Association of Methylenetetrahydrofolate Reductase Polymorphism *C677T* and Dietary Folate with the Risk of Cervical Dysplasia[1]

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Abstract

Epidemiological studies have been inconsistent regarding a role for folate in the etiology of cervical dysplasia. Methylenetetrahydrofolate reductase (*MTHFR*) catalyzes the synthesis of 5-methyltetrahydrofolate, which is involved in the methylation of homocysteine to methionine. A common variant of this enzyme, resulting from a *677C→T* (*Ala→Val*) substitution in the gene, has been shown to have reduced activity and is associated with mild hyperhomocysteinemia. A multiethnic case-control study was used to examine the association of dietary folate and *MTHFR* genotype with the odds ratios (ORs) for cervical dysplasia among women identified from several clinics on Oahu, Hawaii, between 1992 and 1996. We collected blood samples for DNA extraction, cervical smears for cytological diagnosis, exfoliated cervical cells for human papillomavirus (HPV) DNA testing, and personal interviews from 150 women with squamous intraepithelial lesions (SILs) and from 179 women with cytologically normal (Pap) smears. We found a positive, monotonic trend ($P = 0.02$) in the ORs for cervical SILs associated with the number of variant *MTHFR T* alleles, after multivariate adjustment. Women with the heterozygous *CT* genotype had twice the risk of cervical SILs [OR, 2.0; 95% confidence interval (CI), 1.1–3.7], and women with the homozygous *TT* genotype had almost three times the risk of SILs (OR, 2.9; 95% CI, 1.0–8.8) compared to women with the homozygous *MTHFR CC* genotype. The dietary intakes of folate, vitamin B_6, and vitamin B_{12} were inversely related to the ORs for cervical SILs, after adjustment for HPV DNA and other confounders. The OR among women in the highest quartile compared with women in the lowest quartile of folate intake was 0.3 (95% CI, 0.1–0.7; *P* for trend = 0.002). Women with the variant *T* allele and folate intakes below the median were at significantly elevated risk of cervical SILs (OR, 5.0; 95% CI, 2.0–12.2) compared to women with *CC* alleles and folate intakes above the median. HPV infection was a strong risk factor for cervical dysplasia, particularly among women with the variant *T* allele (OR, 46.6; 95% CI, 15.9–136.2). All associations of *MTHFR* genotype with the ORs for cervical SILs were independent of other risk factors under study. These findings suggest that the *MTHFR T* allele and reduced dietary folate may increase the risk for cervical SILs.

Introduction

Research on the hypothesized association of folic acid and related nutrients with the risk of cervical dysplasia is rooted in the observation by Van Niekerk (1) that macrocytic cells from the cervix of pregnant women with folic acid deficiency were cytologically similar to malignant cells, and that this macrocytic atypia could be reversed through vitamin supplementation. In the 1970s, Whitehead *et al.* (2) showed that 10 mg of oral folate supplementation improved the macrocytic changes in cervical cytological specimens associated with oral contraceptive pill use. Subsequent studies (3–9) of the relation of dietary and blood measures of folate to the risk of cervical dysplasia have produced mixed results and are difficult to interpret in the absence of appropriate measurement for the presence of HPV[3] infection, a key risk factor for cervical dysplasia and cancer.

Folate deficiency, defined as erythrocyte folate levels <140 ng/ml, may affect up to 10% of the United States population and a much larger proportion (~50%) of low-income and African-American women who are at the greatest risk of cervical cancer (10). Folate is essential to the synthesis of nucleotides, and low levels of folate have implications for cell replication, DNA excision and repair, and DNA hypomethylation (11). *S*-adenosylmethionine is an important methyl donor, and in folate deficiency, *S*-adenosylmethionine formation compromises the conversion of deoxyuridylate to thymidylate, resulting in thymine starvation. Thymine-starved cells tend to misincorporate uracil into human DNA, and during subsequent DNA repair, transient nicks may be linked on opposite strands, causing chromosome breaks (10). The degree of DNA hypomethylation in biopsied cervical tissue has been correlated to folate concentrations in cervical tissue and serum, suggesting a mechanism for suppressing HPV-mediated cervical carcinogenesis (12).

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[3] The abbreviations used are: HPV, human papillomavirus; CI, confidence interval; MTHFR, methylenetetrahydrofolate reductase; 5-methylTHF, 5-methyltetrahydrofolate reductase; OR, odds ratio; CI, confidence interval; SIL, squamous intraepithelial lesion.

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MTHFR is an enzyme that catalyzes the reduction of 5,10-methylenetetrahydrofolate to 5-methylTHF, the methyl donor for methionine synthesis from homocysteine (13). A common variant of this enzyme with reduced activity and sensitivity to heat inactivation has been shown to be caused by a *677C→T* substitution in the structural gene, resulting in an alanine-to-valine substitution in the enzyme. Homozygotes for this variant have mild elevation of plasma homocysteine levels and nonmethylated folate in their RBCs, both conditions implying impairment in 5-methylTHF synthesis (14). In this case-control study, we tested the hypothesis that women with cervical dysplasia have lower intakes of dietary folate and are more likely to have the variant *MTHFR T* allele.

Materials and Methods

Details of the methodology used in this study have been reported elsewhere (3, 15). Briefly, we conducted a case-control study on the island of Oahu, Hawaii, among women attending three hospital-based clinics for cervical cytological screening between June 1992 and December 1996. Eligible subjects included nonhysterectomized women from 18 to 84 years of age who were residents of Oahu. Women who had been pregnant or lactating within 6 months of enrollment, or who had a diagnosis of cervical abnormalities within the past year, were considered ineligible for study.

Eligible cases were identified through the cytology logs of the participating clinics and included all women with biopsy-confirmed SILs of the cervix according to the Bethesda system (16). Before their return to the clinic for a follow-up examination and cervical smear, potentially eligible women were contacted by letter, followed by a telephone call, about consenting to participate in the study. As part of the study, a colposcopy was performed, and cervical cells were collected for HPV testing. All biopsies were read by one of three pathologists and categorized as normal, "equivocal" SIL (atypical squamous cells of undetermined significance), low-grade SIL ("mild dysplasia"), or high-grade SIL ("moderate" or "severe" dysplasia). Interview information was obtained from 214 of the 303 eligible biopsied women (71%) with a diagnosis of SIL. Potential cases who were not diagnosed with low-grade or high-grade SIL were not included in this analysis. We were able to draw blood from 150 of the 214 biopsy-confirmed SIL cases (70%) who completed interviews, including Caucasian (n = 56), Japanese (n = 26), Native Hawaiian (n = 19), and other Asian/Pacific Island (n = 49) women.

Controls were women with negative cytological smears attending the same clinics as the cases. Eligible women were selected from the admission logs of the participating clinics on a randomly selected day of the month. Potential controls were met at the clinics by one of the interviewers who explained the purpose of the study. An exfoliated cervical cell specimen was obtained at the time of the cervical cytological smear. Of the 318 qualified women with negative cytological results, 271 (85%) were interviewed, and 179 (66%) of these women further consented to have their blood drawn and were used as controls in the present analysis. The control group included 79 Caucasians, 27 Japanese, 27 Native Hawaiian, and 46 additional Asian/Pacific Island women.

Interview. A participant was scheduled for a personal interview at her home or other convenient location. Written, informed consent, as approved by the University of Hawaii and clinic research committees, was obtained from all study subjects. A standardized questionnaire was used to elicit a detailed sexual and reproductive history, including age at first intercourse, number of sexual partners and methods of contraception, diet and dietary supplements, a lifetime history of tobacco and alcohol use, and other demographic and lifestyle information. The food frequency questionnaire used in this study has been described previously (17) and has been validated in our population (18). Almost 250 food items or categories were selected to provide an estimate of the major dietary components. The food sources of these dietary components and common serving sizes for each item were derived from measured food records among a random sample of the major ethnic groups in Hawaii. Specific inquiry was made about added fats and oils, as well as supplements. Alcoholic beverage consumption was also assessed. For each food or beverage item, the respondent indicated the usual frequency consumed per day, week, or month, with yearly frequencies for particular seasonal items, such as cantaloupe. Color photographs illustrating the three most representative serving sizes were used to assist subjects in estimating amounts consumed. Both combination and multiple servings could be selected. The dietary reference period was the year before diagnosis for the cases and the year preceding the interview for controls. The median time between diagnosis and interview was ~4 months for the cases.

Portion sizes were converted to gram weights, and the grams consumed on a daily basis for each food item were calculated as the product of the frequency and serving size. For the nutrient analysis, a food composition database was applied to obtain the daily intake of nutrients from each food item. The food composition database was compiled largely from the United States Department of Agriculture tables (19). Recipes for local ethnic dishes were also included. Values for folate and other nutrients were representative of food composition at the time of the interview, before the widespread folate fortification of the United States food supply. Total daily nutrient intake was calculated as the sum of nutrients from foods and nutrients from supplements.

HPV DNA Detection. Personnel at all laboratories involved in the analyses for this study were blinded to the case-control status of the subjects. Frozen cervical cell specimens (1 ml) were prepared for HPV-DNA detection by PCR amplification and dot-blot hybridization of the amplicons. The PCR amplifies the highly conserved L1 region of the viral genome using degenerate primers (MY09 and MY11; Ref. 20), which amplify >45 types of HPV, including the most common oncogenic types found in the cervix (21). PCR products were assessed through dot-blot hybridization using biotin-labeled oligonucleotide DNA probes.

***MTHFR* Polymorphism.** DNA was purified from peripheral blood leukocytes by SDS/proteinase K treatment and phenol/chloroform extraction. The 677 (*C→T*) substitution alters an alanine into a valine residue, creating a *Hin*fI site. Genotyping for the *C677T* polymorphism followed methods described in by Frosst *et al.* (14).

Quality Control. Quality control testing was performed for both HPV and *MTHFR* analyses and involved strict precautions against PCR contamination. All PCR reagents were made in batch and stored as aliquots, including positive and negative controls, to minimize the handling of reagents. Repeat assays were performed on a random 5% of all laboratory specimens. Gels were interpreted by two individuals.

Statistical Analysis. In previous analyses of these data (3, 15), we reported the following risk factors for cervical SIL: age, ethnicity, tobacco smoking, alcoholic beverage consumption, the number of sexual partners before age 20, and HPV DNA detection in the cervical cell specimens. In this analysis, we have focused on the association of the *MTHFR* genotype, dietary folate, and related B-complex vitamins to the risk of

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Table 1 ORs and 95% CIs for the association of *MTHFR* genotype with the risk of cervical SILs

MTHFR genotype	Cases	Controls	OR[a]	95% CI	P^b for trend	OR[c]	95% CI	*P* for trend
CC	73	93	1[d]			1[d]		
CT	67	75	1.3	0.8–2.1		2.0	1.1–3.7	
TT	10	11	1.1	0.4–2.9	0.62	2.9	1.0–8.8	0.02
CT or *TT*	77	86	1.2	0.8–2.0		2.1	1.2–3.9	

[a] After adjustment by unconditional multiple logistic regression for age and ethnicity.
[b] Based on the likelihood ratio test comparing models with and without a trend variable, assigned values 1, 2, and 3, representing the *CC*, *CT*, and *TT* categories, respectively.
[c] After adjustment by unconditional multiple logistic regression for age, ethnicity, tobacco smoking (ever *versus* never), alcohol drinking (ever *versus* never), number of sexual partners before age 20 (continuous), and HPV detection by PCR dot-blot hybridization (yes *versus* no).
[d] Reference category.

cervical SILs. Other dietary factors were analyzed as potential confounders, but details of these analyses will be reported elsewhere. Partial Pearson correlations (*r*) of continuous log-transformed nutrients and other variables, adjusted for calories and the risk factors above, were calculated to evaluate colinearity (22). The ORs associated with different levels of the exposure variables were evaluated by unconditional multiple logistic regression modeling case-control status (23). ORs and 95% CIs were computed by exponentiating the quantities (coefficient $\pm$ 1.96 $\times$ SE) for the binary indicator variables representing the nutrient quartiles or *MTHFR* genotypes. Adjustment variables included age, as a continuous variable, ethnicity by indicator variables, and the following additional variables: tobacco use (ever *versus* never smoked cigarettes), alcohol use (ever *versus* never drank), number of sex partners before age 20 (continuous), and HPV detection (yes *versus* no) by PCR dot-blot. In the diet analyses, we used the standard multivariate method for energy adjustment in which log-transformed energy intake was included as a covariate, but the inclusion of this variable was not significant in any of the regression models. We also adjusted for plasma nutrients (cryptoxanthin, vitamin C, and tocopherol) that were found to be related to risk in previous examinations of the data (15). The trend variable for nutrients was assigned the median for the appropriate quartile of intake. Gene dosage effects were modeled by assigning the values 1, 2, and 3 to a genotype trend variable, according to the subject's number of variant alleles (zero, one, and two variant alleles, respectively). Logistic regression was used to explore the joint association between the *MTHFR* polymorphism and other variables by modeling each level of interaction between the pairs of variables using subjects who had the homozygous *CC* genotype and unexposed to the second variable (*e.g.,* folate intake above the median 305.5 μg) as the reference category, and comparing this model by the likelihood ratio test to one in which only the main effects were modeled. Power to detect interactions was computed by the method described by Smith and Day (24).

Results

Controls in this population were older (mean age, 39.1 years) than cases (mean age, 32.4 years) and more likely to be Caucasian (43% of controls, 39% of cases) or Japanese (16% of cases, 12% of controls). All models were adjusted for age and ethnicity. The allelic distribution of *MTHFR* in cases and in controls were found to be in Hardy-Weinberg equilibrium. Table 1 shows ORs for the *MTHFR* genotypes after adjustment for age and ethnicity (column 4) and after adjustment for additional confounders (column 7). The variant *T* allele frequency was slightly more common among cervical SIL cases (29.0%) than among controls (27.1%), although age- and ethnicity-adjusted ORs did not vary significantly by genotype. However, after additional adjustment for covariates, the ORs for cervical SILs increased monotonically (p for trend = 0.02) among women with heterozygous (*CT*) and homozygous (*TT*) genotypes. The relationship of the *MTHFR* genotype to cervical SILs was confounded by HPV detection and alcohol drinking but not tobacco smoking or sexual history. The presence of one copy of the *T* allele was associated with an OR of 2.0 (95% CI, 1.1–3.7), and women with homozygous *TT* genotypes had an OR of 2.9 (95% CI, 1.0–8.8), compared with women with homozygous *CC* alleles, after adjustment for all covariates. The adjusted OR for the presence of at least one *T* (*CT* or *TT*) allele was 2.1 (95% CI, 1.2–3.9). Additional stratified analyses were performed by ethnic group with little suggestion of stratum-specific inconsistencies in the ORs (data not shown).

Table 2 shows the association of the dietary (foods + supplements) B-complex vitamins with the ORs for cervical SILs, using women in the lowest quartile of consumption as the reference category. We found a negative monotonic gradient of risk associated with increasing consumption of folate and vitamin B_6. The intake vitamin B_{12} was inversely related to the ORs for of cervical SILs, although the reduction in risk was limited to the fourth quartile. The results also suggested an inverse association of the other B vitamins with the ORs for cervical SILs, but these relationships were weak. The results for the B vitamins by source (supplements or food) were consistent (data not shown). For example, 49 cases (33%) and 75 controls (42%) took folate supplements, and we found a significant inverse dose-response relationship (P = 0.02) between folate supplement intake and cervical SIL risk. There was a high degree of correlation between the dietary intakes of the B-complex vitamins, with r ranging from 0.75 (folate and B_{12}) to 0.96 (thiamin and riboflavin), making it difficult to distinguish the effects of these nutrients on risk. The addition of continuous variables for energy intake and other dietary and plasma nutrients to the logistic models did not change the findings materially but weakened the trends. For example, after additional adjustment for (log) energy intake in the folate model in Table 2, the ORs (95% CIs) for cervical SILs associated with the three upper quartiles compared with the lowest quartile of dietary folate were 1.1 (0.5–2.6), 0.7 (0.3–1.7), and 0.3 (0.1–0.9), respectively (P for trend = 0.007).

Joint analyses were performed to examine the association of cervical SIL risk with the *MTHFR* polymorphism (*CT* or *TT* genotype *versus CC*) and dietary B-complex vitamins. We found no significant interaction between the *MTHFR* genotype and any of the vitamins under study. Results for folate are shown in Table 3 as an example of our findings, although the

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Table 2 OR[a] and 95% CIs for the association of B vitamin intake with the risk for cervical SILs

Nutrient	Quartile of intake[b]							*P* for trend[d]
	1 (low)[c]	2		3		4 (high)		
	OR	OR	95% CI	OR	95% CI	OR	95% CI	
Folate	1	1.0	0.4–2.2	0.6	0.3–1.4	0.3	0.1–0.7	0.002
Vitamin B_6	1	0.6	0.3–1.4	0.5	0.2–1.2	0.4	0.2–0.9	0.04
Vitamin B_{12}	1	0.8	0.3–1.7	0.9	0.4–1.9	0.3	0.2–0.8	0.02
Thiamin	1	0.5	0.2–1.2	0.3	0.1–0.8	0.5	0.2–1.1	0.14
Methionine	1	0.8	0.4–1.9	0.8	0.3–1.8	0.6	0.3–1.3	0.20
Riboflavin	1	0.5	0.2–1.1	0.4	0.2–0.9	0.4	0.2–1.0	0.08
Niacin	1	1.0	0.4–2.3	0.5	0.2–1.1	0.6	0.3–1.4	0.11

[a] After adjustment by unconditional multiple logistic regression for age, ethnicity, tobacco smoking (ever *versus* never), alcohol drinking (ever *versus* never), number of sexual partners before age 20 (continuous), and HPV detection by PCR dot-blot hybridization (yes *versus* no).
[b] The quartile cutpoints for daily nutrient intake were as follows: folate, 220.7, 305.5, 422.6 μg; vitamin B_6, 1.65, 2.66, 4.29 mg; vitamin B_{12}, 3.68, 6.83, 11.39 μg; thiamin, 1.33, 2.06, 3.50 mg; methionine, 1.13, 1.54, 2.21 mg; riboflavin, 1.55, 2.47, 4.13 mg; niacin, 17.49, 27.07, 41.26 mg.
[c] Reference category for all models.
[d] Based on the likelihood ratio test comparing models with and without a trend variable that was assigned the median value for the quartiles.

Table 3 Joint association of *MTHFR* genotype and folate intake with the OR[a] for cervical SILs

Folate intake[b] (μg)	*MTHFR CC*				*MTHFR CT* or *TT*				P^c for interaction
	Cases	Controls	OR	95% CI	Cases	Controls	OR	95% CI	
>305.5	29	55	1[d]		37	43	3.5	1.4–8.6	
≤305.5	44	38	3.9	1.6–9.7	40	43	5.0	2.0–12.2	0.10

[a] After adjustment by unconditional multiple logistic regression for age, ethnicity, tobacco smoking (ever *versus* never), alcohol drinking (ever *versus* never), number of sexual partners before age 20 (continuous), and HPV detection by PCR dot-blot hybridization (yes *versus* no).
[b] Dietary nutrient intake from food and supplements was divided at the median into high and low consumption.
[c] Based on the likelihood ratio test comparing models with and without an interaction term between *MTHFR* genotype and folate intake (degrees of freedom, 1).
[d] Reference category.

Table 4 Joint association of *MTHFR* genotype and HPV DNA test results with the OR[a] for cervical SILs

HPV DNA test[b]	*MTHFR CC*				*MTHFR CT* or *TT*				P^c for interaction
	Cases	Controls	OR	95% CI	Cases	Controls	OR	95% CI	
Negative	17	81	1[d]		31	79	2.2	1.1–4.6	
Positive	56	12	27.7	11.4–67.1	46	7	46.6	15.9–136.2	0.65

[a] After adjustment by unconditional multiple logistic regression for age, ethnicity, tobacco smoking (ever *versus* never), alcohol drinking (ever *versus* never), number of sexual partners before age 20 (continuous), and HPV detection by PCR dot-blot hybridization (yes *versus* no).
[b] HPV detection by PCR dot-blot hybridization.
[c] Based on the likelihood ratio test comparing models with and without the interaction term between *MTHFR* genotype and HPV (degrees of freedom, 1).
[d] Reference category.

power to detect this interaction was modest (44%). Women with the variant *T* allele were at significantly elevated ORs for cervical SILs compared with women who were homozygous *CC*, regardless of dietary folate intake. Women with dietary folate intakes below the median value were also at increased risk of cervical SILs when compared with women with folate intakes above the median, independent of *MTHFR* genotype. Women with low folate intakes and at least one variant *T* allele had a 5-fold greater risk of cervical SILs than women with high folate intakes and the *CC* genotype.

We modeled the interaction between the *MTHFR* polymorphism and HPV test results in Table 4. HPV-positive women with at least one *T* allele had an OR of 46.6 (95% CI, 15.9–136.2) compared with HPV-negative women with homozygous *CC* alleles. We found no evidence for a gene-virus interaction ($P = 0.65$), and the association of the *MTHFR* polymorphism with the ORs for cervical SILs appeared similar among HPV-positive (OR, 46.6/27.7 = 1.7) and HPV-negative (OR, 2.2/1 = 2.2) women.

Discussion

The discovery of a common *MTHFR* variant with reduced catalytic activity and the demonstration that this variant results from a polymorphic point substitution (*C677T*) in the structural gene have stimulated much scientific interest in the possible influence of the *MTHFR* genotype on disease susceptibility (14). Polymorphisms in the *MTHFR* gene have been associated with homocystinuria (25), thromboembolism (26), and neurological disorders (27), depending on the severity of the impairment in enzyme activity, but studies for the most part suggest that this substitution is benign, particularly with regard to cardiovascular disease risk (28). It is no doubt premature to reach any firm conclusions concerning the association of *MTHFR* variants with cancer risk, but it is probable that gene-disease associations will vary by cancer site and histology. Several studies suggest that the *C677T* polymorphism reduces the risk of colon cancer and adenomatous polyps when folate intake is adequate and alcohol consumption is limited (29–32);

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but the polymorphism may have no influence on risk when alcohol consumption is high (29) or may be a risk factor when folate intake is low (30, 31). The *T* allele may also reduce the risk of acute lymphocytic leukemia (33) but not the risk of acute myeloid leukemia (33) nor colorectal hyperplastic polyps (34).

Results from this investigation support the hypothesis that the *MTHFR 677T* allele is a susceptibility factor for cervical dysplasia. Women with one variant *T* allele had a 2-fold increased risk of cervical SILs, and women with the *TT* genotype had almost a 3-fold elevated risk for SILs compared with women with the *CC* genotype. These findings confirm the reported 2.9-fold (95% CI, 1.2–7.9) increased OR for cervical dysplasia among women with the *T* allele reported in a case-control study of 64 cases and 31 controls in Birmingham, AL (35).

Our finding that the *MTHFR 677T* variant is a risk factor for cervical SIL, regardless of folate intake, is consistent with the possibility that adequate folate concentrations in cervical tissue are physiologically maintained, preventing the enzyme variant from increasing efficiency. In instances where the *MTHFR C677T* substitution reduces the ORs for cancer in the presence of adequate folate levels, the *in vivo* synthesis of 5-methylTHF by the enzyme variant may be sufficient to meet the demands for DNA methylation, but the amount of 5-methylTHF synthesized may still be lower than normal, leaving an ample pool of nonmethylated folate (5,10-methylenetetrahydrofolate) for thymidylate and purine synthesis.

Depletion of 5-methylTHF through variant *MTHFR* or low folate levels can decrease levels of *S*-adenosylmethionine, which is responsible for DNA methylation. 5-methylTHF provides the methyl group for *de novo* methionine synthesis and DNA methylation. Kim *et al.* (36) found that global DNA hypomethylation increases progressively with the grade of cervical neoplasia. In a follow-up study, Fowler *et al.* (12) took biopsy samples from 83 patients with varying degrees of cervical intraepithelial neoplasia and assayed the tissues for differences in global methylation by extent of disease. The results suggested that hypomethylation of the cervical epithelium may be an early event in cervical carcinogenesis. Interestingly, concentrations of folate in serum were unrelated to the severity of dysplasia and only weakly, but significantly, associated with degree of cervical hypomethylation. None of the other factors examined, including hormone use, tobacco smoking, or HPV infection, were associated with either DNA methylation or grade of dysplasia.

HPV infection was a strong predictor of cervical SIL risk in this study, in agreement with other investigations of cervical dysplasia and cancer. HPV-positive women who had at least one variant *T* allele had a 47-fold increased OR for SILs compared with HPV-negative women with the *CC* genotype. Although we did not anticipate an increased OR associated with the *MTHFR T* allele in HPV-negative women (Table 4), HPV infections are transient, and women who are negative on a single HPV test cannot be assumed to be at no risk for cervical dysplasia. The HPV regulatory sequences are located within an unmethylated CpG island. List *et al.* (37) postulate that HPV transcriptional activity can be suppressed by hypermethylation of the promoter of the regulatory region. Conversely, hypomethylation at specific promoter sites inhibits the binding of methylation-sensitive papillomavirus transcription factor to the promoter region and facilitates the integration of HPV DNA into host cells (12). It has been demonstrated that CpG methylation directly inhibits the DNA binding activity of the HPV-16 E2 protein (38), and that methylation of cytosines within the HPV-18 CpG island results in the suppression of viral promoter activity (39).

This study confirms an inverse association of dietary folate with the OR for cervical dysplasia that has been reported by other investigators (5–8). The median folate intake in our reference population, 305.5 μg/day, is consistent with the estimated average folate requirements for women in this age group (320 μg/day) and with estimates from the United States National surveys (272 μg/day for women 31–50 years in the Third National Health and Nutrition Examination Survey; Ref. 40), providing some confidence in the dietary assessment method and representativeness of the control group. In a previous analysis, we found no relation of plasma folate levels, based on a single blood sample, to the ORs for cervical SILs (3), whereas dietary folate intake was inversely related to risk in the present analysis. There are several possible reasons for the lack of concordance between the dietary and plasma measures of folate:

(*a*) Plasma folate levels are transient and dependent on recent, rather than long-term, dietary folate intake, so there may be a poor correlation of present plasma folate concentrations to lifetime exposure. Indeed, the partial correlation (adjusted for the covariates described in "Materials and Methods") of plasma folate concentrations to dietary folate intake was only 0.28 in our control population.

(*b*) The *677T* variant may not manifest a reduced enzymatic activity in the presence of adequate folate substrate concentrations, allowing the cellular demands for 5-methylTHF to be accommodated (41).

(*c*) Finally, it is possible that plasma folate concentrations in our study subjects did not adequately reflect concentrations of folate in the cervical target tissue.

The present study had several advantages, including sensitive and specific HPV testing, a validated interviewer-administered food-frequency questionnaire designed specifically for our population, and expert pathological review of all cases. Weaknesses included a relatively small sample size, making it difficult to evaluate the form of the joint relationship between pairs of variables on the ORs for cervical SILs. The power of our study to detect the interaction between *MTHFR* genotype and folate intake in Table 3 as statistically significant was 44%. A sample size of 748 women would have been required to attain a power of 80% in this analysis. Compliance with questionnaire (71% of eligible cases, 85% of eligible controls) and blood sample (50% of eligible cases, 56% of eligible controls) collection was not optimal but compared favorably with other similar molecular studies. A poor response rate may have biased our findings if participants were not representative of all eligible women. We identified few differences in the demographic characteristics and risk factors among interviewed women who participated in the blood sample collection with women who did not participate. We were careful to adjust for age, ethnicity, energy intake, and other variables that might confound the hypothesized dietary and nondietary associations with cervical SILs.

In summary, we found that cervical SIL cases were more likely than controls to have the *MTHFR 677C→T* polymorphism and a low dietary intake of B vitamins. We found no evidence for a statistical interaction between *MTHFR* genotype and folate intake on the ORs for cervical SILs, but because of the small sample size, our study power to examine joint effects between variables was limited. The *MTHFR* polymorphism may have a substantive impact on the population burden of this disease because of its high frequency. In fact, the *T* allele frequency of 27% found in our controls is somewhat lower than that reported in other populations (42). If our results are confirmed, the public health impact of the *MTHFR* polymorphism on the risk of cervical dysplasia may be large in communities with a high prevalence of HPV infection and folate deficiency.

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References

1. Van Niekerk, W. A. Cervical cytological abnormalities caused by folic acid deficiency. Acta Cytol., *10:* 67–73, 1966.

2. Whitehead, N., Reyner, F., and Lindenbaum, J. Megaloblastic changes in the cervical epithelium. J. Am. Med. Assoc., *2126:* 1421–1424, 1973.

3. Goodman, M. T., McDuffie, K., Hernandez, B., Wilkens, L. R., and Selhub, J. Case-control study of plasma folate, homocysteine, vitamin B_{12}, and cysteine as markers of cervical dysplasia. Cancer (Phila.), *89:* 376–382, 2000.

4. Butterworth, C. E., Jr., Hatch, K. D., Macaluso, M., Cole, P., Sauberlich, H. E., Soong, S-J., Borst, M., and Baker, V. V. Folate deficiency and cervical dysplasia. J. Am. Med. Assoc., *267:* 528–533, 1992.

5. Van Eenwyk, J., Davis, F. G., and Colman, N. Folate, vitamin C, and cervical intraepithelial neoplasia. Cancer Epidemiol. Biomark. Prev., *1:* 119–124, 1992.

6. Buckley, D. I., McPherson, R. S., North, C. Q., and Becker, T. M. Dietary micronutrients and cervical dysplasia in Southwestern American Indian women. Nutr. Cancer, *17:* 179–185, 1991.

7. Kanetsky, P. A., Gammon, M. D., Mandelblatt, J., Zhang, Z-F., Ramsey, E., Dnistrian, A., Norkus, E. P., and Wright, T. C., Jr. Dietary intake and blood levels of lycopene: association with cervical dysplasia among non-Hispanic, black women. Nutr. Cancer, *31:* 31–40, 1998.

8. Liu, T., Soong, S-J., Wilson, N. P., Craig, C. B., Cole, P., Macaluso, M., and Butterworth, M. A case control study of nutritional factors and cervical dysplasia. Cancer Epidemiol. Biomark. Prev., *2:* 525–530, 1993.

9. Alberg, A. J., Selhub, J., Shah, K. V., Viscidi, R. P., Comstock, G. W., and Helzlsouer, K. J. The risk of cervical cancer in relation to serum concentrations of folate, vitamin B12, and homocysteine. Cancer Epidemiol. Biomark. Prev., *9:* 761–764, 2000.

10. Blount, B. C., Mack, M. M., Wehr, C. M., MacGregor, J. T., Hiatt, R. A., Wang, G., Wickramasinghe, S. N., Everson, R. B., and Ames, B. N. Folate deficiency causes uracil misincorporation into human DNA and chromosome breakage: implications for cancer and neuronal damage. Proc. Natl. Acad. Sci. USA, *94:* 3290–3295, 1997.

11. Everson, R. B., Wehn, C. M., Erexson, G. I., and MacGregor, J. T. Association of marginal folate depletion with increased human chromosomal damage *in vivo*: demonstration by analysis of micronucleated erythrocytes. J. Natl. Cancer Inst., *80:* 525–529, 1988.

12. Fowler, B. M., Giuliano, A. R., Piyathilake, C., Nour, M., and Hatch, K. Hypomethylation in cervical tissue: is there a correlation with folate status? Cancer Epidemiol. Biomark. Prev., *7:* 901–906, 1998.

13. Bailey, L. B., and Gregory, J. F., III. Polymorphisms of methylenetetrahydrofolate reductase and other enzymes: metabolic significance, risks and impact on folate requirement. J. Nutr., *29:* 919–922, 1999.

14. Frosst, P., Blom, H. G., Milos, R., Goyette, P., Sheppard, C. A., Matthews, R. G., Boers, G. J., den Heijer, M., Kluijtmans, L. A., van den Heuvel, L. P., *et al.* A candidate genetic risk factor for vascular disease: a common mutation in methylenetetrahydrofolate reductase. Nat. Genet., *10:* 11–13, 1995.

15. Goodman, M. T., Kiviat, N., McDuffie, K., Hankin, J. H., Hernandez, B., Wilkens, L. R., Franke, A., Kuypers, J., Kolonel, L. N., Nakamura, J., Ing, G., Branch, B., Bertram, C. C., Kamemoto, L., Sharma, S., and Killeen, J. The association of plasma micronutrients with the risk of cervical dysplasia in Hawaii. Cancer Epidemiol. Biomark. Prev., *7:* 537–544, 1998.

16. Kurman, R. J., and Solomon, D. The Bethesda System for Reporting Cervical/Vaginal Cytologic Diagnoses, pp. 30–38. New York: Springer-Verlag, 1994.

17. Hankin, J. H. A diet history method for research, clinical and community use. 23rd Lenna Frances Cooper Memorial Lecture. J. Am. Diet. Assoc., *86:* 868–872, 1986.

18. Hankin, J. H., Wilkens, L. R., Kolonel, L. N., and Yoshizawa, C. N. Validation of a quantitative diet history method in Hawaii. Am. J. Epidemiol., *133:* 616–628, 1991.

19. U. S. Department of Agriculture (USDA). Nutrient database for standard reference, release 10. Bethesda, MD: Human Nutrition Information Service, USDA, 1992.

20. Ting, Y., and Manos, M. M. Detection and typing of genital human papillomavirus. *In:* M. A. Innis, D. H. Gelfond, J. J. Sninsky, and T. J. White (eds.), PCR Protocol: A Guide to Methods and Applications, pp. 356–367. San Diego: Academic Press, 1990.

21. Bauer, H. M., Ting, Y., Greer, C. E., Chambers, J. C., Tashiro, C. J., Chimera, J., Reingold, A., and Manos, M. M. Genital human papillomavirus infection in female university students as determined by a PCR-based method. J. Am. Med. Assoc., *265:* 472–477, 1991.

22. Snedecor, G. W., and Cochran, W. G. Statistical Methods. Ames, IA: Iowa State University Press, 1967.

23. Breslow, N. E., and Day, N. E. Statistical Methods in Cancer Research. Vol. I. The Analysis of Case-Control Studies. IARC Scientific Publication No. 32. Lyon: IARC, 1980.

24. Smith, P. G., and Day, N. E. The design of case-control studies: the influence of confounding and interaction effects. Int. J. Epidemiol., *13:* 356–365, 1984.

25. Jacques, P. F., Bostom, A. G., Williams, R. R., Ellison, R. C., Eckfeldt, J. H., Rosenberg, I. H., Selhub, J., and Rozen, R. Relation between folate status, a common mutation in methylenetetrahydrofolate reductase, and plasma homocysteine concentrations. Circulation, *93:* 7–9, 1996.

26. Zheng, Y. Z., Tong, J., Do, X. P., Pu, X. Q., and Zhou, B. T. Prevalence of methylenetetrahydrofolate reductase C677T and its association with arterial and venous thrombosis in the Chinese population. Br. J. Haematol., *109:* 870–874, 2000.

27. van der Put, N. M., Steegers-Theunissen, R. P., Frosst, P., Trijbels, F. J., Eskes, T. K., van den Heuvel, L. P., Mariman, E. C., den Heyer, M., Rozen, R., and Blom, H. J. Mutated methylenetetrahydrofolate reductase as a risk factor for spina bifida. Lancet, *346:* 1070–1071, 1995.

28. Brugada, R., and Marian, A. J. A common mutation in methylenetetrahydrofolate reductase gene is not a major risk of coronary artery disease or myocardial infarction. Atherosclerosis, *128:* 107–112, 1997.

29. Ma, J., Stampfer, M. J., Giovannucci, E., Artigas, C., Hunter, D. J., Fuchs, C., Willett, W. C., Selhub, J., Hennekens, C. H., and Rozen, R. Methylenetetrahydrofolate reductase polymorphism, dietary interactions, and risk of colorectal cancer. Cancer Res., *57:* 1098–1102, 1997.

30. Ulrich, C. M., Kampman, E., Bigler, J., Schwartz, S. M., Chen, C., Bostick, R., Fosdick, L., Beresford, S. A., Yasui, Y., and Potter, J. D. Colorectal adenomas and the C677T MTHFR polymorphism: evidence for gene-environment interaction? Cancer Epidemiol. Biomark. Prev., *8:* 659–668, 1999.

31. Levine, A. J., Siegmund, K. D., Ervin, C. M., Diep, A., Lee, E. R., Frankl, H. D., and Haile, R. W. The methylenetetrahydrofolate reductase 677C→T polymorphism and distal colorectal adenoma risk. Cancer Epidemiol. Biomarker. Prev., *9:* 657–663, 2000.

32. Slattery, M. L., Potter, J. D., Samowitz, W., Schaffer, D., and Leppert, M. Methylenetetrahydrofolate reductase, diet, and risk of colon cancer. Cancer Epidemiol. Biomark. Prev., *8:* 513–518, 1999.

33. Skibola, C. F., Smith, M. T., Kane, E., Roman, E., Rollinson, S., Cartwright, R. A., and Morgan, G. Polymorphisms in the methylenetetrahydrofolate reductase gene are associated with susceptibility to acute leukemia in adults. Proc. Natl. Acad. Sci. USA, *96:* 12810–12815, 1999.

34. Ulrich, C. M., Kampman, E., Bigler, J., Schwartz, S. M., Chen, C., Bostick, R., Fosdick, L., Beresford, S. A., Yasui, Y., and Potter, J. D. Lack of association between the C677T MTHFR polymorphism and colorectal hyperplastic polyps. Cancer Epidemiol. Biomark. Prev., *9:* 427–433, 2000.

35. Piyathilake, C. J., Macaluso, M., Johanning, G. L., Whiteside, M., Heimdinger, D. C., and Giuliano, A. Methylenetetrahydrofolate reductase (MTHFR) polymorphism increases the risk of cervical intraepithelial neoplasia. Anticancer Res., *20:* 1751–1757, 2000.

36. Kim, Y-I., Giuliano, A., Hatch, K. D., Schneider, A., Nour, M. A., Dallal, G. E., Selhub, J., and Mason, J. B. Global DNA hypomethylation increases progressively in cervical dysplasia and carcinoma. Cancer (Phila.), *74:* 893–899, 1994.

37. List, H. J., Patzel, V., Zeidler, U., Schopen, A., Ruhl, G., Stollwerk, J., and Klock, G. Methylation sensitivity of the enhancer from the human papillomavirus type 16. J. Biol. Chem., *269:* 11902–11911, 1994.

38. Thain, A., Jenkins, O., Clarke, A. R., and Gaston, K. CpG methylation directly inhibits binding of the human papillomavirus type 16 E2 protein to specific DNA sequences. J. Virol., *70:* 7233–7235, 1996.

39. Rosl, F., Arab, A., Klevenz, B., and zur Hausen, H. The effect of DNA methylation on gene regulation of human papillomaviruses. J. Gen. Virol., *74:* 791–801, 1993.

40. Dietary Reference Intakes for Thiamin, Riboflavin, Niacin, Vitamin B_6, Folate, Vitamin B_{12}, Pantothenic Acid, Biotin, and Choline. A Report of the Standing Committee on the Scientific Evaluation of Dietary Reference Intakes. Food and Nutrition Board, Institute of Medicine, National Academy of Sciences. Washington, DC: National Academy Press, 1998.

41. Stern, L. L., Bagley, P. J., Rosenberg, I. H., and Selhub, J. Conversion of 5-formyltetrahydrofolic acid to 5-methyltetrahydrofolic acid is unimpaired in folate-adequate persons homozygous for the C677T mutation in the methylenetetrahydrofolate reductase gene. J. Nutr., *130:* 2238–2242, 2000.

42. Fodinger, M., Horl, W. H., and Sunder-Plassmann, G. Molecular biology of 5,10-methylenetetrahydrofolate reductase. J. Nephrol., *13:* 20–33, 2000.

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