Null Results in Brief

Methylenetetrahydrofolate Reductase Haplotype Tag Single-Nucleotide Polymorphisms and Risk of Breast Cancer

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Introduction

The folate metabolism pathway contributes to important metabolic processes, such as RNA and DNA synthesis, DNA repair, and DNA methylation (1). Previous observations have suggested a potential relationship between altered folate levels and tumorigenesis (2). Therefore, inherited genetic variation in the gene encoding methylenetetrahydrofolate reductase (*MTHFR*), an enzyme that regulates the main circulating form of folate, 5-methyltetrahydrofolate, as well as the synthesis of *S*-adenosyl-L-methionine, the methyl donor for most methyltransferase reactions, may play an important role in the etiology of cancer. Epidemiologic studies of *MTHFR* and breast cancer have focused on only two common gene variants: the C665T, Ala^{222}Val polymorphism encoding a thermolabile variant allozyme with decreased enzyme activity, and the A1286C, Glu^{429}Ala polymorphism (3). Although significant associations with breast cancer risk have been observed, at least with the 665T variant in premenopausal women (4, 5), the two common polymorphisms studied to date represent only a portion of the sequence variation present in *MTHFR* (6). Therefore, we set out to assess the association of common *MTHFR* polymorphisms and haplotypes with breast cancer using a haplotype-tagging approach.

Material and Methods

Study Population. Cases were women diagnosed with breast cancer within the previous year seen in Medical Oncology at the Mayo Clinic (Rochester, MN). Cases were frequency matched to controls on age (5-year categories) and region of residence. Controls were selected from general medical examination appointments in the Department of Internal Medicine. All subjects were from the states of Illinois, Iowa, Minnesota, North Dakota, South Dakota, or Wisconsin. Both cases and controls were ineligible if they had a previous diagnosis of cancer (except nonmelanoma skin cancer).

***MTHFR* Single-Nucleotide Polymorphism Selection and Genotyping.** As described elsewhere (6), *MTHFR* was resequenced in Coriell DNA samples from randomly selected Caucasian-American subjects. Sixteen single-nucleotide polymorphisms (SNP) that captured most of the genetic variability in the gene were selected either through the haplotype-tagging approach of Stram et al. (7) or through the LD-Select method of Carlson et al. (8). All SNPs selected through the Stram method, except I1C (+128), were in common to both methods. These 16 SNPs, plus four nonsynonymous coding SNPs (C400T Arg^{134}Cys, G1556A Arg^{519}His, G1743A Met^{581}Ile, and C1958T Thr^{653}Met), were genotyped using the SNPstream platform (Beckman Coulter, Fullerton, CA) as described elsewhere (9). *MTHFR* polymorphisms within exons and in 5′ and 3′ untranslated regions were numbered by designating the 'A' in the translation initiation codon for the cDNA encoding the 70-kDa isoform as position (+1). cDNA nucleotides located 5′ to that position were assigned negative numbers, whereas those located 3′ were assigned positive numbers. Positions within introns were numbered relative to splice junctions, with the initial 5′ nucleotide in the intron designated (+1).

Statistical Methods. Genotypes for the controls were assessed for departures from Hardy-Weinberg equilibrium. Single SNP analyses were done using logistic regression, where case-control status was the response, and genotypes were modeled as having a log-additive relationship with breast cancer case status. Empirical adjustment for the multiple SNPs tested was achieved by permuting case-control status 10,000 times, doing all single SNP tests for each permutation, and tallying the number of times the smallest resulting P was lower than the smallest observed P. We assessed effect modification of SNPs with menopausal status using standard tests for interaction and did the same permutation procedure to determine whether there was significant evidence of a *MTHFR*-menopausal status interaction. Finally, a whole-gene test of association between *MTHFR* haplotypes and breast cancer status was done using the haplotype score test of Schaid et al. (10). All analyses were adjusted for age and region of residence. Analyses were done using Statistical Analysis System (SAS Institute, Cary NC) and S-Plus (Insightful, Seattle, WA).

Power Considerations. The minimum odds ratio (OR) that would be detectable in a study of this size was estimated for a variety of minor allele frequencies using power formulae for the Armitage test for trend (11). We set the power at 80% and used two settings for type I error: 0.05 and 0.005. The more

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Table 1. Associations of individual *MTHFR* SNPs with odds of breast cancer

SNP location*		Sequence change	Minor allele frequency		Detectable OR †		OR (95% CI) ‡
			Cases	Controls	α = 0.05	α = 0.005	
	5FR (−1,062)	G→A	0.28	0.26	1.25	1.33	1.13 (0.96-1.34)
	5FR (−201)	G→A	0.06	0.06	1.48	1.62	1.01 (0.73-1.40)
	I1C (+128)	G→T	0.05	0.05	1.53	1.68	1.00 (0.72-1.40)
rs13306561	I1C (+141)	T→C	0.16	0.16	1.30	1.39	0.99 (0.80-1.23)
rs3753584	Exon 1A (−1,127)	A→G	0.16	0.15	1.31	1.40	1.07 (0.86-1.31)
	Exon 1A (−690)	C→T	0.10	0.10	1.37	1.48	1.07 (0.83-1.38)
rs2066470	Exon 1 (117)	C→T	0.11	0.10	1.37	1.48	1.07 (0.84-1.37)
rs13306567	I2 (−86)	C→G	0.06	0.06	1.48	1.62	1.01 (0.73-1.39)
rs2066471	I2 (−79)	G→A	0.17	0.16	1.30	1.39	1.10 (0.90-1.34)
rs1801133	Exon 4 (665)	C→T	0.32	0.33	1.24	1.31	0.91 (0.78-1.06)
rs2066462	Exon 6 (1,056)	C→T	0.11	0.10	1.37	1.48	1.13 (0.88-1.44)
rs1994798	I6 (+31)	T→C	0.46	0.42	1.23	1.29	1.23 (1.06-1.43)
rs12121543	I6 (+115)	G→T	0.27	0.24	1.26	1.33	1.15 (0.97-1.36)
rs1801131	Exon 7 (1,286)	A→C	0.34	0.31	1.24	1.31	1.13 (0.96-1.32)
rs3818762	I10 (−48)	C→G	0.30	0.27	1.25	1.32	1.17 (0.99-1.38)
rs2274976	Exon 11 (1,781)	G→A	0.05	0.05	1.53	1.68	1.11 (0.79-1.56)
	Exon 11 (1,958)	C→T	0.02	0.02	1.87	2.14	1.03 (0.61-1.76)

*Explanations of the numbering scheme for SNP locations can be found in ref. 6.
† OR >1 that is detectable with 80% power, assuming log-additive genetic effects and using formulae for the Armitage test for trend.
‡ Logistic regression analysis, adjusting for the design variables of age and geographic region. The OR represents the estimated increase in the odds.

stringent P of 0.005 was selected following empirical calculations that suggested a Bonferroni correction for 10 independent tests would preserve a whole-gene type I error level of 0.05.

Results

A total of 750 cases and 732 controls were included in these analyses. No genetic variation was observed within the three nonsynonymous coding SNPs C400T Arg^{134}Cys, G1556A Arg^{519}His, and G1743A Met^{581}Ile. All SNPs met the Hardy-Weinberg equilibrium assumption except exon 6, C1056T (P < 0.01, Hardy-Weinberg equilibrium test). Associations of individual SNPs with breast cancer risk are presented in Table 1. The common C665T polymorphism that has previously been shown to confer risk for breast cancer in premenopausal women (4, 5) also showed no association. As with the single SNP main effect tests, tests for interaction between individual SNPs and menopausal status and their associations with breast cancer risk also did not reach statistical significance after accounting for multiple testing (P > 0.05 for each). Haplotype analyses for the LD-selected SNPs revealed eight haplotypes with frequencies >1% (Table 2). None of these haplotypes were significantly associated with breast cancer risk. Similar results were found for the SNPs selected by using the haplotype-tagging approach. We did multivariate logistic regression and haplotype analyses that included the following covariates found to be associated with case status in our cohort: age at menarche, menopausal status, family history, education, activity, and alcohol. Multivariate-adjusted results were similar to those presented in the tables (data not shown).

Discussion

The purpose of this work was to conduct a comprehensive analysis of the possible association between *MTHFR* polymorphisms or haplotypes and risk for breast cancer. MTHFR plays a central role in the regulation of intracellular folate levels and is an important target for epidemiologic studies of folate metabolism and breast cancer. Previous reports of *MTHFR* variants and breast cancer have focused primarily on the first common variant identified, C665T, and those studies yielded inconsistent results. The effect of this polymorphism varied depending on menopausal status and folate levels. Two groups reported an increased risk for breast cancer in premenopausal individuals with the homozygous variant (4, 5). Other groups observed no such association (12, 13). The A1286C variant was associated with increased risk in smaller studies (14, 15) but not in studies with larger population samples (12, 16, 17).

Although informative, prior studies were limited to only one or, at most, two candidate polymorphisms in *MTHFR*. We recently published a report of the systematic resequencing

Table 2. Association of *MTHFR* haplotypes and risk of breast cancer

5FR (−1,062)	5FR (−201)	I1C (+141)	Exon 1A (−1,127)	Exon 1A (−690)	Exon 1 (117)	I2 (−86)	I2 (−79)	Exon 4 (665)	Exon 6 (1,056)	I6 (+31)	I6 (+115)	Exon 7 (1,286)	I10 (−48)	Exon 11 (1,781)	Hap Freq*	Score Test †	P ‡
G	G	T	A	C	C	C	G	T	C	T	G	A	C	G	0.30	−1.07	0.28
G	G	T	A	C	C	C	G	C	C	T	G	A	C	G	0.24	−0.52	0.60
A	G	T	A	C	C	C	A	C	C	C	G	C	G	G	0.02	−0.45	0.65
G	G	C	G	T	T	C	G	C	T	C	T	C	G	G	0.05	0.10	0.92
A	G	T	A	C	C	C	G	C	C	C	G	A	C	G	0.08	0.30	0.77
G	A	C	G	C	C	G	G	C	C	C	G	C	C	G	0.05	0.40	0.68
G	G	C	G	T	T	C	G	C	T	C	T	C	G	A	0.04	1.26	0.21
A	G	T	A	C	C	C	A	C	C	C	T	C	G	G	0.14	1.28	0.20

NOTE: Results adjusted for the effects of the design variables age and geographic region. Global test of significance: P = 0.82.
*Estimated haplotype frequency.
† Score statistics comparing haplotype of interest with all other haplotypes combined. Negative values imply decreased risk of breast cancer, whereas positive values imply increased risk.
‡ P comparing haplotype of interest with all other haplotypes combined.

of all coding exons, exon-intron splice junctions, and the 5′-flanking region of *MTHFR* (6). A total of 65 polymorphisms was identified in the 240 samples examined. The current analysis extends that work. For these analyses, we conducted a systematic selection of tag SNPs from these polymorphisms. We used two methods (7, 8) of SNP selection to determine if one provided greater insight into gene/disease association. Both methods selected essentially the same tagging SNPs.

In our study, we observed no significant association of any of the *MTHFR* variants or haplotypes with breast cancer risk. With 750 cases and 732 controls, this study had 80% power to detect an OR of ≥1.49 with an allele frequency of 0.10. As shown in Table 1, the detectable per-allele ORs for most of the SNPs were <1.5. We also did not observe any significant effect modification of risk by menopausal status.

In conclusion, our study used a broad coverage of the genetic variation in *MTHFR* by the use of haplotype-tagging SNPs chosen from the 65 variants identified during a gene resequencing study. No significant association was observed with any of the tagging SNPs or common *MTHFR* haplotypes after adjustment for multiple comparisons. These results suggest that genetic variation in *MTHFR*, independent of other factors, such as folate levels (which were not available in the current study), may not play a significant role in the development of breast cancer.

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