Multivitamin and Alcohol Intake and Folate Receptor α Expression in Ovarian Cancer

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Abstract

Folate receptor α (FRα) expression in epithelial ovarian cancer may be related to folate intake. We examined this association using multivitamin intake, a proxy for folic acid, and assessed whether the relation was modified by alcohol intake, a folate agonist. Cases (n = 148) with suspected epithelial ovarian cancer, of ages ≥20 years, were seen at Mayo Clinic, Minnesota, between 2000 and 2004; those with tumor specimens (n = 108) were included in analyses. Outpatient controls (n = 148) without cancer and with at least one ovary intact were matched to cases by age (within 5 years) and state of residence. Multivitamin (≥4 pills/wk) and weekly alcohol (≥5 drinks) intakes were assessed. Tumor specimens were analyzed immunohistochemically for FRα. Multivariable rate ratios (RR) and 95% confidence intervals (CI) were estimated using unconditional logistic regression. In case-control analysis, the RRs of multivitamin intake with absent/weak/moderate and strong-expressing FRα tumors were 0.30 (95% CI, 0.12-0.70) and 0.47 (95% CI, 0.24-0.91), respectively. For alcohol, the associations were 0.84 (95% CI, 0.24-2.86) and 1.65 (95% CI, 0.69-3.93), respectively. In case-case analysis, the RR associated with developing strong-expressing versus other FRα tumors was 3.13 (95% CI, 1.14-8.65) for multivitamins and 1.58 (95% CI, 0.45-5.60) for alcohol. The data did not support evidence for an interaction between multivitamin and alcohol intake with risk of developing a strong-expressing FRα tumor. The association of multivitamin intake with ovarian cancer may depend on FRα expression level. (Cancer Epidemiol Biomarkers Prev 2005;14(9):2168–72)

Introduction

Ovarian cancer is the leading cause of mortality among cancers specific to women in the United States (1). Few epidemiologic factors for ovarian cancer are recognized: age, a family history of ovarian or breast cancer, and nulliparity are associated with increased risk, whereas oral contraceptive use is associated with decreased risk (2, 3). These variables, along with a low intake of green vegetables and a high dietary fat score, explained only 50% of ovarian cancer risk in an Italian population (4), highlighting the need to further identify factors associated with the development and progression of this debilitating and often fatal cancer.

In a recent analysis of the 15 year follow-up data of the Iowa Women's Health Study (5), we reported that higher baseline folate intake (≥331 μg/d) was associated with an increased risk of developing ovarian cancer during the first half of follow-up [risk ratio, 1.9; 95% confidence interval (95% CI), 0.9-4.1] compared with women who developed ovarian cancer during the second half of follow-up (risk ratio, 0.9; 95% CI, 0.5-1.6). In performing these analyses, we assumed that women who developed ovarian cancer during the first half were more likely to have undiagnosed tumors in an advanced stage of progression at the time of dietary assessment than those women who developed ovarian cancer with longer follow-up, and that the timing of higher folate intake could be related to tumor progression or prevention as reported elsewhere (6).

From these observations, we hypothesized that the apparent differences in risk may be due to the presence of the folate receptor α (FRα), a single chain glycosyl-phosphatidylinositol–anchored membrane protein (7) with high affinity for binding and transporting into cells physiologic levels (in the nanomolar range) of folic acid, 5-methyltetrahydrofolate (the main circulating form of folate; ref. 8), and, to a lesser extent, antifolates such as methotrexate (9). It differs from the low-affinity reduced folate carrier found in nearly all cells, which mediates the uptake of reduced folates at higher concentrations (10). Compared with normal ovarian cells, FRα is overexpressed in nonmucinous epithelial ovarian tumors (7, 11, 12) and increases in concentration with tumor progression (13). Folate is an essential vitamin required for DNA synthesis in both normal and tumor cells. *In vitro* studies showed that the *FRα* gene expression is up-regulated when cells are exposed to folate-deplete media and down-regulated in folate-replete media (14-17). FRα may confer a growth advantage to the tumor by modulating folate uptake from serum (11) or by generating regulatory signals (18). Alcohol is a folate agonist and may interfere with this process (19, 20).

Although studies have associated tumor characteristics with FRα expression (12), to our knowledge, no study has examined the relation of epidemiologic factors with FRα expression. Therefore, using data from an ongoing case-control study of ovarian cancer with tumor specimens available from the cases, we assessed whether the association of multivitamin intake varies with the level of FRα expression in ovarian tumors, and if this association is modified by alcohol intake.

Materials and Methods

Study Design and Population. This study was carried out in a subset of cases and controls that participated in an ongoing case-control study of ovarian cancer from January 1, 2000 to August 1, 2004 at Mayo Clinic, Rochester, MN. Cases were women over 20 years of age, with histologically confirmed incident epithelial ovarian cancer (borderline or invasive), and enrolled in the study within 1 year of date of diagnosis. Controls without prior history of cancer (other than nonmelanoma skin cancer), with one or both ovaries intact, were matched on age (±5 years), race, and state of residence to cases. Controls were selected from the outpatient clinic in the Department of Internal

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Medicine at Mayo Clinic where they were seen for general medical examinations. Written informed consent was obtained from all participants. All cases consented to providing a tumor specimen under the purview of the Mayo Ovarian Tissue Registry (see below). In addition, we prospectively collected eleven ovarian tissue specimens with surface epithelium from seven postmenopausal women without ovarian cancer who presented to Mayo Clinic for oophorectomies in October 2004. The parent study and the present investigation were approved by the Mayo Clinic Institutional Review Board.

Mayo Ovarian Tissue Registry. Initiated in 1991, the Mayo Ovarian Tissue Registry collects frozen and paraffin-embedded ovarian tissue specimens and is complete with relevant demographic, diagnostic, and treatment data. Patients from the six-state region that defines the primary service population of Mayo Clinic (Minnesota, Iowa, Wisconsin, Illinois, North Dakota, and South Dakota) constitute ~85% of all ovarian cancer cases. A rapid case ascertainment system permits the opportunity to recruit, interview, and obtain specimen samples from patients while they are still in hospital. Participation of patients in the Institutional Review Board–approved protocol is virtually 100% for collection of tumor tissue. Consequently, these women form the sampling frame for the ovarian case-control study described above.

Risk Factor Questionnaire. Both the cases and controls completed an interviewer-administered risk factor questionnaire while in hospital. The seven-page questionnaire asked about known or suspected ovarian cancer risk factors including lifestyle, medical, and reproductive factors. In January 2003, the questionnaire was expanded with questions about vitamin and alcohol intake during the previous year and before cancer diagnosis for the cases. Participants were asked about their regular use of multivitamins and use of single vitamin preparations such as vitamins A, C, and E. Nine category options were provided to describe alcohol consumption and ranged from less than once per month to six or more per day. As of August 1, 2004, 148 cases completed the updated questionnaire.

Exclusion Criteria. Forty cases were excluded for the following reasons: no/insufficient tumor (n = 14), recurrent disease or not primary epithelial ovarian cancer (n = 9), negative biopsy (n = 5), tumor blocks were unavailable from the tissue registry (n = 5), and treatment was received between diagnosis and surgical biopsy or the time between diagnosis and surgery exceeded 2 months (n = 7). The last criterion was specified a priori to minimize any mitigating or potentiating influence on FRα due to dietary change or antifolate therapy, leaving 108 cases available for analysis.

Tissue Microarray Construction. H&E slides were reviewed by the study pathologist (G.L.K.) for tumor histology and location of tumor in the corresponding patient paraffin tumor block for tissue microarray construction. Arrays were constructed using a custom-fabricated device that used a 0.6 mm tissue corer and a 216-capacity paraffin recipient block. Three cores were extracted from each patient paraffin tumor block and transferred into the recipient block in a spreadsheet manner indicating column and row placement of each core. The resulting tissue microarray was cut into 5-μm-thick sections and mounted on charged slides for immunohistochemistry. Both positive and negative tissue controls were also included in the recipient block for immunohistochemical reactions. The normal ovarian tissue specimens were prepared in the same manner, except that a 5-μm-thick cross section was cut from each paraffin block and mounted individually onto charged slides.

Immunohistochemical Staining for FRα. Slides were treated to unmask epitopes in a BioCare Decloaking chamber (BioCare Medical, Walnut Creek, CA). The remainder of the staining process was done on a Dako Autostainer (Carpinteria, CA) using affinity purified, polyclonal anti-folate receptor antibody Pu17 (Endocyte, West Lafayette, Indiana) at a 1:200 dilution and detection with Envision Plus reagents, followed by 3,3′-diaminobenzidine visualization. Slides were counterstained with hematoxylin. Digital imaging of the slides was done using a Bacus Laboratories Inc. Slide Scanner (BLISS) ''Virtual Microscopy'' microscope and computer system (Bacus Laboratories, Lombard, IL) consisting of a Zeiss Axioplan microscope with computer-interfaced electronic stage controls and a high-resolution 3CCD video camera to produce a virtual slide with core images linked to a Microsoft Access database containing relevant tissue core information. Tumors with intense coarse granular staining were defined as strong positive; light and finely granular staining as weak; intermediate staining as moderate; and no stain over background as absent.

Statistical Analyses. We decided, a priori, to use the tissue core with the highest FRα expression level from each case in statistical analyses. We used median values for continuous variables and percentages for categorical variables to examine the distribution of risk factors among cases and controls and by FRα expression among cases only. Tests for trend were computed using the Mantel-Haenszel χ^2 exact statistic. Continuous variables were categorized and treated as indicator variables in logistic regression models (see below). Category cutoff points were modified, where necessary, from previously defined categories derived from an analysis in a similar population (5) by collapsing categories with cell counts less than five. The cutoff points generally followed quartile distributions of controls in the present investigation except for body mass index in kilograms per square meter, which more closely resembled quintile cutpoints. Regular multivitamin use was defined in the questionnaire as ≥4 pills per week during the past year. Weekly alcohol consumption was categorized as ≥5 drinks or approximately ≥0.5 drinks per day (5).

Because the cases and controls selected for this analysis were originally frequency matched on broad categories of age (within 5 years), state of residence, and race (all women were white), we did not preserve the match in the present analyses. Unconditional logistic regression was used to estimate rate ratios (RR) and 95% CIs among cases and controls for risk of developing a FRα-expressing ovarian tumor associated with multivitamin and alcohol use. Using the likelihood ratio test (21), we identified the most parsimonious multivariable model by fitting a model containing all covariates (displayed in Table 1) and excluding in a stepwise manner those variables that were not statistically significant. Multivitamin and alcohol intake, age, and state of residence were retained in all models. Variables that resulted in a change of ≥10% in the estimates of multivitamin use or alcohol on removal from the model were considered confounders and retained (22); they are listed in the footnote of Table 3. We modeled the case-control association as risk of developing either an absent/weak, moderate, or strong-expressing FRα ovarian tumor; however, given the similar estimates between the absent/weak and moderate-expressing FRα tumor models and because of the small number of cases, we grouped this category. Using a case-only analysis, we also estimated RR and 95% CIs for risk of developing a strong-expressing FRα ovarian tumor compared with absent/weak/moderate-expressing FRα ovarian tumors associated with multivitamin and alcohol use.

We evaluated whether the association of multivitamin intake with strong-expressing FRα ovarian tumors depended on alcohol intake. The presence or absence of effect modification was evaluated on the ratio scale using indicator variables to represent the effect of each exposure in the absence of the other, as well as the joint effect of both exposures, using the group not exposed to either factor as the reference (22).

Table 1. Distribution of descriptive variables among 108 cases with ovarian cancer and 148 controls, Mayo Clinic 2003-2004

Characteristic*	Present study		Parent study	
	Controls (n = 148)	Cases (n = 108)	Controls (n = 415)	Cases (n = 603)
Age, y	60.8 (20)	63.2 (21)	61.0 (19)	60.9 (20)
Body mass index, kg/m^2	25.6 (6)	26.8 (7)	26.2 (7)	27.0 (8)
Six-state region	148 (100)	97 (90)	415 (100)	527 (87)
Local (Minnesota)	78 (53)	52 (48)	240 (58)	266 (44)
Regional (Wisconsin, Iowa, Illinois, North Dakota, and South Dakota)	70 (47)	45 (42)	175 (42)	261 (43)
Distant (national)	0	11 (10)	0	76 (13)
Multivitamin use†,‡	97 (67)	55 (51)	242 (63)	142 (55)
Alcohol intake, ≥5 drinks/wk†	20 (14)	16 (15)	67 (17)	34 (13)
Nonsmoker†	96 (66)	65 (60)	260 (64)	285 (49)
Exercise weekly†,§	33 (22)	15 (14)	97 (24)	50 (19)
Education ≤ high school†	39 (26)	46 (43)	122 (30)	195 (44)
Family history of breast or ovarian cancer‖	24 (16)	25 (23)	72 (18)	132 (22)
Age at menarche, y	13.0 (2)	13.0 (2)	13.0 (2)	13.0 (2)
Age at first live birth, y	24.0 (6)	22.0 (4)	23.0 (5)	22.0 (5)
Oral contraceptive use				
Never	57 (40)	57 (55)	151 (38)	280 (49)
1-48 mo	36 (25)	27 (26)	88 (22)	151 (27)
>48 mo	51 (35)	19 (18)	154 (39)	138 (24)
Postmenopausal hormone use				
Never	72 (51)	63 (59)	221 (57)	343 (59)
1-60 mo	30 (21)	21 (20)	80 (21)	108 (19)
>60 mo	38 (27)	22 (21)	84 (22)	127 (22)
Parity				
0	21 (14)	14 (13)	61 (15)	103 (18)
1-2	53 (36)	35 (32)	138 (34)	208 (35)
3+	74 (50)	59 (55)	204 (51)	279 (47)
History of infertility, yes	24 (17)	18 (17)	61 (16)	111 (19)

*Continuous variables are represented by median values (interquartile range) and frequencies are represented by counts (percentage).
†Data based on 415 controls and 258 cases in parent study; these questions were not included on the questionnaire before January 2003.
‡Defined as ≥4 times/wk.
§Defined as ≥2 times/wk of vigorous physical activity during leisure time.
‖In mother or sister.

We also explored whether the RR of multivitamin use with strong-expressing FRα ovarian tumors varied by level of each of the covariates. Analyses were carried out using the Statistical Analysis Software (SAS Institute, Cary, NC) system. Two-sided $P < 0.05$ was considered to be statistically significant.

Results

Cases in the present study were older and heavier than controls, and more cases resided outside the six-state region, and had a family history of ovarian or breast cancer (Table 1). A greater proportion of controls compared with cases took multivitamins and oral contraceptive pills for more than 4 years, had a post-secondary school education, were nonsmokers, and exercised weekly. Cases and controls in the present study were representative of women in the parent study in the distribution of variables except that more cases in the present study did not smoke and had more children (Table 1).

Among cases, the number of malignant tumors that stained strongly for FRα was 49 of 73 (67%) of serous histology, 10 of 15 (67%) of endometrioid histology, 3 of 4 (75%) of clear cell histology, and 0 of 2 mucinous and 0 of 3 other histologies, respectively. Three of 11 (27%) borderline tumors stained strongly for FRα. In comparison, 10 of 11 normal ovarian specimens had surface epithelium and all 10 (100%) stained strongly for FRα (data not shown). The proportion of borderline tumors was inversely associated, and the proportion of tumors of higher grade, stage, and serous histology was positively associated, with increased FRα expression (Table 2). None of the other descriptive variables was significantly associated with FRα tumor expression (Table 2).

In the case-control analyses, multivitamin use was inversely associated with risk of ovarian cancer in age- and state-adjusted analyses regardless of FRα expression level (Table 3), and the associations strengthened following multivariable adjustment. Adjustment for intake of the commonly consumed single vitamin preparations A, C, and E attenuated all associations (data not shown). None of the associations with alcohol intake ≥5 drinks per week was significant. Separate models that excluded borderline tumors did not result in estimates that were meaningfully different than when these tumors were retained in the model (data not shown). Because 10% of cases (n = 11) resided outside of the region represented by our control population, we reran the analyses excluding these cases; all estimates were essentially unchanged (data not shown).

Among cases only, multivitamin intake was associated with an increased risk of developing a strong-expressing FRα ovarian tumor compared with absent/weak/moderate-expressing FRα tumors in multivariable analyses [2.40 (95% CI, 0.96-6.03)]. This relation strengthened to 3.13 (95% CI, 1.14-8.65) following additional adjustment for tumor stage and grade. Adjustment for intake of the single vitamin preparations A, C, and E and exclusion of 11 borderline tumors each attenuated the association. Alcohol intake was associated with an RR of 1.58 (95% CI, 0.45-5.60) of developing a strong-expressing FRα tumor in multivariable analyses after control for tumor stage and grade although the estimate was not precise. There was no evidence of an interaction between multivitamin and alcohol intake (data not shown).

To explore heterogeneity of the RR of multivitamin intake with strong-expressing FRα tumors by level of the other covariates, we did stratified analyses adjusting for age, state of residence, tumor grade, and stage. Only higher body mass index, ≥25 kg/m^2 [4.85 (95% CI, 1.42-16.6)], and nonsmoking [3.51 (95% CI, 0.98-12.5)] were associated with increased risk.

Discussion

In this case-control and case-only analysis of epithelial ovarian cancer, multivitamin intake was associated with a lower risk of ovarian cancer regardless of FRα expression level. Among women who developed ovarian cancer, however, multivitamin intake was associated with a 3-fold increased risk of developing a strong-expressing FRα tumor. No significant association was observed with alcohol intake. To our knowledge, this is the first report to examine the association of epidemiologic, and specifically dietary, factors with expression of FRα in ovarian tumors.

FRα expression was reported to be relatively low or absent in normal ovarian epithelial cells compared with malignant ovarian tumors in earlier studies (8, 11, 23). However, a recent examination of normal and malignant ovarian cells using *in situ* hybridization reported mRNA ratios of FRα to β-actin (an internal standard) that were high in malignant serous tumor subtypes, moderately high in normal surface epithelium and malignant endometrioid tumor subtypes, and relatively insignificant in benign mucinous or serous lesions and in malignant mucinous or clear cell tumor subtypes (24). The pattern of FRα expression in the present analysis agrees with that of Wu et al. (24). The differences between studies may be methodologic. For example, Wu et al. (24) described the limitations of immunohistochemical analysis in earlier studies; our use of a polyclonal antibody to FRα, however, could have offset problems associated with the inability of monoclonal antibodies to recognize epitopes to different FRα isoforms (11). Our finding that FRα is highly expressed in nonmucinous ovarian tumors agrees with that of others (25), as does the positive correlation with tumors of high grade and stage (13, 26).

The present analyses add to recent observations that folate may have a dual modulatory role in carcinogenesis depending on the timing and dose of folate intervention (6). In normal epithelial tissues, folic acid supplementation seemed to suppress tumor development, whereas in established neoplasms supplementation seemed to promote progression (6). During absorption, folic acid is reduced and methylated to 5-methyltetrahydrofolate, the main circulating form of the vitamin. This absorption and biotransformation process is saturated at about 300 μg (27); above this level, folic acid, which is normally found in low concentrations in plasma (28), is transported in an unmodified form that is directly proportional to dose (27, 29). Thus, among multivitamin supplement users, it is plausible that plasma folic acid binds to, and is internalized by, FRα. The observed inverse association of multivitamins in the case-control versus the case-only comparison may reflect the differential binding capacity of the receptor in normal ovarian cells compared with tumor cells. Although expressed in the epithelia of several normal tissues, FRα is situated on the apical membrane, which faces the external milieu, and out of contact with circulating folate (30). Thus, folate may protect against ovarian cancer via mechanisms other than the FRα, but the presence of high levels of the receptor in ovarian tumor cells that possess functional binding capacity may distinguish this tumor type from tumors with lower receptor expression possibly because of, or resulting in, different regulatory patterns. More research is needed to understand the implications of a strong-expressing FRα tumor; if it is associated with tumor aggression (12, 31), then it is possible that multivitamins containing folic acid may influence cancer via the FRα. Nonetheless, we cannot yet exclude components of multivitamins other than folic acid that may explain our associations.

Our study has several strengths. The identification of ovarian cancer cases from Mayo Clinic is virtually complete for Olmstead County and is similar to population-based case identification. Our controls were identified from the outpatient clinic for routine physical exams and are likely representative of the source population that gave rise to the cases. One pathologist assessed FRα expression in a consistent manner. For immunohistochemical analysis, we used a polyclonal antibody to the FRα protein thereby circumventing some limitations associated with monoclonal antibodies used in earlier studies. A molecular epidemiologic study of this nature is less susceptible to confounding by other factors because case recruitment and data collection were independent of FRα status.

Our study also has potential limitations. Ten percent of our cases resided outside the six-state Mayo primary service population compared with none of the controls. Thus, these cases may not be representative of the source population. This limitation did not seem to bias our risk estimates because the associations were inappreciably altered after excluding these cases from the analyses. We were unable to assess folic acid intake directly by dietary questionnaire. Although the risk factor

Table 2. Distribution of descriptive variables by FRα expression among 108 women with ovarian cancer, Mayo Clinic 2003-2004

Characteristic*	FRα-expressing ovarian tumors				P_{trend}
	Absent	Weak	Moderate	Strong	
Number of cases	10 (9)	7 (7)	25 (23)	66 (61)	
Age, y	64.4	55.6	60.8	63.8	0.57
Body mass index, kg/m^2	26.4	25.5	27.9	27.0	0.33
Nonsmoker, *n*	5 (8)	4 (6)	16 (25)	40 (61)	0.61
Exercise weekly †	2 (13)	2 (13)	1 (7)	10 (67)	0.77
Education ≤ high school	7 (15)	2 (4)	10 (22)	27 (59)	0.27
Family history of breast or ovarian cancer ‡	0	2 (8)	7 (28)	16 (64)	0.29
Age at menarche, y	13.0	13.0	13.0	13.0	0.86
Age at first live birth, y	20.0	23.0	22.0	22.0	0.66
Oral contraceptive use, ever§	4 (8)	4 (8)	13 (25)	30 (59)	1.0
Postmenopausal hormone use, ever§	4 (9)	2 (4)	8 (18)	31 (69)	0.36
Parity, *n*	4.0	2.0	3.0	3.0	0.51
History of infertility, yes	0	0	7 (39)	11 (61)	0.24
Borderline tumors	4 (36)	1 (9)	3 (27)	3 (27)	0.002
Serous tumors	3 (4)	5 (7)	16 (22)	49 (67)	0.01
Endometrioid tumors	1 (7)	1 (7)	3 (20)	10 (67)	0.68
Grade 3 and 4 tumors	5 (6)	5 (6)	18 (21)	57 (67)	0.01
Stage III and IV tumors	5 (6)	6 (8)	16 (20)	53 (66)	0.07

*Continuous variables are represented by median values and frequencies are represented by counts (percentage).
†Defined as ≥2 times/wk of vigorous physical activity during leisure time.
‡In mother or sister.
§Former or current use.

Table 3. RRs (95% CIs) of developing FRα-expressing ovarian tumors according to multivitamin and alcohol intake among 108 cases and 148 controls, Mayo Clinic 2003-2004

	Controls (n = 148)	FRα-expressing ovarian tumors	
		None, weak, or moderate (n = 42)	Strong (n = 66)
Multivitamin use, ≥4 pills/wk			
n	97	18	37
Age- and state-adjusted RR	1.0 (reference)	0.38 (0.18-0.78)	0.58 (0.31-1.06)
Multivariable* RR	1.0 (reference)	0.30 (0.12-0.70)	0.47 (0.24-0.91)
Alcohol intake, ≥5 drinks/wk			
n	20	5	11
Age- and state-adjusted RR	1.0 (reference)	0.94 (0.33-2.65)	1.39 (0.61-3.15)
Multivariable* RR	1.0 (reference)	0.84 (0.24-2.86)	1.65 (0.69-3.93)

*Multivariable RR adjusted for age (<50, 50-59, 60-69, 70+), state (Minnesota, Wisconsin, Iowa, other), education (≤high school, some college, bachelor's degree+), family history of ovarian or breast cancer in mother or sister (no, yes), body mass index (<21, 21-22.9, 23-24.9, 25-28.9, 29+), and infertility history (no, yes).

questionnaire did ask about the use of the single vitamin preparation of folic acid, too few cases (3%) and controls (15%) consumed it. Further, we did not ask specifically about intake of multivitamins that contained folic acid. Since 1994, multivitamins containing folic acid in quantities <1 mg are available without a prescription (32, 33), and most over-the-counter supplements now contain between 400 and 800 μg (33). Possibly, other vitamins or minerals in the multivitamins could explain the association with FRα expression in our study. Finally, although we were underpowered to detect a statistically significant association of alcohol intake (consumption prevalence was 14% among controls) and the combined intake of alcohol and multivitamins with FRα expression, we had 81% power to detect the 3.1-fold risk of multivitamin intake among cases using a two-sided test. The attenuation of this estimate following exclusion of borderline tumors may have reflected reduced power to detect the association among the remaining carcinomas or differences in risk factors among these tumor types.

To our knowledge, our study provides the first epidemiologic data of the association between multivitamins and FRα expression in ovarian tumors. It will be interesting to determine whether classification of ovarian tumors by FRα status is functionally important, analogous to how estrogen and progesterone receptor status is viewed in breast cancer. Further study of the implications of our findings is planned in a larger sample using biomarkers of folate status.

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