ORIGINAL ARTICLE: ENVIRONMENT AND EPIDEMIOLOGY

FASN, dietary fat intake, and risk of uterine leiomyomata in the Black Women's Health Study

Lauren A. Wise, Sc.D.,[a,b] Julie R. Palmer, Sc.D.,[a] Lynn Rosenberg, Sc.D.,[a] Stephen A. Haddad, M.Sc.,[a] and Edward A. Ruiz-Narváez, Sc.D.[a]

[a] Slone Epidemiology Center at Boston University; and [b] Department of Epidemiology, Boston University School of Public Health, Boston, Massachusetts

Objective: To replicate results from a previous genome-wide association study of European ancestry women, in which a positive association was found between uterine leiomyomata (UL) and rs4247357, a single-nucleotide polymorphism located near the fatty acid synthase (*FASN*) gene.
Design: Prospective cohort study.
Setting: Not applicable.
Patient(s): African-American women aged 23–50 years, who were premenopausal and had an intact uterus in 1997.
Intervention(s): None.
Main Outcome Measure(s): We genotyped rs4247357 among 2,301 incident UL cases and 3,005 controls from the Black Women's Health Study (1997-2011). Odds ratios (ORs) and 95% confidence intervals (CI) were estimated using logistic regression with control for age, geographic region of residence, and percent European ancestry using a panel of validated ancestry informative markers.
Result(s): Overall, rs4247357 was not associated with UL risk. Relative to the CC genotype, ORs were 1.04 (95% CI 0.92–1.19) for the AC genotype and 1.09 (95% CI 0.93–1.29) for the AA genotype. A positive association was found, however, among those with higher European ancestry (≥40%). Relative to the CC genotype, ORs were 2.03 (95% CI 1.12–3.69) for the AC genotype and 2.44 (95% CI 1.20–4.96) for the AA genotype. Dietary fat intake also appeared to modify the *FASN*–UL association.
Conclusion(s): Although there was little overall association between rs4247357 and UL risk, a positive association was observed among women with ≥40% European ancestry. Direct sequencing of this genomic region might be warranted to determine whether rs4247357, or some other variant, is causally related to UL. (Fertil Steril® 2016;■:■–■. ©2016 by American Society for Reproductive Medicine.)
Key Words: African Americans, fatty acids, genetics, prospective studies, uterine neoplasms

Discuss: You can discuss this article with its authors and with other ASRM members at http://fertstertforum.com/wisel-fasn-fibroids-black-women/

Uterine leiomyomata (UL), or fibroids, are benign neoplasms of the myometrium and are clinically recognized in 25%–30% of reproductive-aged women (1–3). Sequelae of UL include menorrhagia, pelvic pain, infertility, and complications of pregnancy and delivery (4). Studies have documented a two- to threefold higher incidence of UL in African Americans than European Americans (5, 6), and African Americans tend to have younger ages at diagnosis and experience greater symptom severity (7). None of the identified environmental or genetic risk factors fully explain this racial disparity (4).

In a 2012 genome-wide association study (GWAS) among women of European ancestry (1,230 cases and 5,097 controls), a common single-nucleotide polymorphism (SNP), rs4247357, reached genome-wide significance in association with UL (8). This SNP is located on chromosome 17 in a linkage disequilibrium (LD) block that contains three genes, fatty-acid synthase (*FASN*), coiled-coil-domain-containing 57 (*CCDC57*), and solute carrier family 16, member 3 (*SLC16A3*). *FASN* codes for the fatty acid synthase (FAS) enzyme that is responsible for de novo fatty acid

Received February 23, 2016; revised and accepted June 8, 2016.
L.A.W. has nothing to disclose. J.R.P. has nothing to disclose. L.R. has nothing to disclose. S.A.H. has nothing to disclose. E.A.R.-N. has nothing to disclose.
Supported by grants R01-CA58420 (to L.R.), UM1-CA164974 (to L.R.), R01-CA098663 (to J.R.P.), R01-MD007015 (to E.A.R.-N.), and R03-CA169888 (to L.A.W.) from the National Cancer Institute, and grants R01-HD057966 (to L.A.W.) and R03-HD055211 (to L.A.W.) from the *Eunice Kennedy Shriver* National Institute of Child Health and Human Development. The content is solely the responsibility of the authors and does not necessarily represent the official view of the National Institutes of Health.
Reprint requests: Lauren A. Wise, Sc.D., Department of Epidemiology, Boston University School of Public Health, 715 Albany Street, Boston, Massachusetts 02118 (E-mail: lwise@bu.edu).

Fertility and Sterility® Vol. ■, No. ■, ■ 2016 0015-0282/$36.00

http://dx.doi.org/10.1016/j.fertnstert.2016.06.019

synthesis. Levels of FAS were shown to be substantially higher in UL tissue than in matched myometrium (8). It is unknown whether any of these SNPs are causally associated with UL or whether they are in LD with the causal variant. The National Institute of Environmental Health Sciences Uterine Fibroid Study (6), which analyzed 574 African American and 394 non-Hispanic European American women aged 35–51 years with DNA, did not replicate *FASN* findings in either ethnic group (9), but power was low to detect an association.

Overexpression of the *FASN* gene is common in many types of cancers (10, 11). Higher levels of the FAS enzyme and increased de novo lipogenesis confer a selective advantage to cancer cells by meeting the diverse demands of energy, membrane generation, and protein modification (10). We postulate that if the association between rs4247357 and UL is mediated by overexpression of the *FASN* gene and increased de novo lipogenesis, then the genetic association would be stronger among subjects with the lowest dietary intake of total fat. Among these persons, de novo lipogenesis instead of fat intake would be a major contributor of lipid precursors, making more evident the association of rs4247357 with UL risk via *FASN* overexpression. In a previous report from the Black Women's Health Study (BWHS), we found that greater dietary intake of total fat was not appreciably associated with UL risk overall (12).

Whether genetic variation in *FASN* predicts UL risk among African American women is unclear. In the present study we sought to replicate the association between rs4247357 and UL risk among African Americans from the BWHS, and assess whether the association varied by percent European ancestry or by dietary fat intake.

MATERIALS AND METHODS

Study Population

The BWHS is an ongoing prospective cohort study of 59,000 women who self-identify as "black" (13). The study began in 1995 when women aged 21–69 years from across the United States completed a 14-page postal health questionnaire. Follow-up questionnaires have been completed by participants every 2 years, and cohort retention has exceeded 88% of potential person-years through 2011. During 2004–2007 we obtained saliva samples as a source of DNA from 26,814 participants using the mouthwash-swish method (14). Participants who provided DNA were slightly older than those who did not (49.7 vs 47.7 years) but were similar with respect to education, region, body mass index, and family history of UL. The present analysis includes 5,306 premenopausal women aged 23–50 years in 1997. The study protocol was approved by the institutional review board of Boston University Medical Center.

Assessment of Uterine Leiomyomata

Every 2 years, beginning in 1999, women reported whether they had been diagnosed with "uterine fibroids," the calendar year of first diagnosis, and whether their diagnosis was confirmed by ultrasound or surgery. We assessed the accuracy of self-report in a random sample of 248 incident cases and confirmed the diagnosis for 96% (122 of 127) by medical record (15). There were no systematic differences in characteristics according to the release of medical records (15).

Analyses were restricted to premenopausal women because new UL diagnoses are rare after menopause (3). The case group (n = 2,301) consisted of premenopausal women with incident UL diagnosed during 1997–2011 who provided DNA and had not been diagnosed with cancer or autoimmune disease. Controls (n = 3,005) were a random sample of similarly aged premenopausal women who provided DNA and had never been diagnosed with UL, cancer, or autoimmune disease through 2011.

Assessment of Covariates

Baseline and biennial follow-up questionnaires collected data on reproductive, contraceptive, and medical history, height, current weight, Papanicoloau testing, smoking, alcohol, physical activity, geographic region, and various indicators of socioeconomic status. Recency of pelvic ultrasound was reported in 2007 ("never, <5, 5–9, ≥10 years ago"), as well as 2009 and 2011 ("previous two years"). Family history of UL ("Has your mother or any of your sisters ever been diagnosed with uterine fibroids?") was ascertained in 2009.

Usual diet in the past year was estimated in 2001 with an 85-item modified version of the National Cancer Institute–Block food frequency questionnaire (FFQ) (16, 17). The 2001 FFQ was an expanded version of the 1995 FFQ validated in our cohort (17) and included items that women had written in on the 1995 questionnaire. The 2001 FFQ contained a greater number of items about fatty foods, including dark-meat fish vs other fish and seafood, permitting a more valid assessment of fat intake. The frequency responses for food items ranged from "never or <1 serving/month" to "≥2 servings/day." Participants were asked to specify a "small," "medium," "large," or "super size" portion size. A medium portion size was defined for each item (e.g., ½ cup [102 g] of tuna fish), and small, large, and "super-size" servings were weighted as 0.5, 1.5, and 2 times a medium serving size, respectively. Nutrient intake was computed by multiplying the frequency of consumption of each food by the nutrient content of the specified portion. We used the National Cancer Institute's DIET*CALC software (version 1.4.1) (18) to estimate consumption (in grams) of individual types of fatty acids. In a validation study of 400 BWHS participants (17), energy-adjusted and deattenuated Pearson correlations comparing nutrient estimates from the FFQ with averages from the combined recall/record data ranged from 0.5 to 0.8 (17).

Genotyping and Quality Control

DNA isolation and amplification. Deoxyribonucleic acid was isolated from mouthwash swish samples at the Boston University Molecular Core Genetics Laboratory using the QIAAMP DNA Mini Kit (Qiagen). Whole-genome amplification was performed with Qiagen RePLI-g Kits using the method of multiple displacement amplification. Amplified samples underwent purification and PicoGreen quantification before being plated for genotyping.