidence of worsening hypertension, a systolic blood pressure of $\geq$160 mm Hg or a diastolic blood pressure of $\geq$110 mm Hg. Preeclampsia was considered severe if the patient had a systolic blood pressure $\geq$160 mm Hg or a diastolic blood pressure $\geq$110 mm Hg, thrombocytopenia, impaired liver function, progressive renal insufficiency, pulmonary edema, or new-onset visual or cerebral dysfunction.[1] HELLP syndrome (hemolysis with a microangiopathic blood smear, elevated liver enzymes, and low platelet count) in pregnancy was considered present when a physician made the diagnosis contemporaneously.

In addition to evaluating whether or not pathological changes consistent with preeclampsia were noted on the pathology report, two independent pathologists, masked to the fetal *APOL1* genotype, evaluated 19 placental samples from births with the fetal *APOL1* HR genotype and 17 samples with the fetal *APOL1* LR genotype. Placental pathology slides were examined for the presence or absence of thrombosis, hypertrophic changes, atherosis, fibrinoid necrosis, retroplacental hemorrhage or hematoma, chronic villitis, infarction, intervillous thrombi, distal villous hypoplasia, intervillous fibrin, accelerated maturation (for less than 34 weeks gestation, greater than 20% syncytial knots was considered increased, and for those 34 to 38 weeks gestation, greater than 30% syncytial knots was considered increased), and villous edema or dysmaturity.[33–35] Decidual vasculopathy was evaluated by (1) the presence or absence of decidual vaculopathy in fetal membranes or basal plate and (2) with an overall decidual vasculopathy score, from 0 to 4 (0 = none, 4 = most severe).

DNA Isolation and Genotyping

DNA was isolated from formalin-fixed, paraffin-embedded pathology specimens. Fetal DNA was extracted from umbilical cord and/or fetal membranes. These tissues contain fetal DNA only.

Maternal DNA was extracted from the maternal aspect of the placental tissue.

DNA was genotyped using TaqMan assays (ThermoFisher Scientific). *APOL1* G1 allele is comprised of two missense variants, rs73885319 (G1g) and rs60910145 (G1m); however, the presence of only the G1g variant is sufficient to define the G1 risk allele. The *APOL1* G2 allele consists of a 6 bp in-frame deletion, rs717185313. High-risk genotypes were defined as those containing two high-risk alleles (G1/G1, G1/G2, or G2/G2); low-risk genotypes were defined as those containing zero or one risk allele. In order to assess whether an excess of G2 homozygosity in the fetal tissue was the result of allele dropout, we performed additional quality control measures that included repeat PCR with and without a pre-amplification step,[36] Sanger sequencing, and manual visualization of genotype clusters. These additional steps indicated that our genotype assignments were robust and reproducible. These steps, in addition to the fact that we did not have any wild-type mothers with two risk allele infants, argued against allele drop out.

Statistical Analysis

Results were reported as proportions for categorical variables, and median with interquartile range for continuous variables. Significance was evaluated using a two-tailed Fisher exact test for categorical variables and the Wilcoxon rank sum test with continuity correction for continuous variables. Odds ratios were calculated using a median-unbiased estimation along with associated exact 95% confidence intervals using the mid-p method.[37] Allele distribution was tested for deviation from Hardy-Weinberg equilibrium using an exact test.[38] We used logistic regression to examine the relationship of genotype (high or low risk) to specific changes on placental histopathology, and linear regression analyses to examine the relationships of genotype (high or low risk) to gestational age and birth weight, controlling for maternal age, delivery type, maternal obesity, and infant gender as covariates. Linear regression analyses were performed using SPSS Statistics v.24.0 (IBM); all other statistical analyses were performed using R (Web Resources).

UTHSC Case-Control Study

Study Population

Data and patient samples were obtained from the University of Tennessee Health Science Center (UTHSC) CANDLE project (Conditions Affecting Neurocognitive Development and Learning in Early Childhood), a longitudinal study of 1,503 mothers and infants including 999 black women. In the CANDLE project, healthy women of any gravidity in the second trimester of pregnancy were approached for enrollment from an urban hospital obstetric clinic or from several community obstetric practices.[30] Women with chronic diseases requiring medications (e.g., hypertension, diabetes, sickle cell disease), known pregnancy complications at time of enrollment (e.g., complete placenta previa, oligohydramnios, or preeclampsia), or plans to deliver at a nonparticipating hospital were excluded.

Because the primary purpose of the CANDLE project was focused on early life neurodevelopment, there was a broad collection of data related to socio-demographic risk factors, maternal health, family dynamics, and also pregnancy characteristics. The group of 93 black pregnancies with preeclampsia included all pregnancies with a physician diagnosis of preeclampsia in the maternal pregnancy medical record, and the control group of 793 black pregnancies included all pregnancies without a diagnosis of preeclampsia. The nature of the data collection process did not allow us to further assess whether the cases fully met the American College of Obstetrics and Gynecology diagnostic criteria for preeclampsia, as was done in the EMC population.

Design and Data Collection

Blood was collected from women and children at repeated intervals, starting in the second trimester of pregnancy and continuing for 4 years following birth. Participants provided information on race and ethnicity via self-administered questionnaires. Gestational age was determined by ultrasound or by the mother's report of the last menstrual cycle. Birth weight, delivery type, and pregnancy complications, including preeclampsia, were abstracted from the mother's medical record.

DNA Isolation and Genotyping

Fetal DNA was isolated from the umbilical cord or cord blood. Maternal DNA was isolated from blood specimens. DNA was genotyped as described above for the EMC case-only study. TaqMan genotype calls were validated on a subset of subjects by Sanger sequencing for both groups of subjects.

Assessment of Serum Markers of Preeclampsia

Serum samples were analyzed from 33 mothers, 15 with preeclampsia and 18 without, at two time points, the second trimester (termed T1) and at birth (termed T2). Approximately half of the mothers in each group had the fetal high-risk genotype and half had the fetal low-risk genotype. Serum soluble markers of preeclampsia, sFlt-1 (which is elevated in preeclampsia), PlGF (which is depressed in preeclampsia), and soluble endoglin (s-Eng, which is elevated in preeclampsia),[40] were measured with a standard ELISA assay performed using a fully automated and custom multiplexed ELISA on the ELLA platform (ProteinSimple). Per manufacturer quality control the linear ranges for the ELISA targets were as follows: s-Eng 21.8–147,150 mg/mL, PlGF 4.28–4,410 pg/mL, sFlt-1 3.38–4,650 pg/mL. Per manufacturer, overall coefficient of variation (CV) $< 10\%$ (our average CV was less than 5%) and assays were benchmarked against Quantikine ELISA kit with $R^2 > 0.9$. The levels observed in the samples were within the anticipated ranges for other ELISA formats in current clinical use.

Statistical Analysis

Descriptive analyses were performed using univariate and bivariate analyses, with the primary outcome and exposure variables and covariates reported as proportions for categorical variables, and median and interquartile range (IQR) for continuous variables. In the control population, allele distribution was tested for deviation from Hardy-Weinberg equilibrium using an exact test.[38] Additive and genotypic analyses were conducted using linear and logistic models with the recoded haplotypes to assess the association of preeclampsia, birth weight, and gestational age with maternal and child *APOL1* genotype. Point estimates along with associated 95% confidence intervals were calculated and two-sided statistical significance used for all statistical inferences. Analyses were done on the entire group of case subjects with preeclampsia along with control subjects, for subgroups excluding women with elective induction or elective Cesarean section, and finally excluding all women with elective induction or Cesarean section of any type. Differences in serum marker ratios were determined by a two-tailed, non-parametric Kruskal-Wallis test performed with GraphPad Prism 6.0 (Graphpad Prism Software). All other data analyses were conducted using R.

Principal components analysis was performed in Eigenstrat[41] on a subset of samples with high-quality genome-wide association study (GWAS) data available. Data were pruned for LD and filtered to a minor allele frequency of 0.2. Re-analysis was performed using

Table 1. *APOL1* Genotype Association with Preeclampsia, by Maternal and Fetal *APOL1* Genotype

Study Population	Total (n)	Low-Risk Genotype (n) (%)		High-Risk Genotype (n) (%)	Odds Ratio (95% CI)
		0 RA	1 RA	2 RA	
EMC Case-Only Study					
Cases with Preeclampsia					
Fetal genotype	121	42 (35%)	55 (45%)	24 (20%)	1.84 (1.11, 2.93)[a]
Maternal genotype	24	11 (46%)	11 (46%)	2 (8%)	0.72 (0.11, 2.49)
Control, General Population	1,099	486 (44%)	482 (44%)	131 (12%)	
UTHSC Case-Control Study					
Fetal Genotype					
Cases, with preeclampsia	73	29 (40%)	28 (38%)	16 (22%)	1.92 (1.03, 3.42)[a]
Controls, without preeclampsia	666	288 (43%)	293 (44%)	85 (13%)	
Maternal Genotype					
Cases, with preeclampsia	93	38 (41%)	49 (53%)	6 (6%)	0.54 (0.21, 1.17)
Controls, without preeclampsia	793	330 (42%)	373 (47%)	90 (11%)	

RA refers to *APOL1* risk allele, either G1 (p.Ser342Gly mutation) or G2 (a 6 bp deletion p.Asn388_Tyr389del). In the EMC cohort, the crude odds ratio was calculated by median-unbiased estimation and exact confidence interval were calculated using the mid-p method. In the UTHSC cohort, odds ratios were performed using multivariate analyses and were adjusted for maternal age, household income, and child gender.
[a]p value < 0.05

the same linear model including the top ten eigenvectors as covariates in the entire subset only.

Results

EMC Case-Only Study

For all 121 pregnancies in the EMC study, the average age at delivery was 29.2 years. The mean gestational age was 34.6 weeks. Forty (33%) of the infants were born full term ($\geq$37 weeks). Ninety-four (78%) were born at <38 weeks and 67 (55%) at <36 weeks; 70 (58%) infants had a birth weight of <2,500 g, and 27 (22%) were born at <1,500 g.

Of the 121 infants, 42 (35%), 55 (45%), and 24 (20%), had 0, 1, or 2 risk alleles, respectively. Of the 24 mothers, 11 (46%), 11 (46%), and 2 (8%) had 0, 1, or 2 risk alleles, respectively (Table 1). The genotype distribution for the fetal G1 allele (52.9% (+/+), 41.3% (G1/+), 5.8% (G1/G1)) did not deviate from Hardy-Weinberg equilibrium expectations ($p = 0.51$). However, the genotype distribution for the fetal G2 allele (75.2% (+/+), 17.4% (G2/+), and 7.4% (G2/G2)), there was an excess of G2/G2 homozygotes, with 9 observed versus 3 expected ($p < 0.0001$). Similarly, the maternal genotype distribution had an excess of G2 alleles (Table S7).

Black infants in pregnancies with preeclampsia were 1.8 times more likely to carry fetal *APOL1* HR genotypes compared to the control black population, 20% versus 12% (OR 1.84; 95% CI 1.11, 2.93) (Figure 1). The percentage of black mothers with preeclampsia carrying maternal HR genotypes did not differ significantly from the general black population, 8% versus 12% (OR 0.72; 95% CI 0.11, 2.50).

Of the 121 pregnancies from EMC, all had a physician diagnosis of preeclampsia and 113 (93%) met the American College of Obstetrics and Gynecology diagnostic criteria for preeclampsia. Of the subjects, 46% had early-onset preeclampsia; timing of preeclampsia onset did not vary by fetal *APOL1* genotype. One hundred and sixteen (96%) had changes on placental pathology observed in the setting of preeclampsia such as decidual vasculopathy and villous dysmaturity. The presence of changes in pathology did not vary with fetal or maternal *APOL1* genotype (Table S4), nor did the prevalence of preeclampsia risk factors (maternal age, nulliparity, obesity, maternal history of kidney disease, hypertension, diabetes, systemic lupus erythematosus, anti-phospholipid syndrome, history of prior pregnancy with preeclampsia, or history of spontaneous abortion) (Tables 2 and S3, respectively). One hundred and nineteen births (98%) had severe preeclampsia and 12 (10%) had hemolysis, elevated liver enzymes, and low platelets (HELLP) syndrome (Table S5). The presence of severe preeclampsia or HELLP did not vary by fetal or maternal *APOL1* genotype. However, mothers with the fetal *APOL1* HR genotype were more likely than those with *APOL1* LR genotype to experience cerebral or visual disturbances (63% versus 37%, p value 0.04). Perinatal outcomes such as infant gender, gestational age, birth weight, and APGAR score at 1 min did not vary by maternal or fetal *APOL1* genotype (Table 2). However, pregnancies with the fetal *APOL1* HR genotype had lower APGAR scores at 5 min (9.0 versus 8.0, p value 0.01). In linear regression analyses that included genotype (high or low risk), maternal age, delivery type, maternal obesity, and infant gender, none

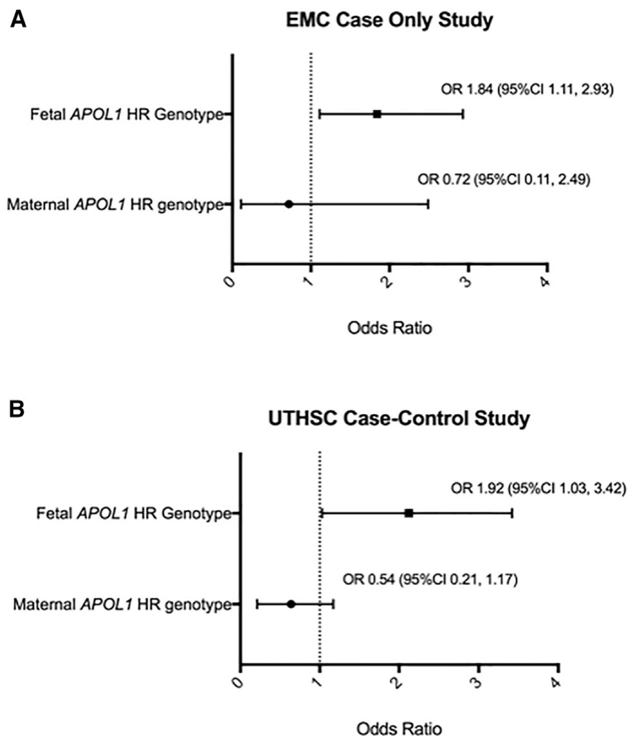

Figure 1. APOL1 Genotype Association with Preeclampsia, by APOL1 Maternal and Fetal Genotype
(A) Odds ratio and confidence intervals in the EMC, case-only population by fetal and maternal genotype.
(B) Odds ratio and confidence intervals for the UTHMC, case-control population by fetal and maternal genotype.
In both the EMC and UTHSC study populations, the presence of the fetal HR genotype resulted in a greater risk of developing preeclampsia. High-risk APOL1 genotypes are those with two risk alleles (G1/G1, G2/G2, and G1/G2). In the EMC cohort the crude odds ratio was calculated by median-unbiased estimation and exact confidence interval were calculated using the mid-p method. In the UTHSC cohort, odds ratios were performed using multivariate analyses and were adjusted for maternal age, household income, and child gender.

of the independent variables was significantly associated with either gestational age or birth weight.

UTHSC Case-Control Study

Genotype data were available from 921 of 999 black women and 747 black children in the CANDLE cohort. Of those with genotype data, preeclampsia status was known for only 886 mothers and 739 children. For the 921 pregnancies, the average age at delivery was 24.7 years. A total of 813 (88%) infants were born full term ($\geq$37 weeks), 81 (9%) were born at $\leq$36 weeks, and the mean gestational age was 38.7 weeks. There were 83 (9%) infants with a birth weight of $<$2,500 g and 15 (1.6%) were born at $<$1,500 g (Table S2). Full details of the complete CANDLE study population are discussed elsewhere.[30]

Of the 921 mothers for whom genotype data were available, 384 (41.7%), 437 (47.4%), and 100 (10.9%) had 0, 1, or 2 risk alleles, respectively. Among the 747 children, 318 (42.6%), 326 (43.6%), and 103 (13.8%) had 0, 1, or 2 risk alleles, respectively (Table S2). Tests for deviation from Hardy-Weinberg equilibrium were performed separately in mothers and children, and neither group showed significant deviation from expected genotype proportions ($p > 0.1$ for both groups, Table S7).

Multivariate analyses were adjusted for maternal age, household income, and child sex. An increased risk for preeclampsia was seen in association with fetal *APOL1* high-risk genotype. Among all pregnancies, the high-risk genotype was associated with an increased risk for preeclampsia (OR 1.92; 95% CI 1.03, 3.42) (Table 1). When electively induced labor and elective Cesarean sections were excluded, leaving 68 cases for analysis, the HR genotype was associated with a greater than 2-fold increased odds ratio for preeclampsia (OR 2.09; 95% CI 1.10, 3.97). When the analysis was repeated with exclusion of all Cesarean sections, leaving 41 case subjects for analysis, the increase in risk was not statistically significant (OR 1.54; 95% CI 0.60, 3.97). Based on our main finding of an odds ratio of 1.92, the population attributable risk of the fetal HR genotype for preeclampsia is 12.3%, suggesting that 1 in 8 pregnancies with preeclampsia among blacks is attributable to the presence of the fetal *APOL1* HR genotypes.

In addition to evaluating the risk for preeclampsia under a recessive model, we also evaluated the independent effects of the G1 and G2 alleles, comparing 1 versus 0 allele, 2 versus 0 alleles, and 2 versus 1 alleles. Under these models we did not find a significant association of fetal *APOL1* genotype with preeclampsia, supporting a recessive model of inheritance (Table S8).

There was no association between maternal *APOL1* status and gestational age among all pregnancies, in the analysis excluding mothers with electively induced labor or elective Cesarean sections, or in the analysis that excluded all Cesarean sections. No association was observed between fetal *APOL1* status and gestational age among all pregnancies, or when the analysis excluded electively induced labor or elective Cesarean sections, or all Cesarean sections.

Similarly, no association was observed between maternal or fetal *APOL1* status and birth weight among all pregnancies, when the analysis excluded mothers who had electively induced labor or elective Cesarean sections, or when the analyses excluded all Cesarean sections. The one exception to this was in the analysis of fetal *APOL1* status and gestational age among all pregnancies, where the high-risk genotypes were associated with a 103.7 g decrease in birth weight compared to children with 0 or 1 risk alleles ($p < 0.0108$). No association was observed between maternal or fetal *APOL1* genotype and gestational age when the analysis excluded those with electively induced labor or elective Cesarean sections or when it excluded all Cesarean sections (Table S2).

Reanalysis of the data including available principal components on 24 case subjects and 279 control subjects with

Table 2. Characteristics of Preeclamptic Births in EMC Case-Only Study, by Fetal *APOL1* Genotype

Fetal *APOL1* Genotype	All (n = 121)	*APOL1* LR (n = 97)	*APOL1* HR (n = 24)	p Value
Maternal Risk Factors for Preeclampsia				
Maternal age, year (IQR)	29.0 (11.0)	28.0 (10.0)	31.5 (8.5)	0.09
Nulliparity, n (%)	25 (21%)	21 (22%)	4 (17%)	0.78
Obesity, n (%)	30 (25%)	26 (27%)	4 (17%)	0.43
History of hypertension, n (%)	34 (28%)	27 (28%)	7 (29%)	1.00
History of diabetes, n (%)	11 (9%)	9 (9%)	2 (8%)	1.00
History of prior preeclampsia, n (%)	22 (18%)	17 (18%)	5 (21%)	0.77
History of spontaneous abortion, n (%)	23 (19%)	16 (16%)	7 (29%)	0.16
Perinatal Outcomes				
Early-onset preeclampsia, n (%)	56 (46%)	43 (44%)	13 (54%)	0.50
Cesarean delivery, n (%)	78 (64%)	62 (64%)	16 (67%)	1.00
Gestational age, weeks (IQR)	35.4 (5.6)	35.7 (5.0)	34.4 (6.2)	0.29
Birth weight, g (IQR)	2,360 (1,335)	2,415 (1,280)	2,193 (1,064)	0.35
Apgar score, 1 min (IQR)	8.0 (3.0)	8.0 (3.0)	7.0 (5.0)	0.07
Apgar score, 5 min (IQR)	9.0 (1.0)	9.0 (1.0)	8.0 (1.0)	0.01[a]

RA refers to *APOL1* risk allele, either G1 (p.Ser342Gly mutation) or G2 (a 6 bp deletion p.Asn388_Tyr389del). The HR genotype consists of 2 RA. The LR genotype consists of 0 or 1 RA. Continuous variables are reported as median and interquartile range in parentheses. The high-risk *APOL1* genotype group was compared to the low-risk *APOL1* genotype group using the Wilcoxon rank sum test with continuity correction continuous variables and a two-tailed Fisher exact test for categorical variables.
[a]p value $\leq$ 0.05

available high-quality GWAS data strongly supported the unadjusted analysis (OR 4.65; 95% CI 1.66, 12.97). Given the already reduced sample size, the subgroups were not examined.

Serological markers altered in the setting of preeclampsia including sFlt-1, PlGF, and sENG were assessed in the second trimester and at birth (Figure 2).[40,42] At birth, PlGF was significantly suppressed in women with preeclampsia and the fetal *APOL1* HR genotype (p = 0.01). All women with preeclampsia (with either the fetal HR or LR genotype) had elevated sFlt-1/PlGF ratios at birth when compared to women without preeclampsia, but the sFlt-1/PlGF ratio was significantly higher in mothers with the fetal *APOL1* HR genotype when compared to those with the LR genotype at birth (p = 0.04) (Figure 2). In current practice, sFlt-1/PlGF ratios >38 are associated with suspicion for pre-eclampsia[43] and a cut-off of 85 aids in the diagnosis of pre-eclampsia.[44] At birth, 5/10 (50%) women with preeclampsia and the fetal LR genotype had ratios greater than 38 and only 2/10 (20%) had ratios above 85. In comparison, all women with preeclampsia and the fetal HR genotype had ratios greater than 38 and 4/7 (57%) had ratios above 85. Serum levels of sFlt-1 and PIGF measured in the second trimester did not vary among the four groups of women with the fetal *APOL1* HR or LR genotype and with or without preeclampsia (Figure 2). Serum sEng did not vary between the four groups of women at either time point.

Discussion

In two independent studies, pregnancies in black women with fetal *APOL1* high-risk genotypes were more likely to be complicated by preeclampsia, with data suggesting that up to 1 in 8 case subjects of preeclampsia in black women may be attributable to the fetal *APOL1* HR genotype. This would account for approximately 12%–14% of the increased rate of preeclampsia in African Americans. These women were also more likely to experience visual and cerebral dysfunction. These findings add to our understanding of the pathogenesis of preeclampsia and could have implications for the clinical care of pregnant women of African descent.

There is one prior study, by Robertson et al., which contains data regarding *APOL1* genotype and risk for preeclampsia.[45] That study's primary focus was the association of *APOL1* genotype with prematurity. In the part of the study by Robertson et al. that addressed preeclampsia, fetal *APOL1* genotyping was available from only one of the two cohorts, a composite outcome was assessed (maternal hypertension, preeclampsia, and eclampsia) rather than preeclampsia only, and the G2 allele data were based on imputation only—all aspects that may have reduced their power to detect an association.

An association of fetal *APOL1* genotype with preeclampsia is consistent with findings within the transgenic mouse model reported by Bruggeman et al., where mice expressing *APOL1* G0 or, to a greater extent, G2 transgenes

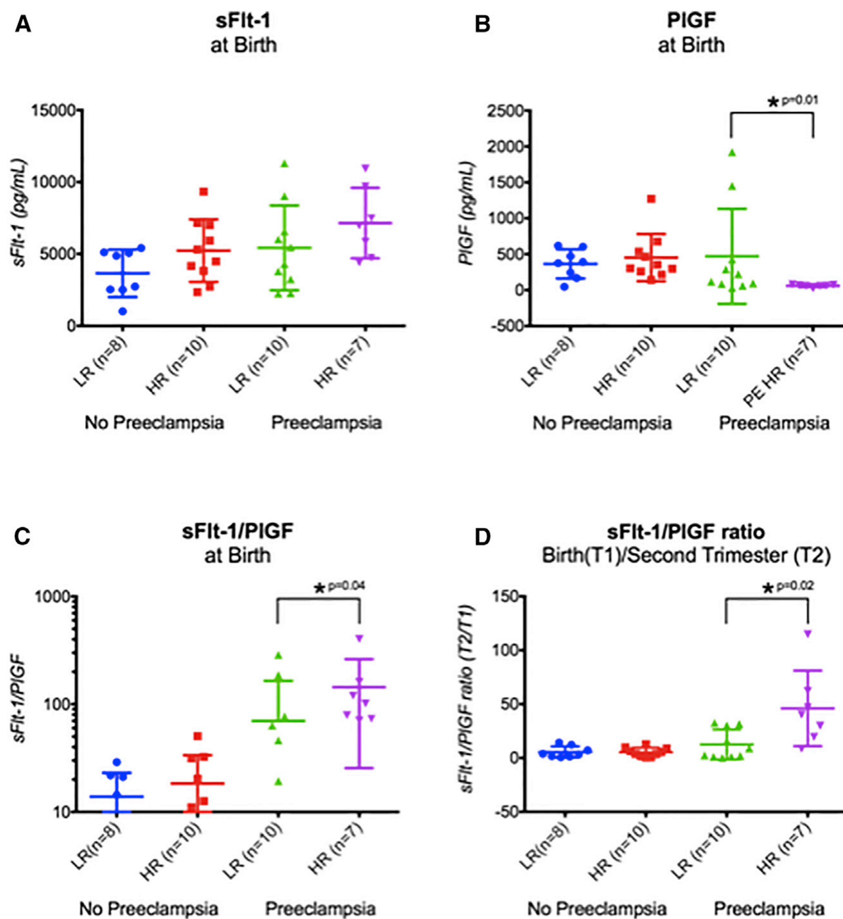

Figure 2. Soluble Serum Markers of Preeclampsia in Women with and without Preeclampsia in the UTHSC Case-Control Study, by Fetal APOL1 Genotype

(A) At birth, mean sFlt-1 in women with preeclampsia and the fetal HR genotype was higher, the difference was not significant.
(B) At birth, PlGF was markedly suppressed in women with preeclampsia and the fetal HR genotype (p value = 0.01).
(C) At birth, the sFlt-1/PlGF ratio was significantly elevated in all women with preeclampsia, compared to all women without preeclampsia. However, the sFlt-1/PlGF ratio was significantly higher in preeclamptic mothers and the fetal APOL1 HR genotype when compared to preeclamptic mother's with the fetal LR genotype at birth (p value 0.04).
(E) There was an even more markedly elevated ratio of sFlt-1/PlGF at birth to the second trimester (or T1/T3) in these same mothers (p value 0.02).
Scatterplot shows the mean and the standard deviation within each group. p values determined using a two-tailed, non-parametric Kruskal-Wallis test. *p value < 0.05.

developed preeclampsia; G1 mice were not available for study.[27] APOL1 orthologs are not present in most mammals, including mice. In that study, preeclampsia was seen not only in dams carrying the *APOL1* transgenes, but also in dams lacking the *APOL1* transgene carrying pups expressing the *APOL1* G0 or G2 transgenes. Further evidence that high-risk *APOL1* plays a role in preeclampsia is strengthened by our finding that the ratio of sFlt-1-1/PlGF not only is elevated in the pregnancies with preeclampsia, but is even higher in pregnancies with the fetal *APOL1* high-risk genotype.

The fact that it is the fetal and not the maternal *APOL1* genotype associated with higher risk for preeclampsia is consistent with the fact that preeclampsia is thought to arise from abnormalities in placentation, a process contributed to by both the mother and the developing fetus.[6] Based on inheritance patterns and epidemiological data, it is thought that up to 50% of the risk for preeclampsia may be due to genetics and that both maternal and fetal genetic factors seem to play a role.[6,46] In fact the first genome-wide significant susceptibility locus for preeclampsia was recently discovered near *FLT* in a genome-wide association study (GWAS) of offspring and not mothers from births with preeclampsia. Of note, similar to prior studies, this GWAS contained only samples of European descent.[13] Our study is important in that it identifies a genetic risk factor within the AA population.

The deleterious effects of *APOL1* expression within in the kidney are thought to be caused by locally expressed and not circulating *APOL1*, with greater cytotoxicity from G1 and G2 than G0.[17,47–52] Preeclampsia is triggered by inadequate invasion of trophoblasts into maternal spiral arteries, resulting in relative placental hypoxia and the release of factors such as inflammatory cytokines and pro-oxidant molecules into maternal circulation, which leads to widespread endothelial dysfunction.[6] The reasons for this underlying abnormal trophoblast invasion is not known; however, it does appear that there is an increase in apoptotic cells with release of syncytiotrophoblast fragments into the maternal bloodstream.[6,53] Our data suggest that APOL1 is associated with trophoblast dysfunction given the uniform suppression of serum PlGF in preeclamptic women with the fetal *APOL1* HR genotypes. Future studies should assess expression of *APOL1* G1 and G2 variants within invading trophoblasts and whether expression levels are associated with cellular stress and/or impaired trophoblast invasion or death. In addition, future studies should further assess the effects of environmental and maternal factors that may serve as an underlying trigger for the development of preeclampsia in those with the fetal APOL1 HR genotype. In our study, available maternal factors (e.g., maternal age, income, smoking, BMI) did not interact with fetal HR genotype; however, there may be other factors not in our dataset (e.g., viral infections and maternal interferon levels),

which might upregulate or otherwise impact fetal APOL1 expression. Similarly, future studies should assess the (1) interaction between maternal and infant genotype, as we were underpowered to evaluate their relationship, and (2) paternal genotype and ancestry, as paternal demographic and genetic data were not available in our study.

In terms of limitations, our case size was limited due to the paucity of preeclampsia births with fetal DNA available for study in the two study groups. However, the replication of our findings in two geographic regions and with two different study designs suggests that our findings are not due to type I (false positive) errors. Due to limited amounts of DNA and other technical reasons, we were unable to perform ancestry informative markers analysis (AIMs) on the entire dataset. Nevertheless, in the sample for which data were available from GWASs, adjustment for population stratification with principal components supported our initial result, albeit with less power and wider 95% confidence intervals.

Our data show consistent effects in two geographically distinct populations of self-identified black participants, residing in New York City and Memphis, Tennessee, respectively. Confounding by some underlying racial substructure would likely have different impacts in the two populations, whereas our study findings are consistent across sites. Furthermore, the association with preeclampsia was found only with fetal genotype and not maternal genotype. If there were confounding by substructure, there is no reason for the effect to be limited to the fetus. An additional limitation is that we cannot formally rule out that the *APOL1* risk variants are in linkage disequilibrium with other true causal variants. However, the observation that *APOL1* is highly expressed in the placenta and that the expression of *APOL1* G2 causes severe preeclampsia in transgenic mice makes it unlikely that the association of preeclampsia with *APOL1* is due to other variants in linkage disequilibrium. Finally, the EMC study population was compared to a pre-existing control cohort, which may not be representative of the base population which gave rise to the case subjects; however, the UTHSC cohort was not subject to this potential bias and showed similar associations between fetal *APOL1* status and preeclampsia risk in the mothers. Also, this pre-existing control cohort used by EMC is composed of two separate, geographically distinct, populations. The genotype frequencies are remarkably similar between the two control groups and therefore unlikely to confound results (Table S9).

Our findings that fetal HR *APOL1* genotype was associated with (1) maternal visual or cerebral disturbances, (2) lower APGAR scores (8 versus 9) at 5 min, and (3) lower gestational age and birth weight (not statistically significant) are of uncertain clinical significance. They may point toward an effect of the fetal *APOL1* genotype on either preeclampsia severity or birth outcomes, which we were not able to detect given our small sample size. However, the observation that we do not see an elevation in soluble endoglin in our population with the fetal *APOL1* genotype is consistent with a lack of correlation with preeclampsia severity, as elevated soluble endoglin is a marker of severe preeclampsia.[4,42,54]

In summary, we found that *APOL1* high-risk status of the fetus is a risk factor for maternal preeclampsia, likely by adversely affecting placental function. *APOL1* genetic testing may have a clinical role to predict and perhaps improve pregnancy outcomes.

Supplemental Data

Supplemental Data include nine tables and can be found with this article online at https://doi.org/10.1016/j.ajhg.2018.08.002.

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Declaration of Interests

K.J.R. is site PI of a Questcor funded study that funds clinical costs of the study only.

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Web Resources

GraphPad, https://www.graphpad.com/
OMIM, http://www.omim.org/
R statistical software, https://www.r-project.org/
SPSS Statistics, https://www.ibm.com/products/spss-statistics

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