ORIGINAL ARTICLE: GENETICS

Interaction of apolipoprotein E gene polymorphisms on miscarriage risk in black and white American women

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Objective: To evaluate whether [1] apolipoprotein E (APOE) polymorphisms can differentially regulate miscarriage risk and [2] whether this genotype effect could also be modulated by the race within populations.
Design: Data were derived from the Coronary Artery Risk Development in Young Adults (CARDIA), a longitudinal study with black and white participants from four U.S. locations.
Setting: Not applicable.
Patient(s): Women without miscarriages (controls) and women who miscarried at least once (cases).
Intervention(s): None.
Main Outcome Measure(s): A group of women (n = 1,372) successfully followed for 25 years and with their *APOE* alleles identified were analyzed for miscarriage risk throughout their reproductive life. Additionally, a larger longitudinal analysis encompassing all the participants who had their *APOE* characterized (n = 2,140) was also performed for the association between *APOE* and miscarriage risk.
Result(s): In white women followed up for 25 years, the odds ratio for miscarriage associated with *APOE*2* allele presence was 1.61 (95% confidence interval, 1.04–2.50) compared with *APOE*33* carriers. This was a race-dependent phenomenon as no associations between *APOE* alleles and miscarriage was observed in black women. Likewise, Cox regression analysis showed that cumulative miscarriage risk in white women was 37.2% in the *APOE*2* carriers compared with 27.8% and 24.8% in *APOE*33* and *APOE*4* carriers, respectively. With *APOE*33* as the reference, the age-adjusted hazard ratio associated with carrying the *APOE*2* allele was 1.47 (95 confidence interval, 1.06–2.05).
Conclusion(s): This variable miscarriage risk, produced by an interaction between genotype and race, may reconcile, at least partially, the conflicting reports of the association of APOE and miscarriage risk. (Fertil Steril® 2016;■:■–■. ©2016 by American Society for Reproductive Medicine.)
Key Words: Apolipoprotein E, APOE2, race, miscarriage, spontaneous pregnancy loss

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Miscarriage is the most common adverse outcome in women's reproductive life and poses an enormous burden on miscarrying women and their partners. Age, smoking, chromosome abnormalities of the fetus, or preexisting medical diseases of the mother-to-be may explain some pregnancy losses. However, the majority remain unexplained, suggesting that genetic factors may be involved in its etiology (1).

Apolipoprotein E (*APOE* for the gene and APOE for the protein) associates with lipoprotein particles and mediates their binding to receptors, playing a paramount role in the redistribution of cholesterol and other lipids between cells (2). In humans, APOE was first described as a hepatic product but is also synthesized in a number of tissues in the body, especially those that experience high lipid flux. Of particular interest is that the endometrium produces APOE, and its mRNA is dramatically upregulated during the

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implantation window (3). Additionally, APOE-mediated delivery of cholesterol regulates the production of steroid hormones, hence controlling the production of ovarian estrogen and P (4). These aspects of APOE physiology suggest that, in addition to its well-established role in lipoprotein metabolism, APOE may also play an important role in regulating fertility (5).

In humans, *APOE* is polymorphic with three major alleles that differ from one another by two nonsynonymous single nucleotide polymorphisms. These missense mutations cause amino acid substitutions at positions 112 (rs7412) and 158 (rs429358) of the protein: *APOE*2* (cys112, cys158), *APOE*3* (cys112, arg158), and *APOE*4* (arg112, arg158). These alleles give rise *to six genotypes: APOE*22, *23, *33, *34, *24, and *44* (6). The *APOE*34* and *APOE*44* alleles have been associated with an elevated risk of recurrent miscarriage in both U.S. and Turkish populations (7, 8). Additionally, APOE4 also appears to contribute to thrombotic risk, which is associated with recurrent pregnancy loss (9). These findings have been challenged by other studies, which either failed to find an association of APOE polymorphisms with recurrent pregnancy loss in Turkish (10), Italian (11), and North Indian (12) populations or identified APOE*2 as a risk factor for recurrent miscarriage in a Turkish population (13). These disparities may be explained by the high heterogeneity of APOE phenotype frequency among ethnic groups; both *APOE*2* and *APOE*4* alleles occur more frequently in black than in white individuals (14, 15), and it has also been reported that racial differences can affect the linkage between *APOE* alleles and other metabolic traits (16).

Herein we present a targeted hypothesis-driven study aimed to test whether or not [1] *APOE* polymorphisms can differentially regulate miscarriage risk and [2] this genotype effect could also be modulated by the race within populations. To this end, we have studied a cohort of women from the Coronary Artery Risk Development in Young Adults (CARDIA) study, a longitudinal study with a biracial composition from four distinct geographic areas in the United States (17). This cohort facilitates the comparison of effects of *APOE* phenotype on miscarriage risk throughout the reproductive life of women in the two ethnic groups (15). We found that when women's race was taken into account, no effect of *APOE* isoforms on miscarriage risk was observed for black women. Conversely, increased odds for miscarrying were detected in white pregnant women bearing the *APOE*2 allele*. This race-dependent effect may reconcile, at least partially, the conflicting reports of the association of *APOE* polymorphisms and miscarriage risk.

METHODS

The CARDIA study (NCT00005130) is a longitudinal, observational, and multicentric study that examines the development of heart disease in young black and white adults from four geographic areas: Birmingham, Alabama; Chicago, Illinois; Minneapolis, Minnesota; and Oakland, California. Descriptions of the study design, methodology, and cohort characteristics have been extensively reported (17). In brief, the study began in 1985–86 with 5,115 participants (2,787 women), and additional examinations were performed every 2–5 years, including a year 25 examination completed in 2011. Research Materials of the baseline examination (year 0) and the seven follow-up examinations were obtained from the National Heart, Lung, and Blood Institute (NHLBI) Biologic Specimen and Data Repository Information Coordinating Center (BioLINCC). This analysis was approved by the Regional Institutional Review Board of Aragon, Spain (CEIC-A).

Sample and Data Collection

Reproductive history was obtained by self-report. We selected women attending the 25-year examination who completed the pregnancy questionnaire detailing the number of pregnancies and their outcomes throughout the 25 years of follow-up. Women were excluded if they had a missing APOE phenotype. At baseline and at every examination, participants reported whether they were currently pregnant and number of times they had been pregnant since the previous examination, including abortions, miscarriages, and live or stillbirths. Miscarriage was defined as loss of a recognized pregnancy before 24 completed weeks of gestation. Total number of pregnancies and total number of miscarriages were calculated by adding the number of pregnancies/miscarriages reported at every examination.

Information for plasma biochemistry (fibrinogen, TG, HDL-C, LDL-C, and total cholesterol), anthropometry (weight, height, and waist girth [WG]), and sociodemographic data (menopause, cigarette smoking, family income, marital status, and contraceptive usage, employment status, and physical activity) were collected at the 25-year exam, with the exception of fibrinogen, which was measured at the 20-year exam. Body mass index (BMI) was calculated as weight in kilograms divided by squared height in meters. APOE phenotype was determined from plasma samples collected during the 7-year examination (1993–94) by isoelectric focusing followed by immunoblotting as described elsewhere (18). Subsequently, women were classified into three distinct APOE groups: E3 (*APOE *33)*, E2 (*APOE*22 and APOE*23*), and E4 (*APOE*34, APOE*24, and APOE*44*).

Statistical Analysis

Bivariate tables were created to summarize variables of interest, χ^2-tests were used to test associations among categorical variables, and analysis of variance or Kruskal-Wallis were used to compare continuous normal or nonnormal data, respectively. Cochran-Mantel-Haenszel correction for the χ^2 was used when APOE phenotypes were considered as ordinal categories (19). Odds ratios (ORs) of miscarrying for each APOE phenotype were determined by age-adjusted logistic regression weighting the number of miscarriages for each participant.

In survival models, follow-up time was the discrete time variable and the endpoint was either the examination year in which the first miscarriage was referred or the follow-up time for the women who did not miscarry. Cumulative probabilities of the first miscarriage for three categories of APOE