*Null Results in Brief*

# No Association between *MTHFR* 677 C→T or 1298 A→C Polymorphisms and Endometrial Cancer Risk

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## Introduction

The enzyme 5,10-methylenetetrahydrofolate reductase, encoded by *MTHFR*, catalyzes the reduction of 5,10-methylenetetrahydrofolate to 5-methyltetrahydrofolate. The *MTHFR* 677 C→T polymorphism results in an amino acid change, Ala→Val. The Val allele is associated with decreased enzyme activity (1), and may potentially influence carcinogenesis through somatic DNA methylation or through uracil misincorporation in DNA synthesis and repair. Another common non-synonymous polymorphism *MTHFR* 1298 A→C (Glu→Ala) has been associated with lower blood folate and higher homocysteine levels in some (e.g., 2) but not all (e.g., 3) studies. There has been one study previously published examining the *MTHFR* 677 C→T polymorphism and endometrial cancer risk (4). Esteller et al. (4) reported in their study of 80 cases and 60 controls that women with a "T" allele had an increased risk of endometrial cancer compared with "CC" homozygotes [odds ratio (OR) = 2.8; 95% confidence interval (CI), 1.36-6.14]. Dietary folate, the substrate of *MTHFR*, has been associated with a decreased risk of endometrial cancer (5). We hypothesized that *MTHFR* polymorphisms may affect endometrial cancer risk, and that the risk may be modified by folate intake.

## Materials and Methods

Detailed information about this nested case-control study of endometrial cancer in the Nurses' Health Study cohort (cases, *n* = 222; controls, *n* = 666) has been reported previously (6). Genotyping was done using the Taqman system. Taqman primers, probes, and conditions for genotyping assays are available on request. All genotyping was done with laboratory personnel blinded to case-control status of the samples, which included quality control samples for validation. Concordance for quality control samples was 100%. Dietary information was collected prospectively for 201 cases and 603 controls, including energy-adjusted total folate from dietary intake and vitamin supplements, and weekly alcohol intake in grams (7). ORs and 95% CIs were calculated using conditional logistic regression and were adjusted for established endometrial cancer risk factors. Haplotype estimation and linkage disequilibrium measures were done using SAS/Genetics version 8.2 (SAS Institute, Cary, NC).

## Results

Both *MTHFR* polymorphisms were in Hardy-Weinberg equilibrium in the cases and the controls (*P* > 0.05). Comparing cases to controls, the prevalence of the variant allele was 32.8% versus 32.6% for 677 C→T and 33.3% versus 33.2% for 1298 A→C. We found little or no association between *MTHFR* genotype and endometrial cancer risk (Table 1). For the *MTHFR* 677 C→T polymorphism, we observed an adjusted OR = 1.10, 95% CI (0.77-1.57) comparing those having a "T" allele to the "CC" homozygotes. For the *MTHFR* 1298 A→C polymorphism, we observed no association comparing those with the "C" allele to the "AA" homozygotes (OR = 0.85; 95% CI, 0.61-1.20).

Among our sample of 201 cases and 603 controls for whom we had prospectively collected dietary information, we observed a marginally reduced risk of endometrial cancer among women with energy-adjusted total folate ≥400 μg/d (OR = 0.74; 95% CI, 0.52-1.07) compared with women with <400 μg/d. Women with alcohol intake ≥15 g/d (approximately one alcoholic beverage) had an estimated endometrial cancer risk of OR = 1.36, 95% CI (0.77-2.40). We observed a modest inverse association between jointly high folate and low alcohol intake and endometrial cancer risk when we compared those with high folate (≥400 μg/d) and low alcohol intake (<15 g/d) to all others (OR = 0.72; 95% CI, 0.49-1.05). There was no statistically significant interaction of dietary factors with either *MTHFR* genotype. Our study had >99% power to detect an OR = 2.8 for either locus, and >80% power to detect OR = 1.6. We had limited power, however, to detect an interaction between genotype and folate and alcohol intake, assuming $OR_{genotype}$ = 2.8 and $OR_{low\ folate}$ = 1.5; we had 41% power to detect θ = 2.0 and 29% power to detect θ = 0.5 (8).

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**Table 1. Associations between *MTHFR* 677 C→T and 1298 A→C genotypes and endometrial cancer risk**

| | | Cases *n* (%)* | Controls *n* (%)* | OR (95% CI)† |
|---|---|---|---|---|
| *MTHFR* 677 C→T (Ala→Val) | Ala/Ala | 97 (44.5) | 299 (45.1) | 1.00 (reference) |
| | Ala/Val | 99 (45.4) | 296 (44.6) | 1.10 (0.75-1.60) |
| | Val/Val | 22 (10.1) | 68 (10.3) | 1.11 (0.62-1.99) |
| *MTHFR* 1298 A→C (Glu→Ala) | Glu/Glu | 102 (46.6) | 302 (45.4) | 1.00 (reference) |
| | Ala/Glu | 88 (40.2) | 285 (42.9) | 0.85 (0.59-1.22) |
| | Ala/Ala | 29 (13.2) | 78 (11.7) | 0.88 (0.51-1.52) |

*Numbers do not add to 222 cases and 666 controls due to missing genotypes.
†ORs and 95% CIs calculated using conditional logistic regression accounting for the matching factors and additionally adjusted for body mass index prior to diagnosis, weight gain since age 18, age at menarche, ages at first birth and last birth, age at menopause, parity, pack-years of smoking, and first-degree family history of endometrial cancer or colon cancer.

We observed significant linkage disequilibrium between the two *MTHFR* loci, $D' = 0.99$, $P < 0.01$. Haplotype estimation predicts three of four possible haplotypes: 677C/1298A, 34.3%; 677C/1298C, 33.1%; and 677T/1298A, 32.4%. There was no evidence of a different distribution of these haplotypes between cases and controls ($P = 0.99$).

We obtained tumor staging information for 205 of the 222 cases included in this study: 111 were classified as well differentiated, 69 as moderately differentiated, and 25 as poorly differentiated. Genotype frequencies did not differ significantly across tumor stages.

## Discussion

In this study, we sought to determine whether *MTHFR* gene variants 677 C→T or 1298 A→C influence endometrial cancer risk. We did not observe any association between *MTHFR* genotypes and endometrial cancer risk. We did not detect significant evidence of a gene-environment interaction between *MTHFR* genotype and dietary folate; however, larger studies will be needed to assess this interaction.

A previous study by Esteller et al. reported an increased risk of endometrial cancer, comparing *MTHFR* 677 "T" allele carriers to "CC" homozygotes. They noted that the association of *MTHFR* 677 C→T with endometrial cancer risk appeared to be strongest among women with poorly or moderately differentiated tumors compared with those with well-differentiated tumors, with 50% of their cases classified as having poorly or moderately differentiated tumors. We considered the possibility of our study population having a different distribution of poorly or moderately differentiated tumors; however, 46% of our cases were classified as having poorly or moderately differentiated tumors. There is not a sufficient difference in distribution of tumor differentiation to account for the differences observed in the two reported studies of *MTHFR* genetic variation and endometrial cancer risk.

We note that the study subjects in the Esteller et al. study were recruited in Barcelona, Spain, whereas our study subjects were recruited from across the United States. Though controls from both studies of predominantly Caucasian populations are in Hardy-Weinberg equilibrium, the estimated allele frequency of the *MTHFR* "T" allele differs in our study (32.6%) compared with that of Esteller et al. (26.7%). Additionally, there may exist unrecognized environmental factors that modify the genetic association of *MTHFR* and endometrial cancer risk and vary widely between populations. A likely explanation for the different results between the two studies may be due to sample size. The Esteller et al. study was a hospital-based case-control study with 80 cases and 60 controls; ours is a case-control study nested within a large prospective cohort, with 222 cases and 666 controls.

## References

1. Frosst P, Blom HJ, Milos R, et al. A candidate genetic risk factor for vascular disease: a common mutation in methylenetetrahydrofolate reductase. Nat Genet 1995;10:111-3.
2. van der Put NM, Gabreels F, Stevens EM, et al. A second common mutation in the methylenetetrahydrofolate reductase gene: an additional risk factor for neural-tube defects? Am J Hum Genet 1998;62:1044-51.
3. Friso S, Girelli D, Trabetti E, et al. A1298C methylenetetrahydrofolate reductase mutation and coronary artery disease: relationships with C677T polymorphism and homocysteine/folate metabolism. Clin Exp Med 2002;2:7-12.
4. Esteller M, Garcia A, Martinez-Palones JM, Xercavins J, Reventos J. Germ line polymorphisms in cytochrome-P450 1A1 (C4887 CYP1A1) and methylenetetrahydrofolate reductase (MTHFR) genes and endometrial cancer susceptibility. Carcinogenesis 1997;18:2307-11.
5. McCann SE, Freudenheim JL, Marshall JR, Brasure JR, Swanson MK, Graham S. Diet in the epidemiology of endometrial cancer in western New York (United States). Cancer Causes Control 2000;11:965-74.
6. Setiawan VW, Hankinson SE, Colditz GA, Hunter DJ, De Vivo I. HSD17B1 gene polymorphisms and risk of endometrial and breast cancer. Cancer Epidemiol, Biomarkers & Prev. 2004;13:213-9.
7. Zhang S, Hunter DJ, Hankinson SE, et al. A prospective study of folate intake and the risk of breast cancer. JAMA 1999;281:1632-7.
8. Garcia-Closas M, Lubin JH. Power and sample size calculations in case-control studies of gene-environment interactions: comments on different approaches. Am J Epidemiol 1999;149:689-92.

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