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Maternal apolipoprotein E genotype as a potential risk factor for poor birth outcomes: The Bogalusa Heart Study

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Abstract

Objective—To assess the association between apolipoprotein E (apoE) genotype and preterm birth (PTB) and small for gestational age (SGA).

Study Design—ApoE phenotyping was performed on 680 women linked to 1 065 births. Allele frequencies were compared and PTB and SGA risk was estimated using log-binomial regression.

Results—The ε2 allele was more common in SGA births ($p < 0.01$). SGA risk was increased among ε2 carriers compared to genotype ε3/ε3, though associations were attenuated following adjustment for maternal age, education, race, smoking, and prenatal visits. Stronger associations were observed for term SGA (first birth: aRR = 1.78, 95% CI 1.06 – 2.98; any birth: aRR = 1.52, 95% CI 0.96 – 2.40) and among whites specifically (first: aRR = 2.88, 95% CI 1.45 – 5.69; any: aRR = 2.75, 95% CI 1.46 – 5.22).

Conclusions—Associations between maternal apoE genotype and SGA may represent decreased fetal growth in women with lower circulating cholesterol levels.

Introduction

Low birthweight (LBW) and preterm birth (PTB) are relatively common pregnancy complications that place a significant burden on the medical system.[1] Although a number of

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social, medical, and environmental risk factors for poor birth outcomes have been investigated, none fully explain the variation in risk, leading researchers to hypothesize that risk for poor birth outcomes may be a combination of environmental and genetic factors.[2] One mechanism by which a normal pregnancy may be compromised is abnormal lipid levels. Cholesterol plays a key role in fetal development; it is an essential component of cell membranes, and is required for cell proliferation, playing an important role in cell differentiation and cell-to-cell communication.[3, 4]

Many studies investigating lipid effects on pregnancy describe a “u-shaped” relationship between lipid levels and poor birth outcomes, with low levels associated with increased risk of PTB and decreased birthweight, and increased lipids potentially leading to macrosomia and preeclampsia.[5-14] However, other studies have shown general dyslipidemia and higher lipid levels to be associated with PTB, LBW, small for gestational age (SGA), decreased length, and smaller head circumference.[15-19] Consequently, these findings suggest that low and high maternal lipid levels likely represents two distinct pathways leading to abnormal fetal development.[11] Accordingly, genes that could influence maternal cholesterol concentrations, such as apolipoprotein E (apoE) may be good candidates for further investigation with regard to birth outcomes.

ApoE is a multifunctional protein integral to the metabolism of cholesterol and triglycerides.[20] Encoded by a gene located on the long arm of chromosome 19 at position 13.2, the apoE protein binds to receptors on the liver to facilitate clearance of chylomicrons and very low density lipoproteins (VLDL) from the bloodstream.[20, 21] The apoE gene has three distinct alleles, epsilons 2 (ε2), 3 (ε3), and 4 (ε4), which, in combination, produce six identifiable genotypes (ε2/ε2, ε2/ε3, ε2/ε4, ε3/ε3, ε3/ε4, ε4/ε4) conferring differential impacts on circulating cholesterol levels.[20-22] Compared to those with genotype ε3/ε3, carriers of the ε4 allele generally demonstrate higher total cholesterol and low density lipoprotein (LDL) levels, while ε2 carriers have decreased lipid levels; less of an impact has been seen on high density lipoprotein (HDL) levels.[21]

The most commonly assessed pregnancy-related outcomes with regard to apoE are preeclampsia and recurrent pregnancy loss, though results of these studies have been mixed. An early investigation suggested increased ε2 allele frequency among preeclamptic women,[23] though others have failed to replicate this finding.[24, 25] Studies have also found increased risk of recurrent pregnancy loss among ε4, and to a lesser extent, ε2 allele carriers,[21, 22, 26] though some have failed to find an association,[27] or shown decreased risk of spontaneous abortion among ε4 carriers.[28, 29] No existing studies have investigated the association between apoE genotype and PTB or SGA, two of the most common pregnancy complications.

The aim of the present study is to assess the association between maternal apoE genotype and preterm birth and small for gestational age outcomes.

Materials and Methods

Study Population

The Bogalusa Heart Study (BHS) is a long-term epidemiologic study of cardiovascular risk factors among children and young adults from the biracial community (65% white, 35% black) of Bogalusa, LA.[30] The study began in 1973–74, recruiting 3,524 children aged 5–14 years from the community (93% of all eligible individuals). Surveys of the town's schoolchildren were repeated approximately every two years through 1994, examining newly enrolled schoolchildren and reexamining those previously enrolled. As participants aged, they were eligible for reexamination in up to four of the ten surveys conducted among adults aged 18-52 between 1977 and 2010. At each study visit, fasting venipuncture blood draws and replicate measures of height and weight were obtained; mean height and weight values were used to calculate body mass index (BMI, kg/m^2), an estimate of adiposity. More detailed study methods have been published previously.[30]

Parental permission and assent of the child was obtained prior to participation in the original study, and informed consent was obtained for participation as an adult. Vital records linkage was conducted under waiver of informed consent. All study procedures received approval from the Institutional Review Board of Tulane University.

Apolipoprotein E Phenotyping

During the 1984-85 study visit (85% of all eligible children), fifty percent of black participants within each age and sex group were randomly selected to undergo apoE phenotyping. A random sample of white participants was then matched to this group by age and sex to provide an equal age-race-sex distribution. Serum samples kept frozen at −70°C for 2 – 3 years were sent to Helsinki, Finland for apoE phenotyping. Phenotyping was performed directly in serum, using a modification[31] of the method described by Havekes et al.,[32] which is based on isoelectric focusing of delipidated serum, followed by immunoblotting using rabbit antihuman apoE antiserum. Using this method, the protein product is assayed from the blood, and the pattern of protein staining is compared with known apoE phenotype banding patterns to determine the phenotype, which is a direct expression of the genotype. Genotype inference based on phenotyping methods is considered highly accurate, with concordance of 97% based on previous studies.[33] An approximate 10% duplicate blood sample was collected on each screening day to evaluate measurement error. Based on 147 pairs undergoing blind duplicate analysis, there was 95% concordance in apoE genotype assignment. Previous investigations of the same study population have documented similar apoE genotype and allele frequencies to the population at large.[34] No other apolipoproteins were considered in the present investigation.

Vital Records Linkage

Vital records linkage was conducted using LinkPro v3.0 (InfoSoft, Inc., Winnipeg, MB) to match female BHS participants to Louisiana state-issued birth records of their children. An exact match of social security number (SSN) was sought for each woman with a non-missing SSN. Alternatively, probabilistic linkage was conducted based on maternal date of

birth, first and last name, and Soundex code (a LinkPro macro that assigns phonetic codes to character variables). More details of the linkage procedure are shown in Figure 1.

Study Sample

The BHS currently includes data on almost 6 000 women. Overall, 1 441 female BHS participants underwent apoE phenotyping. Birth records available for the present analysis were those issued by the state of Louisiana between 1990 and 2009 (n = 1 354 951). Thus, the present study does not include participant births that occurred in Louisiana prior to 1990, or births that may have taken place out of state. In total, the linkage procedure matched 2 770 women to 4 876 infants (see Figure 1). Of these, 688 had apoE genotype data, corresponding to 1 100 live-births. Multiple gestation pregnancies present increased risk for preterm delivery and SGA compared to singleton gestations[35] and were therefore excluded (n = 35). Thus, the present study included 1 065 singleton live-births to 680 women (n = 423 white, n = 257 black).

Statistical Analysis

ApoE allele frequencies were examined both overall and grouped into allelic risk categories based on previous research.[20] Participants were grouped by ε2 (genotypes ε2/ε2 and ε2/ε3) or ε4 (genotypes ε4/ε3 and ε4/ε4) carrier status, while those with genotype ε3/ε3 were considered the referent group. Participants with the ε2/ε4 genotype (n = 15) were excluded from analyses using these groupings.

All birth outcomes for the present study were obtained from individual birth records, and were evaluated dichotomously. Records were categorized as preterm if the clinical estimate of gestational age on the birth certificate was < 37 completed weeks. Births were considered SGA if the birthweight for gestational age was < 10th percentile.[36] Female cut-points were used to provide more conservative estimates of SGA as sex of the baby was unknown. Outcomes were coded among the first birth identified only, as well as for any birth identified. When all births were examined, the first occurrence of the outcome was considered the index pregnancy; otherwise the earliest matched birth was used as the reference for participants who did not have the outcome of interest.

A number of covariates were also obtained from the birth certificate including: maternal age at the time of birth, maternal education level, tobacco use during pregnancy, diabetes during pregnancy, and number of prenatal visits. Maternal age, education, and number of prenatal visits were first treated as continuous variables, and then categorized to evaluate any non-linear associations. Maternal age and education were grouped into generally accepted risk categories (< 20 years, 20 – 24, 25 – 29, 30 – 34, 35 – 39, and 40+ and less than high school, high school diploma, some college/associates degree, college+). Number of prenatal visits was categorized as none, then by quartile among those who had at least one prenatal visit (0 visits, 1 – 8, 9 – 12, 13 – 15, 15+). Tobacco use and diabetes during pregnancy were classified as dichotomous variables according to the birth certificate. The most recent BMI prior to the birth, calculated during a BHS visit when the participant was not pregnant, was considered the measure of maternal BMI (mean time = 5.4 years, SD = 4.0). While slight increases in weight are common, BMI is not expected to change drastically over the course

of five years,[37, 38] and any changes are thought to be non-differential by apoE genotype. Additionally, lower prepregnancy BMI has been identified as a risk factor for SGA and PTB;[39, 40] thus, potential weight increases are not likely to influence study results.

All covariates considered were compared between outcome groups using chi-square for categorical variables and t-test for continuous variables. Linear relationships between maternal age, education, and number of prenatal visits were assessed by examining relative risks estimates obtained using log-binomial regression to evaluate whether risk increased or decreased in a linear fashion. Subsequently, allele frequencies were examined by outcome to determine whether a particular allele was overrepresented among participants with PTB or SGA. Finally, log-binomial regression was used to evaluate risk of PTB and SGA among ε2 and ε4 carriers compared to those with the ε3/ε3 genotype, both alone and controlling for covariates. Secondary analyses of SGA were conducted among term (≥ 37 weeks gestation) births only to examine fetal growth outside the context of preterm birth. The decision was made *a priori* to adjust for a number of established risk factors for PTB and SGA including: maternal age, race, education, and tobacco use, to facilitate comparison with existing studies of poor birth outcomes.[41, 42] In addition, any other covariates significant at $p < 0.05$ in bivariate analyses were included in adjusted analyses. Continuous variables found to have a non-linear relationship with the outcome were treated as categorical covariates in adjusted analyses.

Consistent with previous findings,[20, 43] slight differences in apoE allele frequencies were noted by race [white: ε2 (5.0%), ε3 (80.7%), ε4 (14.3%), black: ε2 (7.0%), ε3(69.6%), ε4 (23.4%), $p = 0.0001$]. Despite these variances, research suggests that apoE functions similarly among racial groups and can be considered as a risk factor across populations.[20, 43, 44] Nonetheless, effect modification by race was evaluated by including a product term in analyses of ε2/ε4 carrier status. Analyses were conducted initially among the first birth identified only, then for any birth identified, using SAS 9.3 for PC; a two-tailed p-value of 0.05 was considered significant for all analyses.

Results

Demographic characteristics of the study sample

Characteristics of the study sample by birth outcome are presented in Table 1. No significant differences in maternal age were noted in the first birth of record, though women coded as having a PTB at any time point were slightly older than those who did not (28.7 vs. 26.8 years, $p = 0.005$). Participants experiencing a SGA birth during both the first birth and any birth were more likely to be black (55.2% vs. 44.8%, $p = 0.0001$ and 56.5% vs. 43.6%, $p = 0.0001$, respectively), and were more likely to have used tobacco during pregnancy (SGA: 19.0 vs. 10.3%, $p = 0.01$ and 21.6 vs. 10.3%, $p = 0.0008$, respectively). Additionally, those having an SGA birth had a slightly lower education level (12.3 vs. 13.3 years, $p = 0.0001$) and approximately two fewer prenatal visits (10.2 vs. 12.3, $p = 0.002$ and 10.2 vs. 12.4, $p = 0.0003$). A non-linear association was detected for number of prenatal visits, with participants who had 1 – 8 prenatal visits having the highest risk of SGA. No other significant differences between groups were noted.

ApoE allele frequency and birth outcomes

As expected, the ε3 allele was the most prevalent allele in the study population (1041/1360 total alleles, 76.5%), followed by the ε4 (241/1360, 17.7%) and ε2 (78/1360, 5.7%) alleles (see Table 2). The ε2 allele was more common among participants who had a SGA infant in both the first birth and overall (10.9 vs. 4.9%, p = 0.003 and 9.8 vs. 4.9%, p = 0.01, respectively). No differences in allelic frequencies were noted for PTB.

ε2 and ε4 allele carrier status and birth outcomes

As shown in Table 3, compared to those with the ε3/ε3 genotype, women who were ε2 allele carriers were 1.80 times more likely to have a SGA infant in the first birth of record (95% CI CI 1.09 – 2.97) though associations were no longer significant following adjustment for maternal age, education level, race, tobacco use during pregnancy, and number of prenatal visits, or when SGA in any birth was considered. More robust associations were observed when analyses were restricted to term (≥ 37 weeks gestation) SGA births (aRR = 1.78, 95% CI 1.06 – 2.98 and aRR = 1.52, 95% CI 0.96 – 2.40 for the first birth and any birth, respectively, controlling for previously identified covariates. Potential effect modification by race was noted for SGA in both the first birth and any birth (p = 0.06 and p = 0.05, respectively); following stratification by race, an association was seen among white ε2 carriers only (aRR = 2.88, 95% CI 1.45 – 5.69 vs. aRR = 1.02, 95% CI 0.46 – 2.25; and aRR = 2.75, 95% CI 1.46 – 5.22 vs. aRR = 0.90, 95% CI 0.41 – 1.95, respectively), controlling for maternal age, education, tobacco use, and number of prenatal visits. No significant associations were observed for PTB.

Discussion

Results of the present study indicate that apoE genotype may be a risk factor for delivering a small for gestational age infant. More specifically, women who are carriers of the ε2 allele were more likely to have had a SGA birth. A possible association between apoE genotype and preterm birth was also noted, though effects did not reach statistical significance. Associations persisted after controlling for known risk factors for poor birth outcomes, such as maternal age, education level, race, tobacco use and number of prenatal visits. These findings suggest that apoE genotype might influence fetal growth, with ε2 carriers at risk for decreased fetal growth during pregnancy.

To our knowledge, this is the first study to evaluate maternal apoE genotype as a possible risk factor for PTB and SGA. One existing study assessed the association between fetal gestation and single-nucleotide proteins (SNPs) in the apoE gene, however, this study made inferences among preterm births only, and the primary focus was on fetal rather than maternal genetics.[2] Nonetheless, findings suggested a maternal SNP in the apoE gene was associated with gestational age overall, though not PTB. Additionally, findings from the present study are in accord with a number of studies of other pregnancy complications, which suggest that ε2 allele carriers may have fewer children and pregnancies overall,[45] and be at increased risk for preeclampsia[23] and miscarriage.[22, 26, 28] Conversely, other studies have not shown an increased risk of pregnancy complications among ε2 carriers,[21, 25, 27, 29]

though many of these studies have been limited in their inclusion of those carrying the ε2 allele or have used unusual or high risk control groups.[27, 29]

Given ε2 carriers are thought to have decreased lipid levels,[20] it seems biologically plausible that ε2 allele carriers may experience poorer fetal growth during pregnancy. Previous examinations of the current study population confirm this association, finding lower total, LDL, and VLDL cholesterol levels among ε2 carriers.[34, 46, 47] Furthermore, this decrease in LDL levels was shown to be more marked in white ε2 carriers compared with black,[34] which may help explain the differential SGA findings by race in the current study. Other studies have also noted differential lipid effects by race; preterm birth was previously associated with low total cholesterol levels among white mothers only, though lower birthweights among term infants to all women with low cholesterol levels were noted.[6] Adequate maternal cholesterol levels are necessary for proper placental development and fetal nutrient transfer,[3] and fetal plasma cholesterol levels are significantly correlated with maternal concentrations, especially in early gestation.[3, 4] Thus, placental development and fetal growth may be compromised among women with a propensity for lower lipid levels.

In support of this, studies have demonstrated a linear association between maternal lipid levels during pregnancy and birthweight,[9] as well as lower pre-pregnancy lipid levels among women with intrauterine growth restricted pregnancies, especially with regard to low and very-low density lipoprotein levels.[12] Likewise, placental trophoblast cells have been shown to express both VLDL and apoE receptors, as well as LDL receptor–related proteins,[3, 4] and limited evidence has suggested decreased LDL receptor-related proteins in the placentas of SGA infants born to preeclamptic mothers.[48] Additionally, a recent study also found decreased risk of term SGA among women with higher intermediate density lipoprotein (IDL) levels during pregnancy,[17] which is a lipoprotein similar to LDL formed during the degradation of VLDL, whose apoE receptor ligands permit bonding to LDL receptors.

Research indicates the greatest increase in plasma triglycerides during gestation corresponds to VLDL triglycerides.[3] Essential fatty acids (EFA), which are obtained from maternal diet, are transported in maternal plasma as triglycerides in triglyceride-rich lipoproteins, such as VLDL and LDL. Maternal triglycerides in plasma lipoproteins are hydrolyzed to be taken up by the placenta, where they are then reesterified to provide a reservoir of fatty acids for the fetus.[3, 4] In support of this process, a linear correlation has been found between maternal and fetal plasma triglycerides in rats, and a direct relationship between maternal triglycerides and newborn weight has been found in humans.[3] Thus, women with decreased LDL levels, as has been found in carriers of the ε2 allele, may provide lower levels of bioavailable nutrients to their developing fetuses, resulting in diminished fetal growth. Though we were not able to evaluate cholesterol levels during pregnancy and directly assess the proposed pathway between apoE and decreased fetal growth, existing research supports this potential mechanism and warrants further study.

A number of limitations and strengths of the present study should be noted. Although we had access to live-births that occurred in Louisiana over the course of 19 years, it is likely that some of the women in the Bogalusa Heart Study gave birth prior to 1990 or in a state other than Louisiana. Thus, it is possible that some of the women included in our

investigation may have had earlier births that were not included in the present study. Therefore, our group of “first births” are likely not true first births. Nevertheless, it is thought to be unlikely that this would have influenced our results as findings persisted when any birth detected was included. Additionally, the study relied on vital statistics data gathered from the birth certificate, which has inherent reporting limitations. Still, reliability studies suggest that birthweight data from the birth certificate is highly accurate,[49, 50] and estimates of gestational age have been steadily improving since changes to the United States birth certificate were implemented in 1989.[51] Further, we were unable to account for medical conditions that may have impacted fetal growth or gestational age, though number of births with these types of disorders are thought to be low, and conditions may be intermediaries on the pathway between apoE and poor birth outcomes (for example, hypertension). We were also unable to assess preeclampsia, which has been the focus of previous investigations, due to low prevalence reported on the birth certificate (eclampsia, 0.9%). Though much of the data contained on the birth certificate is considered suitable for research use, maternal complications are notoriously underreported.[49, 50] Finally, since sex of the baby was unknown, SGA cut-points were based on female growth curves, which may have resulted in underestimation of SGA births. However, misclassification is thought to be non-differential by apoE genotype; therefore, if affected at all, findings are likely to be biased towards the null.

Despite these limitations, the study is strengthened by the relatively large number of participants and the cohort nature of the study design. With the exception of a meta-analysis of recurrent pregnancy loss,[22] only one other study had a sample size similar to the one in the present investigation (n = 616 vs. n = 680).[28] Additionally, the vast majority of existing studies of apoE genotype and pregnancy complications are case-control studies, and have been limited by their use of potentially inappropriate control groups.[21, 23, 25, 27-29] The present study also permitted evaluation of the ε2 allele, which has been limited in other investigations.[27, 29, 45, 52] Moreover, a major strength of the present study is the ability to control for known risk factors for PTB and SGA, which has not been done previously. The inability to account for patient-level characteristics has been noted as a weakness in previous studies, given the potential for gene-environment interactions with regard to cholesterol levels.[2]

In summary, the present study supports an association between maternal apoE genotype and risk of having a small for gestational age birth. Compared to women with the most common apoE genotype (ε3/ε3), women who were carriers of the ε2 allele were more likely to have given birth to a SGA infant at some point during adulthood. This association may represent decreased fetal growth in women with lower circulating total and LDL cholesterol levels. More studies are needed to fully examine the influence of maternal apoE genotype on fetal growth.

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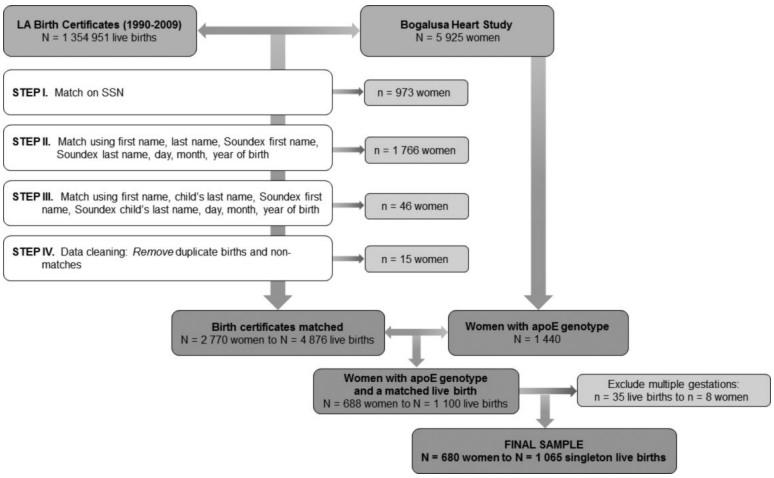

Figure 1.

Steps to create the final study sample: matching Bogalusa Heart Study women who underwent apoE phenotyping to singleton live-births in Louisiana (1990 – 2009)

Table 1

Demographic characteristics of the study sample by preterm birth and small for gestational age birth outcomes (n = 680)

Characteristic	First Birth			Any Birth		
	Preterm Birth (< 37 weeks)					
	No (n = 633)	Yes (n = 47)	p-value[a]	No (n = 607)	Yes (n = 73)	p-value[a]
Mother's age (mean)	26.7	28.0	0.10	26.8	28.7	**0.005**
Mother's race (%)						
White	62.7	55.3	0.31	63.4	53.4	0.10
Black	37.3	44.7		36.4	46.6	
BMI – closest prior to pregnancy (mean)	24.1	23.9	0.82	24.2	23.6	0.41
Mother's education level (mean)	13.1	13.5	0.22	13.1	13.5	0.20
Tobacco use during pregnancy (% yes)	11.3	14.9	0.45	11.4	12.3	0.82
Diabetes during pregnancy (% yes)	2.8	4.3	0.64	3.0	4.1	0.59
Number of prenatal visits (mean)	12.0	11.7	0.77	12.0	11.7	0.66
Categorical number of visits (%)			0.12			0.42
0	0.8	2.1		0.8	1.4	
1 – 8	24.7	40.4		24.3	32.9	
9 – 12	30.1	19.2		30.3	26.0	
13 – 15	27.6	23.4		27.8	28.8	
15+	16.8	14.9		16.8	10.9	
	Small for Gestational Age (<10th percentile)					
	No (n = 584)	Yes (n = 96)	p-value[a]	No (n = 563)	Yes (n = 117)	p-value[a]
Mother's age (mean)	26.9	25.8	0.06	27.1	26.2	0.09
Mother's race (%)						
White	65.1	44.8	**0.0001**	66.1	43.6	**0.0001**
Black	34.9	55.2		33.9	56.4	
BMI – closest prior to pregnancy (mean)	24.1	23.8	0.64	24.1	24.0	0.84
Mother's education level (mean)	13.3	12.3	**0.0001**	13.3	12.3	**0.0001**
Tobacco use during pregnancy (% yes)	10.3	19.0	**0.01**	10.3	21.6	**0.0008**
Diabetes during pregnancy (% yes)	3.1	2.1	1.00	3.2	1.7	0.55
Number of prenatal visits (mean)	12.3	10.2	**0.002**	12.4	10.2	**0.0003**
Categorical number of visits (%)			**0.0005**			**0.0002**
0	0.8	1.1		0.7	2.6	
1 – 8	22.8	44.2		22.2	40.5	
9 – 12	30.4	23.2		30.0	25.0	
13 – 15	28.7	18.9		29.2	19.8	
15+	17.3	12.6		17.9	12.1	

[a] p-value based on chi-square or Fisher's exact test for categorical variables and t-test for continuous variables

Table 2

ApoE allele frequencies by birth outcome in the first birth or any birth (n = 680)

Allele[a]	ALLELE FREQUENCIES[b]						
	Overall	Preterm Birth			Small for Gestational Age		
		No n (%)	Yes n (%)	p-value[c]	No n (%)	Yes n (%)	p-value[c]
First Birth							
ε2	5.7	69 (5.4)	9 (9.6)	0.24	57 (4.9)	21 (10.9)	**0.003**
ε3	76.5	973 (76.9)	68 (72.3)		898 (76.9)	143 (74.5)	
ε4	17.7	224 (17.7)	17 (18.1)		213 (18.2)	28 (14.6)	
Any Birth							
ε2	5.7	66 (5.4)	12 (8.2)	0.32	55 (4.9)	23 (9.8)	**0.01**
ε3	76.5	935 (77.0)	106 (72.6)		866 (76.9)	175 (74.8)	
ε4	17.7	213 (17.5)	28 (19.2)		205 (18.2)	36 (15.4)	

[a] ApoE genotype distribution: ε2/ε2 (4), ε2/ε3 (55), ε2/ε4 (15), ε3/ε3 (397), ε3/ε4 (192), ε4/ε4 (17)

[b] Denominator is number of alleles

[c] p-value based on chi-square

Table 3

Association between ε2 and ε4 carrier status and preterm birth and small for gestational age in the first birth or any birth: results of log-binomial modeling (n = 665)

Single allele influences[a]	FIRST BIRTH				ANY BIRTH			
	Unadjusted		Adjusted[b]		Unadjusted		Adjusted[b]	
	RR	95% CI	aRR	95% CI	RR	95% CI	aRR	95% CI
	PRETERM BIRTH							
Any ε2 allele	1.55	0.67 – 3.61	1.46	0.63 – 3.41	1.38	0.68 – 2.81	1.22	0.60 – 2.48
Any ε4 allele	0.94	0.50 – 1.81	0.89	0.46 – 1.70	1.12	0.69 – 1.82	1.02	0.63 – 1.67
	SMALL FOR GESTATIONAL AGE							
Any ε2 allele	**1.80**	**1.09 – 2.97**	1.55	0.95 – 2.52	1.56	0.98 – 2.50	1.38	0.90 – 2.15
Any ε4 allele	0.75	0.47 – 1.19	0.65	0.41 – 1.03	0.77	0.51 – 1.16	0.72	0.49 – 1.04
	SMALL FOR GESTATIONAL AGE (TERM)[c]							
Any ε2 allele	**1.96**	**1.17 – 3.29**	**1.78**	**1.06 – 2.98**	**1.67**	**1.03 – 2.70**	1.52	0.96 – 2.40
Any ε4 allele	0.72	0.44 – 1.18	0.65	0.39 – 1.06	0.75	0.49 – 1.15	0.70	0.47 – 1.04

[a] Compared to genotype ε3/ε3

[b] Adjusted for: maternal age, education level, race, and tobacco use during pregnancy; SGA also adjusted for number of prenatal visits

[c] n = 620 for analyses limited to term births