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FASN, Dietary Fat Intake, and Risk of Uterine Leiomyomata in the Black Women's Health Study

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Abstract

Objective—To replicate results from a previous genome-wide association study (GWAS) of European ancestry women, in which a positive association was found between uterine leiomyomata (UL) and rs4247357, a single nucleotide polymorphism (SNP) located near the fatty-acid synthase *(FASN)* gene.

Design—Prospective cohort study.

Setting—United States.

Patients—African American women aged 23–50 years, who were premenopausal and had an intact uterus in 1997.

Interventions—None.

Main outcome measures—We genotyped rs4247357 among 2,301 incident UL cases and 3,005 controls from the Black Women's Health Study (BWHS). 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.

Results—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 (*P*-trend=0.281). 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 (*P*-trend=0.012; *P-interaction*=0.136). Dietary fat intake also appeared to modify the *FASN*-UL association.

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Conflict of interest: none.

Conclusions—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.

CAPSULE

Rs4247357 was related to an increased risk of uterine leiomyomata in the Black Women's Health Study, but only among African American women with ≥40% European ancestry. The association was strongest among women in the lowest tertile of dietary fat intake.

Keywords

prospective studies; African Americans; fatty acids; genetics; uterine neoplasms

INTRODUCTION

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 2–3-fold 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 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 with DNA, did not replicate *FASN* findings in either ethnic group (9) but power was low to detect an association.

Over-expression 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 over-expression 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* over-expression. In a previous report from the Black Women's Health Study, 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 report, we sought to replicate the association between rs4247357 and UL risk among African Americans from the Black Women's Health Study, and assess whether the association varied by percent European ancestry or by dietary fat intake.

MATERIALS AND METHODS

Study population

The Black Women's Health Study (BWHS) is an ongoing prospective cohort study of 59,000 women who self-identify as "black" (13). The study began in 1995 when women 21–69 years of age from across the United States completed a 14-page postal health questionnaire. Follow-up questionnaires have been completed by participants every two 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 two 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% (122/127) 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 (Pap) 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 (NCI)-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 versus 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 (102g) 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 ranging from 0.5 to 0.8 (17).

Genotyping and quality control

DNA isolation and amplification—DNA was isolated from mouthwash swish samples at the Boston University Molecular Core Genetics Laboratory using the QIAAMP DNA Mini Kit (Qiagen, Valencia, CA). 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.

Genotyping and QC—Genotyping was carried in two batches. Most of our samples were genotyped at the Broad Institute (Cambridge, MA) using a Sequenom iPLEX assay (Sequenom, San Francisco, CA) as previously described (19). The second batch was genotyped at the Affymetrix laboratory (Santa Clara, CA) on an Axiom custom array. We excluded samples with a calling rate <80%. We used blinded duplicates to assess reproducibility, and HapMap samples to assess concordance. An average reproducibility of 96% was obtained, and mean concordance with HapMap samples was 99%. The analytic dataset combining both batches included 2,301 incident UL cases and 3,005 controls.

We genotyped the top 30 ancestral informative markers (AIMs) from a list of 1,536 validated SNPs to estimate percent European ancestry and adjust for population stratification due to European admixture. These 30 AIMs had allele frequency differences between Africans and Europeans ≥75% (20). We used a Bayesian approach as implemented in the ADMIXMAP software (21, 22) to estimate individual admixture proportions. In our cohort, the correlation between percent European admixture determined by the reduced panel of 30 AIMs as compared with the full panel of 1,536 AIMs was significant (r=0.87, P=0.0001), confirming the validity of the reduced panel (23).

Data analysis

We used PLINK software version 1.07 (24) to compute summary statistics for the genetic data. We tested for allele frequency differences between cases and controls using a 1-df chi-square test that does not assume any particular genetic model, correcting for multiple testing through 10,000 permutations. We used logistic regression to estimate odds ratios (OR) and 95% confidence intervals (CI) for the association between rs4247357 and UL, with adjustment for age in 1997 (<30, 30–34, 35–39, 40–44, ≥45 years), percent European ancestry (continuous variable), and region of residence (Northeast, South, Midwest, and West) (25). We also constructed models that further controlled for UL determinants, including age at menarche (years), parity (births), and BMI (<20, 20–24, 25–29, 30–34, ≥35 kg/m^2); however, because multivariable models gave slightly stronger results than models adjusted for age, region, and ancestry, and it is unlikely that these "downstream" factors are true confounders or mediators of the observed association, we presented the more parsimonious models. We stratified the data by % European ancestry, with *a priori* cut points determined by the variable's distribution (<10, 10–19, 20–39, ≥40%). Likewise, total dietary fat intake was divided into tertiles. In secondary analyses, we stratified the data by age at baseline, family history of UL, and surgery because early diagnosis and/or a family history of UL may reflect a genetic predisposition to disease, and surgically-confirmed cases often have more symptomatology (26). We also restricted controls to those with a recent pelvic ultrasound ("5 years ago" in 2007, or within the "previous two years" in 2009 or 2011). Two-sided p-values were calculated from tests for trend using the number of minor alleles for rs4247357.

RESULTS

Characteristics of cases and controls are shown in Table 1. Mean age at the start of follow-up (1997) was 34.3 years for UL cases and 34.5 years for controls. Mean age of UL cases at diagnosis was 38.4 years. As expected, cases had lower parity, lower percent European ancestry, earlier ages at first birth, and greater years since last birth than controls (4). Total dietary fat intake (median, interquartile range) was not appreciably different across strata of percent European ancestry: <10% (52.6, 33.2–73.4 g/day), 10–19% (56.1, 38.3–81.2 g/day), 20–39% (57.1, 38.5–81.3 g/day), and ≥40% (56.5, 41.4–79.6 g/day).

Overall, rs4247357 was not associated with UL risk (Table 2). Relative to the CC genotype (29.6%), the OR was 1.04 (95% CI: 0.92–1.19) for the AC genotype (52.2%) and 1.09 (95% CI: 0.93–1.29) for the AA genotype (18.3%) (*P*-trend=0.281).

Although the association between rs4247357 and UL risk was slightly stronger among women aged 35 and older at baseline (Table 3), age-specific associations were not statistically different (*P*-interaction by age: 0.658, when using a general genotypic model for rs4247357 (i.e., separate categories for each of the three genotypes). Likewise, results were similar when the case group was restricted to surgical cases, surgical cases aged <35 at baseline, or those with a family history of UL (data not shown). When the control group was restricted to those with a recent pelvic ultrasound, thereby reducing potential for misclassification of UL cases as non-cases, results were similar to the overall results: relative

to CC genotype, ORs were 0.99 (95% CI: 0.84–1.17) for the AC genotype and 1.07 (95% CI: 0.87–1.33) for the AA genotype (*P*-trend: 0.581).

When we stratified the data by percent European ancestry (Table 3), we observed a positive association between rs4247357 and UL risk among the 7% of women with mean European ancestry ≥40%. Relative to the CC genotype (25.7%), the OR was 2.03 (95% CI: 1.12–3.69) for the AC genotype (55.0%) and 2.44 (95% CI: 1.20–4.96) for the AA genotype (19.3%) (*P*-trend=0.012; *P*-interaction by % European ancestry: 0.137, when using a general genotypic model; 0.066, when using a dominant model for the risk A-allele with AC or AA genotypes combined).

We assessed the potential modifying effect of total dietary fat intake on the *FASN*-UL association (Table 4). Among women in the bottom tertile of total fat intake (<40 g/day), ORs were 1.26 (95% CI: 0.97–1.64) for the AC genotype and 1.58 (95% CI: 1.13–2.19) for the AA genotype, relative to CC genotype (*P*-trend: 0.006); no material associations between rs4247357 and UL risk were observed among the middle (40–64 g/day) and upper (≥50 g/day) tertiles of dietary fat intake. Differences in association across strata of dietary fat intake were statistically significant (*P*-interaction: 0.042). Numbers were too small to examine the *FASN* association within joint levels of fat intake and % European ancestry.

When we grouped women with at least 1 risk allele (AA or AC) and compared them with women who had no risk allele (CC), results were similar to the original results. Overall, the main effect of rs4247357 was 1.06 (95% CI: 0.94–1.19); the ORs within strata of age <35 and ≥35 years were 1.01 (95% CI: 0.86–1.18) and 1.16 (95% CI: 0.98–1.37), respectively (p-interaction: 0.390); the ORs within strata of mean % European ancestry <10%, 10–19%, 20–39%, and ≥40% were 1.22 (95% CI: 0.85–1.74), 1.01 (95% CI: 0.85–1.22), 0.97 (95% CI: 0.80–1.18), and 2.14 (95% CI: 1.20–3.79), respectively (p-interaction: 0.066); and the ORs within strata of total dietary fat intake (lowest, middle, and highest tertiles) were 1.34 (95% CI: 1.04–1.72), 0.92 (95% CI: 0.73–1.16), and 0.95 (95% CI: 0.77–1.17), respectively (p-interaction: 0.055).

DISCUSSION

This is the first well-powered study to assess rs4247357 and UL incidence among African American women. Although there was little evidence for an association overall, UL risk was positively associated with the AA genotype of SNP rs4247357 among the 7% of women with ≥40% European ancestry. In addition, the association between rs4247357 was stronger among women in the lowest tertile of dietary fat intake (<40g/day) relative to women in the middle (40–64 g/day) and upper (≥65 g/day) tertiles of dietary fat intake. The interaction between total dietary fat intake and *FASN* supports the hypothesis that *FASN* affects risk of UL through increased *de novo* lipogenesis. Among women with low total fat intake, *de novo* lipogenesis would be a major contributor of fatty acid supply to meet the diverse metabolic needs of tumor cells.

A previous publication from our cohort reported a strong inverse association between percent European ancestry and UL incidence (27), indicating that genetic ancestry is an

important determinant of UL risk. The observation of an association between rs4247357 and UL risk among women with ≥40% European ancestry supports findings from the recent 2012 GWAS among women of European descent (8). Our results do not agree with those from a smaller ultrasound screening study of European and African American women (9). The average percent European ancestry among African American women is about 20%. If we assume that there is a single causal variant in *FASN*, then our results might indicate that rs4247357 would be tagging that variant among European ancestry subjects and among African Americans with high European admixture with a similar LD structure (i.e. large haplotype blocks) to European subjects. On the other hand, African American women with low European admixture would have smaller LD blocks around the causal variant, which could indicate that rs4247357 is not a good tag of this variant. The LD block around rs4247357, defined as $r^2 \geq 0.8$, extends over a region of 134 kb in European ancestry populations and over a smaller region of 82 kb in African ancestry populations from 1000 Genomes Project.

Limitations of our investigation included the restriction of our analysis to the index SNP reported in the Eggert et al. study (8). The frequency of the risk A-allele of rs4247357, 48%, is the same in African and European ancestry populations from 1000 Genomes Project. With our overall sample size, we had >90% power to detect an OR=1.30 (i.e., the effect size found in the GWAS of women of European descent (8)). However, we may have missed other important neighboring SNPs in the same gene and our actual power could be lower than 90% if the true OR was less than 1.30 (i.e. the original GWAS may be overestimating the true effect due to the “winner’s curse” phenomenon). Analyses of gene-environment interactions require large sample sizes to detect differences in genotype by environmental factors. Despite relatively small numbers of cases and controls, there was a suggestion in the data that percent European ancestry and dietary fat intake modified the genotype effect in the expected direction. The 2001 FFQ, instead of the 1995 FFQ, was used for estimation of total dietary fat intake because of its more detailed ascertainment of fatty foods. Misclassification of total dietary fat was possible if intake in 2001 was not reflective of average total fat intake in the preceding years.

Strengths of our study included adjustment for age, geographic region, and ancestry, potential confounders of the *FASN*-UL association. Observed allele frequencies were consistent with 1,000 Genomes Project data for populations of African ancestry (28). Cohort retention was high, reducing potential for differential loss to follow-up. Because we did not screen all women for UL, we may have misclassified true cases as non-cases (6). However, our validation study of UL indicated high accuracy in reporting (>96%) and results were similar when we restricted the control group to women with a recent pelvic ultrasound. Although misclassification of UL was likely, any misclassification would be non-differential (unrelated to genes under study) and lead to attenuation of associations. The large sample size conferred high statistical power to detect relatively small associations. Because 87% of cases had UL-related symptoms or a palpable tumor on pelvic exam (15), our results likely apply to symptomatic UL, which represents the disease burden in reproductive-aged women.

In summary, our data indicate that *FASN* may be involved in the etiology of UL among women of European ancestry, but not African ancestry. Total dietary fat intake may modify

this association. If the observed association is real among African American women with high European ancestry, it remains to be shown whether rs4247357, or some neighboring SNP, is the true causal variant. Additional studies involving direct sequencing of this region among women of African and European ancestry are warranted.

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Table 1

Baseline characteristics of cases and controls. Black Women's Health Study, USA, 1997–2011

Characteristic[a]	Cases	Controls	*P-value*
Number of women	2,301	3,005	
Age, y (mean)	34.2 (±6.2)	34.1 (±7.1)	*0.497*
BMI, kg/m^2 (mean)	28.0 (±7.1)	28.4 (±7.4)	*0.098*
Age at menarche, y (mean)	12.2 (±61.6)	12.4 (±1.6)	*0.003*
Parous (%)	53.5	58.3	*<0.001*
Age at first birth, y (mean)[b]	23.2 (±4.9)	25.4 (±5.4)	*0.003*
Time since last birth, y (mean)[b]	12.6 (±6.5)	15.5 (±7.0)	*0.041*
Papanicoloau test <2 y ago (%)	95.0	95.0	*0.261*
% European ancestry (mean)[c]	20.0 (±10.6)	21.8 (±11.5)	*<0.001*

Abbreviations: UL, uterine leiomyomata; BMI, body mass index.

[a] Means (± SD) and percentages are standardized to age distribution of sample in 1997.

[b] Restricted to parous women only.

[c] Based on panel of ancestry informative markers (AIMs) designed to estimate % European ancestry.

Table 2

Association between single nucleotide polymorphism rs4247357 and UL.

Subgroup	Genotype[a]	Cases/controls	Unadjusted model	Adjusted model[b]	*P-trend*
			OR (95% CI)	OR (95% CI)	
All women					
	CC	664/905	1.00 (ref.)	1.00 (ref.)	*0.281*
	AC	1,205/1,563	1.05 (0.93–1.19)	1.04 (0.92–1.19)	
	AA	432/537	1.10 (0.93–1.29)	1.09 (0.93–1.29)	
	Per one A-allele increase		*1.05 (0.97–1.13)*	*1.05 (0.96–1.13)*	

[a]Order of genotype categories is as follows: top row = homozygous for the reference allele, middle row =heterozygous, bottom row = homozygous for the risk allele.

[b]Adjusted for age in 1997, geographic region, and percent European ancestry.

Table 3

Association between single nucleotide polymorphism rs4247357 and UL, by age and percent European ancestry.

	Genotype[a]	Cases/Controls	Adjusted OR (95% CI) [b]	*P-trend*
Age at baseline, years				
<35	CC	365/502	1.00 (ref.)	*0.568*
	AC	628/868	0.99 (0.84–1.16)	
	AA	238/307	1.07 (0.87–1.32)	
	Per one A-allele increase		*1.03 (0.93–1.14)*	
≥35	CC	299/403	1.00 (ref.)	*0.093*
	AC	577/695	1.15 (0.96–1.37)	
	AA	194/230	1.20 (0.95–1.50)	
	Per one A-allele increase		*1.10 (0.98–1.23)*	
Mean European ancestry, %				
<10	CC	81 /89	1.00 (ref.)	
	AC	195/170	1.27 (0.88–1.85)	
	AA	60/61	1.06 (0.66–1.71)	
	Per one A-allele increase		*1.05 (0.83–1.33)*	*0.669*
10–19	CC	306/395	1.00 (ref.)	
	AC	517/647	1.02 (0.84–1.23)	
	AA	170/216	1.00 (0.77–1.28)	
	Per one A-allele increase		*1.00 (0.88–1.13)*	*0.991*
20–39	CC	158/347	1.00 (ref.)	
	AC	529/611	0.93 (0.76–1.14)	
	AA	176/216	1.09 (0.84–1.41)	
	Per one A-allele increase		*1.03 (0.91–1.17)*	*0.656*
≥40	CC	19/74	1.00 (ref.)	
	AC	64/135	2.03 (1.12–3.69)	
	AA	26/44	2.44 (1.20–4.96)	
	Per one A-allele increase		*1.56 (1.10–2.20)*	*0.012*

[a] Order of genotype categories is as follows: top row = homozygous for the reference allele, middle row =heterozygous, bottom row = homozygous for the risk allele.

[b] Adjusted for age in 1997, geographic region, and percent European ancestry.

P-value, interaction test (Likelihood ratio) for age: 0.658 (when coding FASN as polytomous variable) and 0.390 (when coding FASN as binary variable with AC or AA genotypes combined). P-value, interaction test (Likelihood ratio) for % European ancestry: 0.137 (when coding FASN as polytomous variable) and 0.066 (when coding FASN as binary variable with AC or AA genotypes combined).

Table 4

Association between *FASN* and UL, by total dietary fat intake

	Intake of total dietary fat (g/day)					
	Bottom tertile (<40 g/day)		Middle tertile (40–64/g/day)		Upper tertile (≥65 g/day)	
	Cases/ controls	Adjusted model[b] OR (95% CI)	Cases/ controls	Adjusted model[b] OR (95% CI)	Cases/ controls	Adjusted model[b] OR (95% CI)
Genotype[a]						
CC	154/222	1.00 (ref.)	191/230	1.00 (ref.)	222/290	1.00 (ref.)
AC	288/325	1.26 (0.97–1.64)	315/431	0.88 (0.69–1.13)	402/533	0.99 (0.79–1.23)
AA	124/114	1.58 (1.13–2.19)	127/153	1.01 (0.74–1.37)	113/174	0.84 (0.62–1.13)
P-trend		*0.006*		*0.895*		*0.309*

[a]Order of genotype categories is as follows: top row = homozygous for the reference allele, middle row =heterozygous, bottom row = homozygous for the risk allele.

[b]Adjusted for age in 1997, geographic region, and percent European ancestry.

P-value, interaction test (Likelihood ratio) between total fat intake (coded as ordinal categorical variable) and FASN: 0.042 (when coding FASN as polytomous variable in genotypic model) and 0.055 (when coding FASN as binary variable in genotypic model).