European Journal of Clinical Nutrition (2011) 65, 1133–1140

0954-3007/11
www.nature.com/ejcn

ORIGINAL ARTICLE

Folate and related micronutrients, folate-metabolising genes and risk of ovarian cancer

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Background/Objective: Folates are essential for DNA synthesis and methylation, and thus may have a role in carcinogenesis. Limited evidence suggests folate-containing foods might protect against some cancers and may partially mitigate the increased risk of breast cancer associated with alcohol intake, but there is little information regarding ovarian cancer. Our aim was to evaluate the role of folate and related micronutrients, polymorphisms in key folate-metabolising genes and environmental factors in ovarian carcinogenesis.
Subjects/Methods: Participants in the Australian Ovarian Cancer Study (1363 cases, 1414 controls) self-completed risk factor and food-frequency questionnaires. DNA samples (1638 cases, 1278 controls) were genotyped for 49 tag single-nucleotide polymorphisms (SNPs) in the methylene tetrahydrofolate reductase (*MTHFR*), methionine synthase (*MTR*) and MTR reductase (*MTRR*) genes. Logistic regression models were used to generate adjusted odds ratios and 95% confidence intervals.
Results: We saw no overall association between the intake of folate, B vitamins or other methyl donors and ovarian cancer risk, although increasing folate from foods was associated with reduced risk among current smokers ($P_{trend} = 0.03$) and folic acid intake was associated with borderline significant increased risks among women who consumed $\geqslant 1$ standard alcoholic drinks/day (odds ratio (OR) = 1.64; 95% confidence interval (CI) 1.05–2.54, $P_{trend} = 0.05$). Two SNPs (rs7365052, rs7526063) showed borderline significant inverse associations with ovarian cancer risk; both had very low minor allele frequencies. There was little evidence for interaction between genotype and micronutrient intake or for variation between different histological subtypes of ovarian cancer.
Conclusions: Our data provide little evidence to support a protective role for folate in ovarian carcinogenesis but suggest further evaluation of the joint effects of folic acid and alcohol is warranted.
European Journal of Clinical Nutrition (2011) **65,** 1133–1140; doi:10.1038/ejcn.2011.99; published online 1 June 2011

Keywords: ovarian cancer; folate; vitamin B; MTHFR

Introduction

There is a strong link between folate deficiency during pregnancy and neural tube defects and, as a result, many countries have introduced mandatory or voluntary fortification of grain products with folic acid. Folate also has a key role in DNA synthesis and methylation, thus, adequate intake is essential to maintain DNA integrity, and its deficiency may increase the risk of mutation, and hence cancer. A report from the World Cancer Research Fund concluded that there was some evidence that folate-containing foods might protect against pancreatic and colorectal cancer, and might also partially mitigate the increased risk of breast cancer associated with alcohol intake (WCRF/AICR, 2007). However, DNA synthesis is also integral to the process of tumour formation, and for many years anti-folates have been used to treat some cancers. Recent studies have suggested that rather than preventing cancer, high folate levels might promote progression of pre-neoplastic lesions to cancer (Cole *et al.*, 2007), and that the introduction of

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Received 1 February 2011; revised 6 April 2011; accepted 25 April 2011; published online 1 June 2011

mandatory folate fortification in Canada and the United States of America may have led to increases in colorectal cancer rates (Mason *et al*., 2007).

Folate availability for DNA synthesis and as a methyl donor for methylation depends not only on intake, but also on the activity of enzymes, including methylene tetrahydrofolate reductase (MTHFR), methionine synthase (MTR) and MTR reductase (MTRR), and the presence of co-factors including vitamins B_2, B_6 and B_{12}. Other factors, such alcohol, which blocks folate uptake (Halsted *et al*., 2002) and smoking are also important. Polymorphisms in the *MTHFR* gene, including rs1801133 (C677T) and rs1801131 (A1298C), affect MTHFR activity and the *MTHFR* 677TT variant genotype, and to a lesser extent the 1298CC variant, have been associated with reduced risks of colorectal cancer (Curtin *et al*., 2004; Sharp and Little, 2004).

There is currently insufficient evidence to determine whether folate has a role in ovarian carcinogenesis (WCRF/AICR, 2007). Early case–control studies gave inconsistent results but did not consider the effects of alcohol intake, whereas two prospective studies (147 and 266 cases) reported reduced risks of ovarian cancer associated with high folate intake among women who also drank alcohol (Kelemen *et al*., 2004; Larsson *et al*., 2004). One of these studies also found that higher intake of methionine, another methyl donor, was associated with a reduced risk of ovarian cancer among women with low folate intake (Kelemen *et al*., 2004). There have been few studies of vitamin B and ovarian cancer. One hospital-based study reported no association with vitamin B_6 (Bidoli *et al*., 2001), but a second study saw a nonsignificant reduction in risk among women who had used B-complex supplements for 10 years or more (Pan *et al*., 2004). Aside from one study, which reported an interaction between polymorphisms in the *DNMT3A* gene and multivitamin use (Kelemen *et al*., 2008), no studies have considered the joint effects of genotype, diet and lifestyle in relation to ovarian cancer risk. Our aim was, therefore, to evaluate the role of folate and related micronutrients, polymorphisms in key folate-metabolising genes and environmental factors in ovarian carcinogenesis.

Material and methods

The Australian Ovarian Cancer Study was an Australia-wide population-based case–control study (Merritt *et al*., 2008). Cases were women aged 18–79 years with histologically confirmed epithelial ovarian, fallopian tube or primary peritoneal cancer diagnosed between January 2002 and June 2005. A total of 2744 women with a clinically suspected diagnosis of cancer were invited to participate (before surgery, to facilitate fresh tissue collection) and, of these, 2320 (85%) agreed to take part. After surgery, 590 women were excluded because their final diagnosis was not primary epithelial ovarian cancer, 19 because they were first diagnosed before 2002 and 1 was not an Australian resident. Of the final 1710 women, 1458 (85%) completed the risk factor and food frequency (FFQ) questionnaires, 1387 (81%) had DNA for genotyping and 1208 (71%) had both. An additional 285 cases were recruited between July 2005 and June 2006 to increase the numbers of biospecimens collected; of these, 251 (88%) had DNA and 246 (98%) also completed a shortened risk factor questionnaire (they were not asked to complete the FFQ).

Controls were randomly selected from the electoral roll (enrolment is compulsory) and frequency matched by age (5-year bands) and state of residence to the case group. Of the 3442 eligible women contacted, 1614 (47%) returned a risk factor questionnaire; 105 reported a history of ovarian cancer or bilateral oophorectomy and were thus excluded leaving 1509 population controls. Of these, 1461 (97%) returned a FFQ, 1278 (85%) had DNA and 1259 (83%) had both questionnaire data and DNA.

Risk factor information was collected by a self-administered questionnaire that included questions about demographic, medical, hormonal, reproductive and family history. The FFQ was based on the instrument developed by Willett *et al*. (1985), and it was adapted and validated for the Australian population (Marks *et al*., 2006a, b). Respondents were asked to recall how often, on average, they had consumed a standard serving size of 135 separate food items in the previous 12 months (controls) or the 12 months before their cancer diagnosis (cases) or, if their diet had changed in the last 12–24 months, their usual diet. Nutrient intake was estimated by multiplying daily intake of each food item in grams by the nutrient content of that food using the Australian Food Composition Tables (Food Standards Australia New Zealand, 2007) for total energy, folate, vitamins B2, B6 and B12, and methionine and the United States Department of Agriculture database for choline content of common foods (USDA, 2008) for choline and betaine, two other methyl donors. Values were adjusted for total energy intake using the residual method (Willett and Stampfer, 1986). Women were also asked to report frequency of use of vitamin supplements, and this information was used to estimate nutrient intake from supplements. We excluded women if $>10\%$ of FFQ items were missing (29 cases; 3 controls) or if their estimated caloric intake was very extreme (<2930 or $>16\,750$ kJ: 63 cases, 44 controls). Three additional cases were excluded because they were missing parity information leaving a final group of 1363 cases and 1414 controls for nutritional analyses.

Genetic analyses focussed on *MTHFR*, *MTR* and *MTRR* because single-nucleotide polymorphisms (SNPs) in these genes have been shown to affect enzyme activity, and there is most evidence for a potential role in cancer development (Ebbing *et al*., 2009). We included the most frequently studied SNPs (*MTHFR*: A1298C, rs1801131 and C677T, rs1801133; *MTR*: A2756G, rs1805087 and *MTRR*: A66G, rs18011394) and selected other SNPs for study using the TAMAL online tag SNP selection tool (Hemminger *et al*., 2006), using default parameters with the TAGGER method of

selection from the Caucasian HapMap population. Genotyping was performed using the Sequenom MassARRAY system as described previously (Beesley *et al*., 2007).

As folate, which occurs naturally in foods, differs from the more active folic acid used for fortification and in vitamin supplements, we considered these both separately and combined. For other nutrients we considered total intake from foods and supplements. Nutrient variables were divided at the quartiles for analysis (Q1 = lowest 25% to Q4 = highest 25%). Multivariable logistic models were used to adjust for potential confounders including age, education, parity and hormonal contraceptive use; nutritional analyses also included terms for alcohol and total energy intake. Other potential confounders, including family history of breast or ovarian cancer, body size, smoking, hysterectomy, tubal sterilisation, breastfeeding and talc use, were not included in the final models, as they did not substantially alter risk estimates.

We conducted analyses for all tumour types combined, and then by subtype, simultaneously comparing each subtype to controls using polynomial logistic regression. We examined the effect modification by hormonal contraceptive use, menopausal status and parity. The statistical significance of any observed stratum-specific differences was assessed by including a cross-product term in regression models. Analysis was performed using SAS version 9.1 (SAS Institute Inc., Cary, NC, USA).

This study was approved by the Human Research Ethics Committees at the Peter MacCallum Cancer Centre, Queensland Institute of Medical Research, University of Melbourne, and all participating cancer registries and hospitals. All participants provided informed consent.

Results

Table 1 shows the characteristics of case and control women. On average, cases were slightly older and less educated than controls and, as expected, they were significantly more likely to have a family history of breast or ovarian cancer, or to be nulliparous, but were less likely to have used hormonal contraceptives. Women with and without valid dietary and/or genotype data did not differ appreciably with respect to their age, subtype and stage of cancer, family history of breast and ovarian cancer, level of education, body size, parity or oral contraceptive use.

We saw no association between the intake of folate from foods (excludes folic acid from fortified foods), folic acid (from fortified foods and supplements) or total folate and ovarian cancer risk (Table 2). We also saw no association when we considered dietary folate equivalents (data not shown). Likewise, there was no association with intake of vitamins B_2, B_6 and B_{12} from food and supplements; restricting these analyses to include only food sources did not alter the results. Our results were also unchanged when we excluded women who reported changing their diet in the year before diagnosis/recruitment (22% of cases, 18% of controls) and did not vary appreciably across the different histological subtypes of ovarian cancer (invasive serous,

Table 1 Characteristics of cases and controls with valid data for dietary analyses and/or DNA for genotyping

	Cases (N = *1877*)	*Controls* (N = *1469*)	P-*value*[a]
Age (years)			
Mean (s.d.)	58.1 (12.0)	56.4 (12.4)	0.0001
Cancer type			
Invasive serous	1008 (53.7%)		
Borderline serous	160 (8.5%)		
Mucinous[b]	226 (12.0%)		
Invasive	161 (8.6%)		
Endometrioid	101 (5.4%)		
Invasive clear cell	110 (5.9%)		
Mixed histology	111 (5.9%)		
Other[c]			
Valid data available for dietary analysis[d]	1363 (83.8%)	1414 (96.3%)	
DNA available for genotyping	1638 (87.2%)	1278 (87.0%)	
Valid data and DNA available[d]	1125 (69.2%)	1223 (83.3%)	
Education			
High school	976 (54.2%)	719 (48.9%)	
Certificate/diploma	587 (32.6%)	536 (36.5%)	
University	238 (13.2%)	214 (14.6%)	0.009
Breast or ovarian cancer in first-degree relative	312 (17.3%)	192 (13.1%)	0.0008
Body mass index (kg/m^2)			
<25	695 (42.1%)	677 (46.7%)	
25–29.9	546 (33.1%)	439 (30.3%)	
$\geqslant 30$	411 (24.9%)	335 (23.1%)	0.03
Hormonal contraceptive use			
Never	579 (32.5%)	311 (21.2%)	
<5 years	502 (28.1%)	357 (24.4%)	
$\geqslant 5$ years	703 (39.4%)	796 (54.4%)	<0.0001
Parity			
0	341 (19.0%)	172 (11.7%)	
1–2	730 (40.7%)	636 (43.3%)	
$\geqslant 3$	722 (40.3%)	661 (45.0)	<0.0001
Ever breastfed (parous women only)	1086 (77.7%)	1082 (84.4%)	<0.0001
Use of talc in perineal region	856 (48.0%)	652 (44.6%)	0.05
Energy intake (kJ)			
Mean (s.d.)	9014 (2711)	8693 (2530)	0.6

[a]From *t*-test (age, energy intake), χ^2-test (cancer history, talc use) or χ^2-test for trend.
[b]Combines invasive and borderline tumours, as they are believed to have similar aetiology.
[c]Other epithelial tumours, for example, carcinosarcoma and transitional cell tumours.
[d]Dietary data were not collected in the final year of case recruitment, thus the maximum number that could have data is 1626.

Table 2 ORs[a] (95% CI) for risk of ovarian cancer associated with intake of folate, B vitamins, methionine, betaine and choline in 1363 cases and 1414 controls

Nutrient	*Q1*[b]	*Q2*	*Q3*	*Q4*	*Trend P-value*
Folate from foods[c]					
μg/day	<252	252–307	308–366	>366	
OR (95% CI)	1.0	0.94 (0.76–1.16)	0.87 (0.70–1.08)	1.00 (0.80–1.24)	0.9
Folic acid[c]					
μg/day	<36.3	36.3–95.9	96.0–204	>204	
OR (95% CI)	1.0	1.22 (0.98–1.51)	1.13 (0.91–1.41)	1.13 (0.91–1.40)	0.6
Total folate[d]					
μg/day	<334	334–422	423–546	>546	
OR (95% CI)	1.0	1.14 (0.92–1.41)	1.07 (0.86–1.33)	1.05 (0.84–1.30)	0.9
Total vitamin B_2[d]					
mg/day	<2.19	2.19–2.79	2.80–4.60	>4.60	
OR (95% CI)	1.0	0.98 (0.79–1.22)	1.01 (0.81–1.25)	0.92 (0.75–1.15)	0.4
Total vitamin B_6[d]					
mg/day	<1.19	1.19–1.47	1.48–3.15	>3.15	
OR (95% CI)	1.0	1.01 (0.81–1.25)	1.22 (0.99–1.52)	1.00 (0.80–1.24)	0.5
Total vitamin B_{12}[d]					
μg/day	<1.29	1.29–1.93	1.94–3.99	>3.99	
OR (95% CI)	1.0	0.98 (0.79–1.22)	1.15 (0.95–1.47)	1.07 (0.87–1.33)	0.7
Total methionine[d]					
mg/day	<1.16	1.16–1.36	1.37–1.60	>1.60	
OR (95% CI)	1.0	1.01 (0.81–1.25)	0.94 (0.76–1.17)	0.97 (0.78–1.20)	0.7
Total betaine[d]					
μg/day	<92.9	92.9–114	115–143	>143	
OR (95% CI)	1.0	1.08 (0.87–1.34)	0.85 (0.69–1.06)	1.09 (0.88–1.36)	0.7
Total choline[d]					
μg/day	<433	433–622	623–1022	>1022	
OR (95% CI)	1.0	0.87 (0.70–1.09)	0.92 (0.74–1.14)	0.89 (0.71–1.10)	0.5

Abbreviations: CI, confidence interval; OR, odds ratio.
[a]ORs and 95% CIs adjusted for age (years), level of education (school, diploma/certificate and university), oral contraceptive use (never, <5 and ⩾5 years), parity (0, 1–2, ⩾3), alcohol intake (quartiles) and total energy intake (kJ).
[b]Nutrient intake was categorised at the quartiles such that Q1 (reference group) is the lowest 25% of intake range and Q4 the highest 25%.
[c]Folate from foods is energy adjusted and excludes folic acid from fortified foods; folic acid includes folic acid from fortified foods (energy adjusted) and supplements.
[d]Total intake from foods (energy adjusted) and supplements.

borderline serous, mucinous, endometrioid and clear cell; data not shown). There was also no suggestion that higher intake of any of the nutrients studied was more beneficial in women with low folate intake (<400 μg/day) than for those with adequate intake (⩾400 μg/day). We saw no significant associations between intake of the other one-carbon donors, methionine, choline and betaine, and ovarian cancer (Table 2).

When we stratified by alcohol intake, we saw a borderline significant increase in risk with increasing folic acid intake among women who consumed ⩾10 g alcohol (approximately one standard drink) per day, with no effect among women drinking less than this ($P_{\text{heterogeneity}} = 0.04$, Table 3). No such difference was seen for folate from food sources. The results were similar when we stratified women based on alcohol consumption above and below the median level (2.25 g/day). In contrast, stratification by smoking status (current versus never/ex-smokers) suggested that increased intake of folate from foods was associated with a reduced risk of ovarian cancer among smokers ($P_{\text{trend}} = 0.03$) with no effect seen among never/ex-smokers ($P_{\text{heterogeneity}} = 0.01$) or for folic acid or total folate (Table 3).

Further analyses explored the joint effects of folate, vitamins B_2, B_6 and B_{12}, and use of vitamin supplements by including cross-product terms in the models and by jointly classifying women as to their intake of two nutrients, and comparing groups with a common reference group with low intake of both nutrients. Although we observed some statistically significant interactions, inspection of the stratum-specific estimates did not identify any clear patterns, and it is likely that these occurred by chance. The most notable was an observation that the use of vitamin B

Table 3 Odds ratios[a] and 95% confidence intervals for the association between folate intake and ovarian cancer, stratified by level of alcohol consumption and smoking status

Nutrient	*Q1*[b]	*Q2*	*Q3*	*Q4*	P_{trend}	$P_{heterogeneity}$
Alcohol intake						
Folate from foods[c]						
<10 g/day	1.0	0.91 (0.71–1.17)	0.84 (0.66–1.09)	0.97 (0.76–1.24)	0.8	
⩾10 g/day	1.0	1.02 (0.67–1.57)	0.94 (0.61–1.46)	1.06 (0.68–1.66)	0.9	0.7
Folic acid[c]						
<10 g/day	1.0	1.16 (0.91–1.49)	0.99 (0.77–1.27)	0.98 (0.77–1.26)	0.5	
⩾10 g/day	1.0	1.33 (0.86–2.07)	1.67 (1.08–2.60)	1.64 (1.05–2.54)	0.046	0.04
Total folate[c]						
<10 g/day	1.0	1.06 (0.83–1.37)	0.96 (0.75–1.23)	0.94 (0.73–1.21)	0.5	
⩾10 g/day	1.0	1.37 (0.89–2.10)	1.40 (0.90–2.21)	1.41 (0.91–2.18)	0.2	0.1
Smoking status						
Folate from foods[c]						
Never/ex	1.0	1.01 (0.80–1.29)	0.98 (0.78–1.25)	1.16 (0.91–1.47)	0.2	
Current	1.0	0.93 (0.53–1.63)	0.59 (0.33–1.05)	0.59 (0.37–1.05)	0.03	0.01
Folic acid[c]						
Never/ex	1.0	1.19 (0.94–1.50)	1.19 (0.94–1.50)	1.12 (0.89–1.42)	0.6	
Current	1.0	1.60 (0.85–3.00)	0.93 (0.51–1.60)	1.18 (0.67–2.08)	0.9	0.8
Total folate[c]						
Never/ex	1.0	1.25 (0.98–1.58)	1.19 (0.94–1.51)	1.16 (0.92–1.47)	0.4	
Current	1.0	0.88 (0.49–1.58)	0.72 (0.41–1.26)	0.74 (0.42–1.32)	0.3	0.1

[a]Odds ratios and 95% confidence intervals adjusted for age (years), level of education (school, diploma/certificate and university), oral contraceptive use (never, <5 and ⩾5 years), parity (0, 1–2, ⩾3), total energy intake (kJ).
[b]Nutrient intake was categorised at the quartiles such that Q1 (reference group) is the lowest 25% of intake range and Q4 the highest 25%.
[c]Folate from foods is energy adjusted and excludes folic acid from fortified foods; folic acid includes folic acid from fortified foods (energy adjusted) and supplements; total folate includes energy-adjusted folate and folic acid from foods and folic acid from supplements.

supplements was associated with an increased risk of ovarian cancer (OR = 1.42, 95% CI 1.01–1.99) among women with below median intake of folate from food sources, but a borderline significant reduced risk among women with folate intake above the median level (OR = 0.75, 95% CI 0.53–1.06; $P_{\text{interaction}} = 0.03$). This pattern was not seen for folic acid from supplements and fortified foods or total folate (folate plus folic acid).

Given recent suggestions that high folate intake might promote progression of pre-cancerous lesions to cancer, we looked separately at women who reported a history of endometriosis, as this is a strong risk factor for endometrioid and clear cell ovarian cancers. Although the risk estimates for folic acid intake were generally higher among women with endometriosis (2.0, 1.1 and 1.7 for Q2, Q3 and Q4 versus Q1) than for those without (1.2, 1.2 and 1.1), the number of women with endometriosis was small and none of the associations was statistically significant.

Of the 49 SNPS genotyped, 18 were excluded from analysis because they had a minor allele frequency <1.5% or were highly correlated with another SNP in the set; and 1 was excluded because it was out of Hardy–Weinberg equilibrium (*MTHFR* rs2066462, χ^2 $P<0.0001$ in cases and controls; see Supplementary Table). Table 4 shows the results for the most commonly studied SNPs and any others that gave significant or borderline significant ($P<0.1$) results (results for the other SNPs are shown in the Supplementary Table). We saw no significant associations with any of the *MTHFR* SNPs tested, although women homozygous for the variant alleles of rs1801131 (A1298C) and rs1476413 had nonsignificant 20–30% increased risks of ovarian cancer, as did those homozygous for both variant alleles (OR = 1.33, 95% CI 0.99–1.80). In contrast, the variant allele of the *MTR* rs7365052 and rs7526063 polymorphisms was associated with significantly reduced risks of ovarian cancer, although both of these variants were very rare (minor allele frequency <0.02) and only one woman carried both. None of the *MTRR* SNPs studied was associated with ovarian cancer risk. The results were essentially unaltered when we restricted the analyses to Caucasian women (95% of the study population) and/or women with invasive serous cancers (62% of cases), the most common histological subtype. We did not see any convincing evidence of effect modification by age (< and ⩾50 years), alcohol intake, intake of folate and other nutrients or multivitamin use (data not shown).

Discussion

In summary, we found no evidence for an overall association between folate intake and risk of ovarian cancer, although there were suggestions of a modest increase in risk with increasing intake of folic acid among women who consumed ⩾10 g alcohol/day and of a reduction in risk with increasing intake of naturally occurring folate from foods among current smokers. We found little evidence for interaction

Table 4 Odds ratios and 95% confidence intervals for the association between genotype and ovarian cancer risk for polymorphisms previously reported in the literature or with a borderline significant association[a]

Gene and polymorphism	*Cases*[b]			*Controls*[b]			OR_{Het}[c]	OR_{Hom}[c]	$OR_{per\ allele}$[c]	$P_{heterogeneity}$/ P_{trend}
	WT (%)	*Het (%)*	*Hom (%)*	*WT (%)*	*Het (%)*	*Hom (%)*				
MTHFR										
rs1476413	54.1	37.0	8.9	53.1	39.9	7.0	0.92 (0.78–1.08)	1.28 (0.95–1.72)	1.03 (0.92–1.17)	0.09/0.6
rs1801131	47.0	42.3	10.7	46.8	43.9	9.3	1.02 (0.87–1.19)	1.20 (0.92–1.57)	1.07 (0.95–1.20)	0.4/0.3
rs1801133	45.4	43.3	11.3	44.7	44.4	10.9	0.95 (0.81–1.11)	1.00 (0.78–1.30)	0.98 (0.88–1.10)	0.8/0.8
MTR										
rs1805087	66.5	29.3	4.2	66.0	30.1	3.9	0.96 (0.81–1.13)	1.05 (0.71–1.55)	0.98 (0.86–1.12)	0.8/0.8
rs1802059	40.0	46.7	13.2	42.4	46.0	11.6	1.21 (0.94–1.56)	1.13 (0.95–1.33)	1.11 (0.99–1.24)	0.2/0.08
rs7365052	96.8	3.2	—	95.0	5.0	—	0.61 (0.41–0.91)			0.01
rs7526063	96.8	3.2	—	95.2	4.7	—[d]	0.66 (0.45–0.99)			0.04
MTRR										
rs1801394	31.1	47.3	21.6	30.4	49.7	19.9	0.94 (0.79–1.13)	1.01 (0.81–1.25)	1.00 (0.90–1.11)	0.7/1.0

Abbreviations: Het, heterozygous; Hom, rare homozygous; MTHFR, methylene tetrahydrofolate reductase; MTR, methionine synthase; MTRR, methionine synthase reductase; OR, odds ratio; WT, common homozygous.
[a]See Supplementary Table for a list of the other single-nucleotide polymorphism tested.
[b]% WT, % Het and % rare Hom.
[c]OR and 95% confidence intervals adjusted for age, level of education, oral contraceptive use and parity.
[d]One control carried the homozygous variant genotype and was combined with the heterozygous group for analysis.

between intake of folate and other micronutrients aside from a somewhat paradoxical observation that the use of vitamin B supplements was associated with increased risk among women with low intake of folate from foods but reduced risk among those with high folate intake, and it is likely this is a chance finding. We also found no convincing evidence for an association between genetic variants in the *MTHFR*, *MTR* and *MTRR* genes, and risk and no interaction between genotype and age, diet or other lifestyle variables.

Early studies reported no significant association between folate intake and ovarian cancer risk but none included intake from supplements or considered the effects of alcohol intake (Kushi *et al*., 1999; Bidoli *et al*., 2001; Salazar-Martinez *et al*., 2002; McCann *et al*., 2003). Three reports have suggested that increasing folate intake is associated with reduced risks of ovarian cancer among women with moderate alcohol consumption (>20–28 g/week; Kelemen *et al*., 2004; Larsson *et al*., 2004; Navarro Silvera *et al*., 2006), although others have not confirmed this (Pelucchi *et al*., 2005; Tworoger *et al*., 2006). To further complicate things, cohort studies have suggested that when intake from supplements is included, the risks of ovarian cancer may increase with increasing folate intake, and studies that have observed little or no effect overall have reported increased risks in the first few years of follow-up (Kelemen *et al*., 2004; Tworoger *et al*., 2006). This suggests a possible adverse effect of folic acid as opposed to folate from foods, and among women with undiagnosed pre-clinical disease.

Our data are largely consistent with these latter studies, especially in women with higher alcohol intake. Taken together, this suggests that higher folate intake is not associated with lower risks of ovarian cancer, but that higher consumption of folic acid might be associated with slight increases in risk. When coupled with the results of folate supplementation trials that have reported increased incidence of advanced lesions in participants with a recent history of colorectal adenoma (Cole *et al*., 2007) and increased cancer incidence and mortality among patients with ischaemic heart disease (Ebbing *et al*., 2009), this adds further evidence that high folate/folic acid intake might increase cancer risk and raises concerns about the widespread practise of fortifying foods with folic acid to reduce rates of neural tube defects.

Our observation that intake of other B vitamins or methyl donors was not related to ovarian cancer risk is consistent with other studies that have assessed this (Pan *et al*., 2004; Tworoger *et al*., 2006; Kotsopoulos *et al*., 2010). Only one previous study has considered genetic variation in folate-metabolising genes and, similar to us, they found no evidence that polymorphisms in the *MTHFR*, *MTR* and *MTRR* genes influenced ovarian cancer risk (Kelemen *et al*., 2008).

Strengths of our study include the population-based design, large numbers and analysis of multiple aspects of the folate–ovarian cancer relationship that have not previously been considered together. A limitation was the participation rate among controls (47%); however the distributions of education level, parity and body mass index among our control women were almost identical to those from the 2004 Australian National Health Survey (Australian Bureau of Statistics, 2006), although current smokers were slightly under-represented in our controls (Pandeya *et al*., 2009). Given that participating controls who were current smokers had lower folate intake than ex- and never smokers,

it is possible that folate intake in our control group might be slightly higher than in the general population. If this is the case, then our estimates would be biased downwards and, consequently, the true estimates might be higher than those we observed thereby strengthening our conclusions that folate intake is not associated with reduced risk of ovarian cancer, and high folic acid intake might be associated with an increased risk among women who consume 10 g or more alcohol per day. Measurement error is always an issue, but to minimise this we used a dietary questionnaire that has been validated for (Marks *et al*., 2006a, b) and has been shown to be reproducible in (Ibiebele *et al*., 2009) the Australian population. Furthermore, among 185 control women, serum folate levels were correlated with total dietary folate equivalents as measured by our FFQ ($r=0.40$, $P<0.0001$). Finally, although it is possible that cases may have changed their diet with the onset of disease, sensitivity analyses excluding women who reported changing their diet in the past year did not alter our results.

In summary, our results add further evidence that folate does not have a major role in the development of ovarian cancer. We did, however, see suggestions of slightly increased risks among women with high intake of folic acid. Given others have reported similar patterns, the emerging evidence of adverse effects of folate supplementation from randomised trials and the widespread practise of folate fortification, this issue warrants further investigation.

Conflict of interest

The authors declare no conflict of interest.

Acknowledgements

We acknowledge the cooperation of the following institutions: New South Wales: John Hunter Hospital, North Shore Private Hospital, Royal Hospital for Women, Royal North Shore Hospital, Royal Prince Alfred Hospital, Westmead Hospital, New South Wales Cancer Registry; Queensland: Mater Misericordiae Hospital, Royal Brisbane and Women's Hospital, Townsville Hospital, Wesley Hospital, Queensland Cancer Registry; South Australia: Flinders Medical Centre, Queen Elizabeth II, Royal Adelaide Hospital, South Australian Cancer Registry; Tasmania: Royal Hobart Hospital; Victoria: Freemasons Hospital, Mercy Hospital For Women, Monash Medical Centre, Royal Women's Hospital, Victorian Cancer Registry; and Western Australia: King Edward Memorial Hospital, St John of God Hospitals Subiaco, Sir Charles Gairdner Hospital, Western Australia Research Tissue Network, Western Australia Cancer Registry. We also acknowledge the contribution of the study nurses and research assistants, and we would like to thank all of the women who participated in the study. These analyses were funded by the Queensland Cancer Council (389880). The Australian Ovarian Cancer Study was supported by the US Army Medical Research and Materiel Command under DAMD17-01-1-0729, the Cancer Council Tasmania and the Cancer Foundation of Western Australia; the Australian Cancer Study was supported by the National Health and Medical Research Council (NHMRC) of Australia (199600). PM Webb and G Chenevix-Trench are supported by Fellowships from the NHMRC.

Full membership of the Australian Ovarian Cancer Study Group is listed at http://www.aocstudy.org/; the Australian Cancer Study Investigators are A Green, P Parsons, N Hayward, P Webb and D Whiteman.

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Supplementary Information accompanies the paper on European Journal of Clinical Nutrition website (http://www.nature.com/ejcn)