Is the Observed Association Between Dairy Intake and Fibroids in African Americans Explained by Genetic Ancestry?

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Uterine leiomyomata are a major source of gynecological morbidity and are 2–3 times more prevalent in African Americans than European Americans. In an earlier report, we found that dairy intake was inversely associated with uterine leiomyomata among African Americans. Because African Americans are more likely to have lactose intolerance and avoid dairy products, the observed association might have been confounded by genetic ancestry. This report reevaluates the dairy-uterine leiomyomata association after accounting for genetic ancestry among 1,968 cases and 2,183 noncases from the Black Women’s Health Study (1997–2007). Dairy intake was estimated by using food frequency questionnaires in 1995 and 2001. Percent European ancestry was estimated by using a panel of ancestry informative markers. Incidence rate ratios and 95% confidence intervals were estimated by using Cox regression, with adjustment for potential confounders and percent European ancestry. Incidence rate ratios comparing 1, 2, 3, and ≥4 servings/day with <1 serving/day of dairy products were 0.95 (95% confidence interval (CI): 0.85, 1.06), 0.75 (95% CI: 0.61, 0.92), 0.77 (95% CI: 0.57, 1.04), and 0.59 (95% CI: 0.41, 0.86), respectively (P_trend = 0.0003). These effect estimates were similar to those obtained without control for ancestry. The findings suggest that the observed inverse association between dairy consumption and uterine leiomyomata in African Americans is not explained by percent European ancestry.

African Americans; dairy products; genetic ancestry; leiomyoma; prospective studies

Abbreviations: AIM, ancestry informative marker; CI, confidence interval; SNP, single nucleotide polymorphism.
obtained DNA from 26,814 participants using the mouthwash-swish method (10). The present analysis includes 4,151 premenopausal women aged 23–50 years in 1997. The institutional review board of Boston University Medical Center approved the study protocol.

Assessment of outcome

On each follow-up questionnaire, participants were asked whether they had been diagnosed with "uterine fibroids," in what year, and whether the diagnosis had been confirmed by pelvic examination, ultrasound, or surgery (5). Self-reported uterine leiomyomata cases confirmed by ultrasound or surgery were included as cases. A validation study confirmed the self-report in 96% of cases (11).

Assessment of diet

Diet was assessed in 2 separate questionnaire cycles (1995 and 2001) by using a modified version of the National Cancer Institute (NCI)-Block short form food frequency questionnaire (12, 13). Data were collected on beverage consumption (and portion size) during the previous year, with frequencies ranging from “never or <1 per month” to “≥6 per day.” Dairy items included skim milk/1% milk/butter-milk, 2% milk, whole milk, milk/cream in coffee or tea, ice cream (1995), regular ice cream (2001), low-fat ice cream (2001), frozen yogurt, yogurt, cheese and cheese spreads (not cottage cheese), and butter. Total dairy intake was calculated by summing servings of all dairy foods except butter, because butter is composed almost entirely of fat. DIETCALC, version 1.4.1, software (National Cancer Institute, Rockville, Maryland) provided estimates of caloric intake and various nutrients. In a validation study (13), mean daily servings of dairy items were 1.0 (within 1.3 standard deviation) for the 1995 food frequency questionnaire and 1.1 (within 1.0 standard deviation) for the average of 3 dietary recalls (14).

Assessment of covariates

In 1995, we collected data on age at menarche, oral contraceptive use, parity, age at each birth, height, weight, alcohol intake, smoking, education, marital status, occupation, and geographical region. We asked about household income in 2003 and about recency of pelvic examination and ultrasound in 2007. Family history of uterine leiomyomata was ascertained in 2009. Reproductive factors, weight, smoking, marital status, and region were updated on follow-up questionnaires.

DNA isolation, amplification, and genotyping

As reported previously (6), we conducted an admixture-based genome-wide scan of incident uterine leiomyomata cases confirmed by ultrasound or surgery during 1997 through 2009. We also genotyped 30 AIMs for diagnosis of a set of premenopausal participants who did not report a diagnosis of uterine leiomyomata through 2009 (6).

DNA was isolated from mouthwash swish samples at the Boston University Molecular Core Genetics Laboratory using the QIAMP DNA Mini Kit (Qiagen, Valencia, California). Whole-genome amplification was performed with Qiagen RePLI-g kits by using multiple displacement amplification. Before genotyping, amplified samples underwent purification and PicoGreen (Thermo/Fisher, Wilmington, Delaware) quantification at the Broad Institute Center for Genotyping and Analysis (Cambridge, Massachusetts).

In the original admixture mapping scan (6), uterine leiomyomata cases were genotyped on the “Phase 3” admixture panel consisting of 1,509 AIMs in an Illumina GoldenGate custom assay in the BeadLab platform (Illumina, San Diego, California) (15). After AIMs were filtered out with a call rate <95% or poor clustering, the final admixture analysis included 1,430 AIMs. Noncases were genotyped for 30 AIMs that were a subset of the single nucleotide polymorphisms (SNPs) in the admixture panel selected to have the greatest difference in frequency between European and West African populations. We previously showed that this reduced set of AIMs produces estimates of European ancestry that are highly correlated with those based on the complete panel (r = 0.89) (16). Genotyping of these 30 SNPs was carried out in a Sequenom iPLEX assay (Sequenom, San Francisco, California). One SNP failed the genotyping, leaving 29 SNPs for analysis.

In the present analysis, cases and noncases shared a common set of 22 SNPs that were successfully genotyped in both groups, which we used to adjust for percent European ancestry in primary analyses. In secondary analyses, we used all available genotyping data to estimate percent European ancestry: 1,430 AIMs for cases and 29 AIMs for noncases. The mean, median, minimum, and maximum values of percent European ancestry were 20.6%, 18.9%, 6.8%, and 57.7% for the 22 AIMs and were 20.9%, 18.8%, 0.9%, and 75.7% for the 1,430 AIMs. The Pearson correlation for the reduced versus full panel was 0.79 (P < 0.0001).

Analytical sample

Our previous report on dairy and uterine leiomyomata was based on 5,871 cases ascertained in the Black Women’s Health Study from 1997 through 2007 (5). The present report was based on the subset of 1,968 uterine leiomyomata cases diagnosed through 2007 that were included in the admixture scan and had complete genotyping data (6). Also included were 2,183 noncases with genotyping data (premenopausal women similar in age to the cases without a family history of uterine leiomyomata and who did not report a diagnosis of uterine leiomyomata through 2007). We excluded noncases with a family history of uterine leiomyomata to increase the specificity of outcome classification (17).

Data analysis

We used restricted cubic splines to summarize the relation between percent European ancestry and uterine leiomyomata incidence (18). We used age- and period-stratified Cox regression to estimate incidence rate ratios and 95% confidence intervals for the associations of interest. Person-years were calculated from March 1997 until uterine leiomyomata diagnosis, menopause, death, loss to follow-up, or March 2007.
We used the same multivariable models from our original dairy analysis (5), which controlled for age, time period, energy intake, age at menarche, parity, age at first birth, years since last birth, ever use of oral contraceptives, age at first oral contraceptive use, body mass index (weight (kg)/height (m)^2), vigorous exercise, smoking, current alcohol use, education, marital status, occupation, household income, geographical region, and diabetes. The present analysis further controlled for “percent European ancestry” as a continuous variable, in quartiles, and in finer categories (<5%, 5–9%, 10–14%, 15–19%, 20–24%, 25–29%, 30–34%, 35–39%, 40–44%, 45–49%, and ≥50%) to assess whether the results varied according to its functional form. Age, childbearing characteristics, and lifestyle factors were modeled as time-varying covariates. Tests for trend were conducted by modeling dairy intake as an ordinal variable (20). Statistical interaction was evaluated by using a likelihood ratio test comparing models with and without cross-product terms between exposure and effect modifiers. The figure was generated by using STATA software (21), and all regression analyses were performed by using SAS software (22).

**RESULTS**

In the present analysis, European ancestry was inversely associated with uterine leiomyomata incidence (Figure 1) and positively associated with dairy intake. Mean percent European ancestry was significantly higher among women consuming ≥4 servings/day with <1 serving/day of dairy products were 0.94 (95% CI: 0.88, 1.00), 0.87 (95% CI: 0.78, 0.98), 0.84 (95% CI: 0.70, 1.01), and 0.70 (95% CI: 0.58, 0.86), respectively (P \text{trend} < 0.001). The present study found a similar inverse association of dairy intake with uterine leiomyomata after control for the same confounders (Table 1): Multivariable incidence rate ratios were 0.94 (95% CI: 0.84, 1.05), 0.74 (95% CI: 0.60, 0.91), 0.77 (95% CI: 0.57, 1.05), and 0.57 (95% CI: 0.39, 0.83), respectively (P \text{trend} = 0.00001). Multivariable incidence rate ratios were similar regardless of how percent European ancestry was controlled, including when ancestry was estimated by using the full panel of 1,430 AIMs in cases and 29 AIMs in noncases (Table 1).

An inverse association between dairy intake and uterine leiomyomata was evident in quartiles 1, 3, and 4 of percent European ancestry, and the association did not differ statistically across quartiles (P \text{interaction} = 0.345) (Table 2).

The SNP rs4988235 has been associated with lactase persistence in northern Europeans (23). Our full panel of AIMs (genotyped only in cases) included rs218174, which is highly correlated with rs4988235 in African Americans (r = 0.84) (23–26). In our cohort, the correlation between rs218174 and percent European ancestry was 0.28 (P < 0.0001). Mean dairy intake increased with increasing number of rs218174 alleles. Relative to the GG genotype (no lactase persistence allele), mean differences in dairy intake (servings/week) were 0.93 (95% CI: 0.33, 1.52) and 2.75 (95% CI: 1.17, 4.32) for those with the AG and AA genotypes, respectively, after controlling for percent European ancestry.

**DISCUSSION**

In our original study (5), multivariable incidence rate ratios comparing 1, 2, 3, and ≥4 servings/day with <1 serving/day of dairy products were 0.94 (95% CI: 0.88, 1.00), 0.87 (95% CI: 0.78, 0.98), 0.84 (95% CI: 0.70, 1.01), and 0.70 (95% CI: 0.58, 0.86), respectively (P \text{trend} < 0.001). The present study found a similar inverse association of dairy intake with uterine leiomyomata after control for the same confounders (Table 1): Multivariable incidence rate ratios were 0.94 (95% CI: 0.84, 1.05), 0.74 (95% CI: 0.60, 0.91), 0.77 (95% CI: 0.57, 1.05), and 0.57 (95% CI: 0.39, 0.83), respectively (P \text{trend} = 0.00001). Multivariable incidence rate ratios were similar regardless of how percent European ancestry was controlled, including when ancestry was estimated by using the full panel of 1,430 AIMs in cases and 29 AIMs in noncases (Table 1).

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**DISCUSSION**

In the present study, control for percent European ancestry did not alter the observed inverse association between dairy intake and uterine leiomyomata risk. The presence of a dose-response between dairy intake and uterine leiomyomata among women in the lowest and highest categories of percent European ancestry suggests that the inverse association is not due to some aspect of European ancestry and its association with dairy consumption.

Although the exact mechanism for the dairy–uterine leiomyomata association is unknown, there is biological plausibility for an effect (5). Briefly, calcium is a major component of dairy foods that may reduce cell proliferation by maintaining intracellular calcium concentrations (27, 28). Vitamin D may also play a protective role: It has been shown to decrease uterine leiomyoma cell proliferation in vitro (29, 30) and shrink uterine leiomyoma volume in the Eker rat (31). Two cross-sectional studies found that higher blood levels of 25-hydroxyvitamin D were associated with reduced uterine leiomyoma risk (32, 33). Finally, given evidence that sex steroid hormones influence uterine leiomyoma growth (34), hormones in American dairy products (35) may also interact with endogenous hormones to influence uterine leiomyoma risk.

A limitation of our study is that the estimation of European ancestry was based on a subset of 22 AIMs in common between cases and noncases. However, we showed that a reduced panel of AIMs was highly correlated with the full
We were unable to directly assess the role of lactose intolerance in explaining our dairy–uterine leiomyomata association. Our full panel of 1,430 AIMs included rs218174, which is highly correlated with the lactase persistence SNP rs4988235 (23–26). Among cases, rs218174 was positively associated with percent European ancestry. Lack of data on rs218174 among noncases precluded us from evaluating its association with uterine leiomyomata. Evidence suggests that not all SNPs predictive of lactase persistence have been identified; none of the currently identified lactase persistence SNPs (rs3778919, rs4988235, rs178174, rs4988237) were associated with uterine leiomyomata in our full panel (16) and that results were not appreciably different when we used 1,430 AIMs for cases and 29 AIMs for noncases.

### Table 1. Risk of Uterine Leiomyomata in Relation to Dairy Intake, With and Without Adjustment for Percent European Ancestry, Black Women's Health Study, 1997–2007

<table>
<thead>
<tr>
<th>Dairy servings/day</th>
<th>No. of Cases</th>
<th>Person-Years</th>
<th>IRR**</th>
<th>95% CI</th>
<th>IRR**</th>
<th>95% CI</th>
<th>IRR**</th>
<th>95% CI</th>
<th>IRR**</th>
<th>95% CI</th>
<th>IRR**</th>
<th>95% CI</th>
</tr>
</thead>
<tbody>
<tr>
<td>&lt;1</td>
<td>1,293</td>
<td>18,460</td>
<td>1.00</td>
<td>Referent</td>
<td>1.00</td>
<td>Referent</td>
<td>1.00</td>
<td>Referent</td>
<td>1.00</td>
<td>Referent</td>
<td>1.00</td>
<td>Referent</td>
</tr>
<tr>
<td>1</td>
<td>490</td>
<td>7,376</td>
<td>0.93</td>
<td>0.84, 1.05</td>
<td>0.94</td>
<td>0.84, 1.05</td>
<td>0.95</td>
<td>0.85, 1.06</td>
<td>0.94</td>
<td>0.84, 1.06</td>
<td>0.95</td>
<td>0.85, 1.06</td>
</tr>
<tr>
<td>2</td>
<td>108</td>
<td>2,224</td>
<td>0.70</td>
<td>0.57, 0.86</td>
<td>0.74</td>
<td>0.60, 0.91</td>
<td>0.75</td>
<td>0.61, 0.92</td>
<td>0.74</td>
<td>0.60, 0.92</td>
<td>0.75</td>
<td>0.61, 0.92</td>
</tr>
<tr>
<td>3</td>
<td>47</td>
<td>902</td>
<td>0.74</td>
<td>0.55, 1.00</td>
<td>0.77</td>
<td>0.57, 1.05</td>
<td>0.78</td>
<td>0.58, 1.05</td>
<td>0.77</td>
<td>0.57, 1.04</td>
<td>0.79</td>
<td>0.58, 1.07</td>
</tr>
<tr>
<td>≥4</td>
<td>30</td>
<td>789</td>
<td>0.57</td>
<td>0.39, 0.83</td>
<td>0.57</td>
<td>0.39, 0.83</td>
<td>0.58</td>
<td>0.40, 0.84</td>
<td>0.58</td>
<td>0.40, 0.84</td>
<td>0.59</td>
<td>0.41, 0.86</td>
</tr>
</tbody>
</table>

Abbreviations: CI, confidence interval; IRR, incidence rate ratio.

** Adjusts for time-varying age, questionnaire cycle, and energy intake.

* Adjusts for age, questionnaire cycle, energy intake, age at menarche, parity, age at first birth, years since last birth, use of oral contraceptives, age at first oral contraceptive use, vigorous exercise, smoking, alcohol, body mass index, diabetes, education, occupation, income, marital status, and geographical region.

\[ P_{\text{trend}} < 0.0001 \]

### Table 2. Risk of Uterine Leiomyomata in Relation to Dairy Intake Within Quartiles of Percent European Ancestry, Black Women's Health Study, 1997–2007

<table>
<thead>
<tr>
<th>No. of Cases</th>
<th>Person-Years</th>
<th>IRR**</th>
<th>95% CI</th>
</tr>
</thead>
<tbody>
<tr>
<td>Quartile 1 (&lt;14%)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Dairy servings/day</td>
<td>1,293</td>
<td>18,460</td>
<td>1.00</td>
</tr>
<tr>
<td>1</td>
<td>490</td>
<td>7,376</td>
<td>0.93</td>
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<tr>
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<td>108</td>
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<tr>
<td>≥4</td>
<td>30</td>
<td>789</td>
<td>0.57</td>
</tr>
</tbody>
</table>

\[ P_{\text{trend}} < 0.0001 \]

Abbreviations: CI, confidence interval; IRR, incidence rate ratio.

* Adjusts for age, questionnaire cycle, energy intake, age at menarche, parity, age at first birth, years since last birth, use of oral contraceptives, age at first oral contraceptive use, vigorous exercise, smoking, alcohol, body mass index, diabetes, education, occupation, income, marital status, geographical region, and percent European ancestry (continuous).

\[ P_{\text{trend}} < 0.0001 \]

* Adjusts for all variables in footnote b and percent European ancestry (quartiles).

* Adjusts for all variables in footnote b and percent European ancestry (5% interval categories).

* Adjusts for all variables in footnote b but uses the fully genotyped panel of 1,430 single nucleotide polymorphisms in cases and 29 ancestry informative markers in noncases.
predict the phenotype in subpopulations of West and East Africa (36–38). Thus, it was difficult to predict the extent to which residual confounding by lactase deficiency affected our results.

Most studies of behavioral and lifestyle determinants of disease lack information on genetic ancestry. Genotyping a small panel of AIMs in such studies may be a cost-effective way to control for differences in genetic ancestry. In addition to the present study, we evaluated the association between hair relaxer use and uterine leiomyomata while controlling for European ancestry (39), but we could find no other studies of this kind.

In summary, we found that control for percent European ancestry did not explain the previously observed inverse association of high dairy intake with uterine leiomyomata among African Americans. Because dairy intake is appreciably lower among black than white Americans (40), differences in dairy intake may contribute to the racial discrepancy in rates of uterine leiomyomata. Confirmation of these findings in other populations is warranted.

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Conflict of interest: none declared.

REFERENCES


