Influence of *Methylenetetrahydrofolate Reductase* Gene Polymorphisms C677T and A1298C on Age-Associated Risk for Colorectal Cancer in a Caucasian Lynch Syndrome Population

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Abstract

Lynch syndrome is caused by germ-line mutations in the DNA mismatch repair (MMR) genes; mutation carriers are predisposed to a variety of cancers, most commonly colorectal and endometrial. The median age of colorectal cancer onset is 45 years and the lifetime risk is ∼80%, but the onset age varies substantially. It is likely that other low-penetrance genes and environmental factors act as modifiers of the risk associated with the highly penetrant MMR gene mutations. Methylenetetrahydrofolate reductase plays a key role in folate metabolism. We investigated the association of C677T and A1298C, two common polymorphisms in the *methylenetetrahydrofolate reductase* gene, with risk for early onset colorectal cancer in Lynch syndrome. Subjects were 185 non-Hispanic whites with confirmed DNA MMR mutations. Kaplan-Meier estimates for the age at colorectal cancer onset according to C677T genotypes were significantly different for the CT and TT genotypes compared with the wild-type CC ($P = 0.014$, log-rank test; $P = 0.004$, trend test). The median ages at onset were 43 years for the CC genotype and 39 years for the combined CC and CT genotypes and the CC+CT genotypes were associated with a reduced age-associated risk for developing colorectal cancer (hazard ratio, 0.55; 95% confidence interval, 0.36-0.85). No differences in ages at onset or risk were found for the A1298C genotypes. This is the first report to our knowledge to provide evidence that the C677T polymorphism modifies the age at onset of colorectal cancer in Caucasian Lynch syndrome subjects with the *677T* allele having a protective effect. (Cancer Epidemiol Biomarkers Prev 2007;16(9):1753–9)

Introduction

Lynch syndrome is an autosomal dominant inherited disorder resulting from germ-line mutations in DNA mismatch repair (MMR) genes *MLH1, MSH2, MSH6*, or *PMS2* (1). It is also commonly known as hereditary nonpolyposis colorectal cancer (HNPCC), which may be a misnomer because cancer predisposition is not restricted to the colon. The term Lynch syndrome has been specifically referred to as individuals diagnosed based on an inherited germ-line mutation in a DNA MMR gene (2, 3) and has been used as such herein. Many types of cancer cluster in families with Lynch syndrome. The most common are colorectal and endometrial but also included in this syndrome are cancers of the stomach, ovary, uroepithelial tract, biliary tract, small intestine, and, rarely, certain cancers of the skin and brain (4). As their name suggests, MMR proteins recognize and repair errors, such as base mismatches and insertion/deletion loops that can arise during DNA replication and recombination and also repair some forms of DNA damage. Deficient DNA MMR can result in accumulation of mutations in cells leading to carcinogenesis (5). The median age at colorectal cancer diagnosis for Lynch syndrome individuals is 45 years and they carry a greatly elevated lifetime risk of ∼80% for colorectal cancer (6, 7). However, age at onset varies substantially and cannot be explained by the type of MMR mutation alone, suggesting that other factors, possibly environmental and genetic, may influence the risk for cancer at an early age.

Methylenetetrahydrofolate reductase (MTHFR) is a key folate-metabolizing enzyme involved in both DNA methylation and DNA synthesis. It catalyzes the methylation reactions of folate, a major methyl group donor and influences the availability of nucleotides for DNA synthesis and repair. MTHFR irreversibly converts 5,10-methylenetetrahydrofolate to its reduced form, 5-methyltetrahydrofolate (the main circulating form of folate) and directs the folate pool toward the remethylation of homocysteine to methionine, at the expense of thymidylate synthesis. Abnormalities in DNA methylation can result in altered gene expression and can

contribute to carcinogenesis (8). Alternatively, reduced availability of thymidylate results in the misincorporation of uracil into DNA and dsDNA breaks can occur during its repair by excision repair processes, which can be potentially carcinogenic (9). dsDNA breaks are commonly seen in colorectal cancer (10). *MTHFR* has two common single nucleotide polymorphisms (SNP) C677T and A1298C, which result in amino acid changes A222V and E429A, respectively. The polymorphisms are known to affect enzyme activity; for example, the *677T* allele results in a more thermolabile enzyme with reduced enzyme activity (30% enzyme activity with TT and 70% with CT compared with the wild-type (WT) CC; ref. 11). The evidence that the *1298C* allele may affect enzyme activity is not as conclusive, although the *1298C* allele has also been associated with low enzyme activity but not the decreased plasma folate or increased plasma homocysteine levels seen with 677T (12, 13).

SNPs in *MTHFR*, particularly C677T, have been widely studied as potential low-penetrance alleles that modify susceptibility to many cancers including sporadic colorectal cancer in the United States (14-20) as well as worldwide (21-35). It has also been suggested that *MTHFR* may modify risk for colorectal tumors with microsatellite instability (MSI[+]), mediated through aberrant CpG island promoter methylation of the *MLH1* gene (24). MSI[+] tumors are a hallmark of HNPCC, although in HNPCC, MSI is largely due to mutations in the MMR genes rather than CpG island methylation of the promoter region and gene silencing. A study by Plaschke et al. (26) examined the association of *MTHFR* and colorectal cancer risk by MSI status and did a subgroup analysis of 60 individuals with suspected or verified hereditary disease (based on patients fulfilling the Bethesda criteria at the time of colorectal cancer diagnosis) but did not find a difference in the C677T or A1298C genotype frequencies between the cases and controls by MSI status or by Bethesda-positive status. In terms of location in the colon, a majority of colon cancers in Lynch syndrome are in the proximal colon (1). Slattery et al. (15) found that the 677TT genotype was associated with reduced risk among individuals with proximal but not those with distal tumors, although these were not specified as sporadic or hereditary tumors. Additionally, on the premise that common low-penetrance genes could contribute to familial aggregation of colon cancer, two other studies by Slattery et al. (36) and Keku et al. (37) examined the prevalence of *MTHFR* and several other at-risk genotypes in persons with and without a family history of colon cancer but did not find any difference in the prevalence of *MTHFR* genotypes between the two groups. The above-mentioned studies examined association between *MTHFR* and markers that could be surrogates for Lynch syndrome−related colorectal cancer, such as MSI[+] tumors, tumors in the proximal colon, or colorectal cancer with positive family history. However, to our knowledge, our study is the first to provide evidence on the influence of *MTHFR* gene polymorphisms on risk of colorectal cancer in patients with Lynch syndrome.

Materials and Methods

Study Subjects. The subjects were 185 Caucasians with confirmed MMR gene mutations from The University of Texas M. D. Anderson Cancer Center's Hereditary Nonpolyposis Colorectal Cancer Registry; details of identification and recruitment of these individuals are described elsewhere (38, 39). Briefly, the Registry patients were from HNPCC or HNPCC-like families or patients who were <45 years of age at colorectal cancer diagnosis or were referred because of a known MMR gene mutation. Study participants included 81 probands and 104 family members (51% with colorectal cancer and 49% without colorectal cancer), who tested positive for an MMR gene mutation. Fifty-six individuals were singletons and the rest had one or more family members enrolled in the study. The proportion of women (52%) was greater than that of men (48%) but more men were colorectal cancer affected (62%) than women (42%). Age of onset was defined as the age at colorectal cancer diagnosis for the cancer cases. For the unaffected carriers, age (at censoring) was based on age at blood draw or age at last follow-up, whichever was later. Individuals who developed a colorectal adenoma or a cancer other than colorectal cancer were also censored at that age as, presumably, the diagnosis of an adenoma or any cancer would alter the course (and age at onset) of colorectal cancer due to factors such as increased screening. MMR mutations were determined at a laboratory certified by the Clinical Laboratory Improvement Act. Informed consent was obtained from all participants. Subject characteristics are summarized in Table 1.

Genotyping. DNA was extracted from 10 mL blood samples drawn from the study subjects in Vacutainer tubes containing EDTA (Becton Dickinson Vacutainer System) by using the AUTOPURE LS Automated DNA Purification Instrument (Gentra Systems, Inc.) according to the manufacturer's instructions. PCR and pyrosequencing were used to genotype the C677T (A222V) and A1298C (E429A) polymorphisms.

PCR fragments were generated by using 10 ng DNA in a 50-μL reaction mixture consisting of 50 mmol/L KCl; 10 mmol/L Tris-HCl (pH 8.3); 2.0 mmol/L MgCl$_2$; 0.125 mmol/L dATP, dCTP, dGTP, and dTTP; and 1.5 units AmpliTaq Gold DNA polymerase (Applied Biosystems). The PCR primers and reaction conditions are listed in Table 2.

For the pyrosequencing reactions, biotinylated PCR products were immobilized on streptavidin-coated Sepharose beads (Amersham Biosciences AB) in 96-well plates. The reaction mix in each well consisted of 2 μL streptavidin-coated Sepharose beads diluted with 38 μL binding buffer [10 mmol/L Tris-HCl, 2 mol/L NaCl, 1 mmol/L EDTA, 1% Tween 20 (pH 7.6)] and 35 μL high-purity water, to which was added 5 μL of PCR product. After vigorous mixing for 5 min, the samples were transferred to a filter plate and biotinylated ssDNA was isolated using 70% ethanol, denaturation solution (0.2 mol/L NaOH), and washing buffer [10 mmol/L Tris-acetate (pH 7.6)]. The ssDNA template was resuspended in a mixture containing 1.2 μL of 5 mmol/L sequencing primer (Table 2) and 10.8 μL of annealing buffer [20 mmol/L Tris-acetate, 2 mmol/L Mg acetate (pH 7.6)]. Sequencing primers were hybridized by incubation at 80°C for 2 min. The SNP sequencing analysis was run on a PSQ 96 System (Biotage, Inc.), with enzymes and reagents from a Biotage SNP Reagent kit, according to the manufacturer's instructions. As a quality

Table 1. Demographic and genetic characteristics of the study population

		Colorectal cancer, $n = 95$	No colorectal cancer, $n = 90$	Total, $N = 185$ (%)
Gender				
Male		55	34	89 (48.1)
Female		40	56	96 (51.9)
Age at onset* (y)				
Mean (SD)		41.7 (11.0)	42.7 (11.8)	42.2 (11.4)
Median		40	42	41
Range		23-75	18-77	18-77
MMR gene mutated				
MLH1		46	40	86 (46.5)
MSH2		49	50	99 (53.5)
MMR gene mutation type				
Missense[†]		21	19	40 (21.5)
Deletion/insertion/ nonsense/splice		74	71	145 (74.4)
MTHFR				
A1298C	AA	49	51	100 (54.0)
	AC	36	30	66 (35.7)
	CC	10	7	17 (9.2)
Unable to genotype				2 (1.1)
(Any C) AC+CC		46	37	83 (44.9)
C677T	CC	43	32	75 (40.5)
	CT	42	44	86 (46.5)
	TT	10	14	24 (13.0)
(Any T) CT+TT		52	58	110 (59.5)

*Age is mean age at colorectal cancer onset for individuals with colorectal cancer and mean age at censoring for unaffected MMR gene mutation carriers (subjects were censored at age at last blood draw or at last contact, or at diagnosis of colorectal adenoma or cancer other than colorectal cancer).

[†] These missense mutations were confirmed to be pathogenic mutations by a Clinical Laboratory Improvement Act–certified laboratory or from the International Collaborative Group-HNPCC InSiGHT database (48) or from the published literature (49, 50).

control measure, genotyping was repeated for 50% of the samples and complete concordance was found between the two sets of pyrosequencing results.

Statistical Analysis. The relationship between *MTHFR* polymorphisms and time to onset of colorectal cancer was assessed using survival analysis, with age at onset of colorectal cancer as the outcome variable and the MTHFR genotype as the exposure (independent) variable. The median age at onset was defined as the age at which 50% of the population remained cancer-free. The Kaplan-Meier product-limit estimator was used to plot time to onset, and the log-rank test was used to test for homogeneity of the survival curves by genotype for

each of the SNPs. Cox proportional hazards regression was used to calculate hazard ratios (HR) and 95% confidence intervals (95% CI) as risk estimates comparing the polymorphic genotypes with the WT genotype to test for differences in risk for colorectal cancer according to genotype. Gender, MMR gene mutated (*MLH1* or *MSH2*), and type of mutation (missense versus truncation/insertion/deletion) were adjusted for as potential confounding factors. To correct for any correlations in time to onset of colorectal cancer that may exist among family members, the Huber-White robust variance correction was applied. The proportional hazards assumption was tested by examining whether the Schoenfeld residuals correlated with age, but no departure from proportional hazards assumption was observed for either of the SNPs. The SNPs were also tested for Hardy-Weinberg equilibrium. Linkage disequilibrium between the two MTHFR SNPs was examined by using 2LD software (40); SAS statistical software version 9.1 (SAS Institute, Inc.) was used to construct haplotypes based on the known genotypes. The analysis for time to colorectal cancer onset and estimation of HRs as described above was repeated for the derived haplotypes. Stata 8.0 (Stata Statistical software, Stata Corp. LP) was used for all other statistical analyses.

Results

The genotypes for the *MTHFR* A1298C and C677T SNPs were distinguished by pyrosequencing, and the frequencies were found to be in Hardy-Weinberg equilibrium for each of the SNPs, both in the total sample (exact $P = 0.3$ and 1.0 for A1298C and C677T) and among colorectal cancer-affected ($P = 0.4$ and 1.0) and unaffected individuals ($P = 0.5$ and 1.0). The frequencies for the homozygous polymorphic genotypes were 9.2% CC and 13% TT. The allelic frequencies for C677T were similar to those reported for Caucasians in the SNP500 database (C = 63% in SNP500 versus 64% in our study and T = 37% versus 36%; ref. 41), but the allelic frequencies for A1298C were somewhat different (A = 63% in SNP500 versus 73% in our study and C = 37% versus 27% in our study). However, the A1298C allelic frequencies did not deviate significantly from other published reports that had a C allele frequency range of 29% to 36% (13, 16, 17, 19, 42). No difference was found in the genotypic frequencies between the colorectal cancer-affected and unaffected individuals for either the *MTHFR* SNP A1298C ($\chi^2 = 0.85$; $P = 0.6$) or C677T ($\chi^2 = 2.19$; $P = 0.33$).

The Kaplan-Meier survival curves for time to onset of colorectal cancer by genotype were statistically

**Table 2. Primers and conditions for PCR**

SNP	DbSNP ID	SNP region	Amino acid change	PCR and pyrosequencing primers	PCR conditions
A1298C	rs1801131	Ex8-62A>C	E429A	Biotinylated 5′-7AGGAGGAGCTGCGAAGATG (F) 5′-CCCCACTCCAGCATCACTCA (R) 5′-AACAAAGACTTCAAAGACAC (S)	Denaturing at 95°C for 6 min 45 cycles of 95°C for 15 s 62°C for 30 s
C677T	rs1801133	Ex5+79C>T	A222V	Biotinylated 5′-7ATCCCTCGCCTTGAACAGGT (F) 5′-TCGGTGCATGCCTTCCACAA (R) 5′-CGTGATGATGAAATCG (S)	72°C for 15 s Extension at 72°C for 6 min

Abbreviations: F, forward; R, reverse; S, sequencing.

significantly different for C677T [χ^2=8.49 (2 degrees of freedom); P = 0.01, log-rank test] but not for A1298C [χ^2=1.86 (2 degrees of freedom); P = 0.39, log-rank test]. Kaplan-Meier curves for C677T are shown in Fig. 1. For C677T, the median age at colorectal cancer onset among those homozygous for the WT C allele (CC genotype) was 4 years younger (39 years) than that for carriers of the T allele (CT and TT genotypes) whose median age of onset was 43 years. The Kaplan-Meier estimates for age at onset of colorectal cancer did not differ by MMR gene mutated (*MLH1* versus *MLH2*), type of MMR mutation (missense mutations versus others), or gender for the main effects of each of these variables (P > 0.05, log-rank test). However, on stratifying by MMR gene mutated, the difference in age at onset by *MTHFR* C677T genotype was significant only among the *MLH1* mutation carriers (P = 0.02) but not the *MSH2* mutation carriers (P = 0.19). Among the *MLH1* mutation carriers, the median ages at onset for the CC, CT, and TT genotypes were 38.5, 44, and 48 years, respectively (44.5 years for CT and TT combined). Kaplan-Meier plots comparing time with onset for colorectal cancer by C677T genotypes, stratified by *MLH1* and *MSH2* mutation carriers, are presented in Fig. 2. No significant difference was found in median age at onset by genotype for A1298C.

Using Cox proportional hazards regression analysis to estimate the association between the *MTHFR* genotypes and age at onset of colorectal cancer, we found that for *MTHFR* C677T, subjects with the CT and TT genotypes had a lower risk of colorectal cancer in any age interval than those with the homozygous WT genotype CC. Specifically, the HR for the CT genotype was 0.61 (95% CI, 0.40-0.94; P = 0.024) and the HR for the TT genotype was 0.42 (95% CI, 0.21-0.87; P = 0.019), with CC as the referent. A test for linear trend to examine a dose effect of the T allele was statistically significant (P = 0.004). Further, the HR for the combined CT and TT genotypes (with CC as the referent) was 0.57 (95% CI, 0.37-0.87; P = 0.009). Adjusting the risk estimates for gender, MMR gene mutated, and MMR mutation type in the Cox model did not appreciably alter the HRs (Table 3). The A1298C *MTHFR* genotypes were not associated with risk of colorectal cancer. In combined analysis of the A1298C with the C677T genotypes, no significant change was found in the HRs obtained by univariate analysis for each of the SNPs (data not shown).

As established in other studies (13, 17), the two SNPs were in significant linkage disequilibrium (D' = 0.95; R^2 = 0.19; P < 0.0001). The *MTHFR* haplotypes derived from the 677C/677T and 1298A/1298C alleles were 677C-1298A (frequency, 36.5%), 677C-1298C (frequency, 27.3%), 677T-1298A (frequency, 35.9%), and only one 677T-1298C (frequency, 0.3%). The age at onset of colorectal cancer was significantly different among these four haplotypes (P = 0.028 and 0.018 if T-C, the rare haplotype was omitted, log-rank test). Using the haplotype constituted by the WT allele of both SNPs, 677C-1298A, as the referent, we found a decreased risk of colorectal cancer associated with the 677T-1298A haplotype (HR, 0.64; 95% CI, 0.46-0.89; P = 0.008) but no difference in risk for the 677C-1298C haplotype (Table 3). Any risk estimate for 677T-1298C haplotype, polymorphic for both alleles, would not be meaningful as it was found in only one individual.
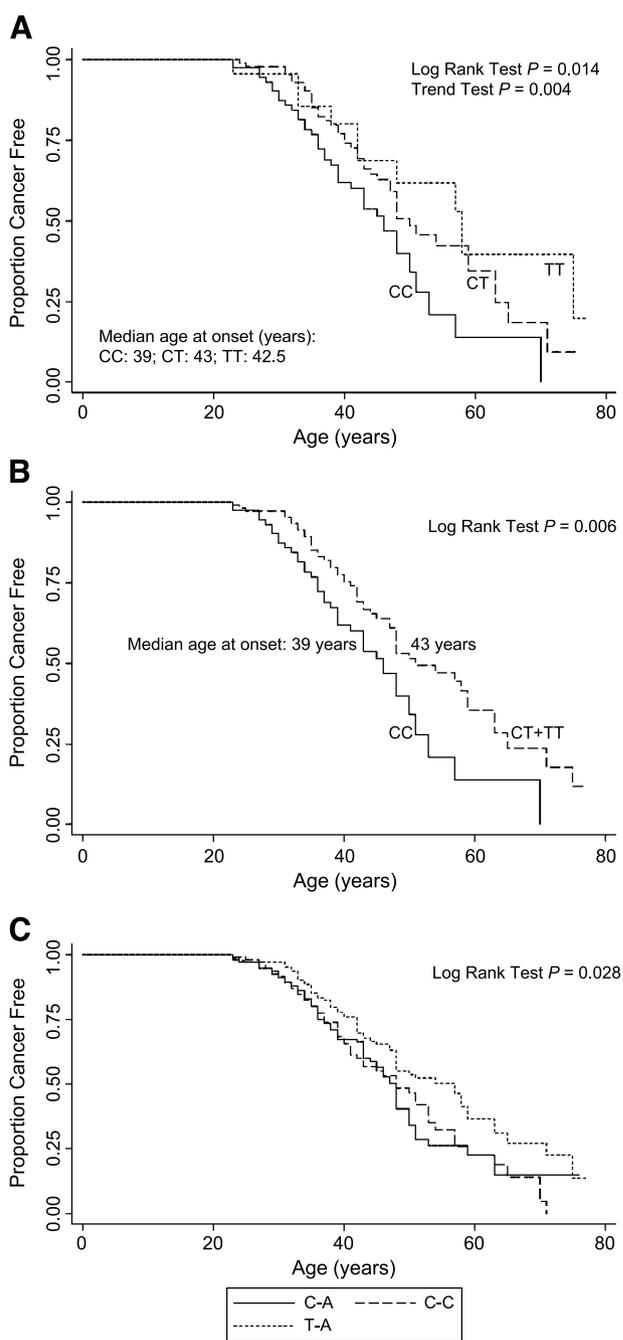
Figure 1. Kaplan-Meier survival plots comparing time to onset of colorectal cancer according to the three MTHFR C677T genotypes CC, CT, and TT (**A**); according to CT+TT versus CC (**B**); and according to haplotypes C-A, C-C, and T-A, derived from C677T and A1298C (**C**). Haplotype T-C was omitted in (**C**) because it was present in only one subject.

Discussion

Our findings suggest that Caucasian individuals with Lynch syndrome, carrying an inherited MMR gene mutation and the WT genotype (CC) for a common polymorphism in *MTHFR* C677T, develop colorectal

cancer earlier than individuals with one or two copies of the polymorphic 677T allele. Our findings on the association of this polymorphism with reduced age-associated risk of colorectal cancer in a Lynch syndrome population are consistent with its well-documented protective effect in sporadic colorectal cancer populations. A recent meta-analysis by Hubner and Houlston (43), examining *MTHFR* C677T and risk of sporadic colorectal cancer in 25 independent populations, concluded that compared with CC, the TT genotype was associated with a reduced risk of colorectal cancer (odds ratio, 0.83; 95% CI, 0.75-0.93; $P = 0.001$), although the heterozygous CT genotype did not seem to influence colorectal cancer risk. Another meta-analysis by Huang et al. (44) on 20 worldwide studies similarly found a protective effect for the 677T allele compared with the 677C allele (odds ratio, 0.93; 95% CI, 0.89-0.98; $P = 0.0003$). They also reported a significantly decreased risk of colorectal cancer for the 1298C allele among Caucasians (odds ratio, 0.81; 95% CI, 0.57-0.99; $P = 0.04$) based on a smaller number of studies that had examined A1298C. In addition to influencing risk for sporadic colorectal cancer as evidenced in the aforementioned studies, our study shows that *MTHFR* C677T can also modify the effect of the highly penetrant gene mutations in *MLH1* and *MSH2* on age-associated risk of colorectal cancer in Lynch syndrome.

A possible link between MTHFR and carcinogenesis has been postulated through the pathways of DNA methylation and DNA damage. MTHFR regulates folate metabolism by directing folate groups toward these two important pathways. On the one hand, the MTHFR substrate 5,10-methylenetetrahydrofolate is required for thymidine synthesis, which is vital for DNA synthesis; on the other, MTHFR irreversibly converts 5,10-methylenetetrahydrofolate to 5-methyltetrahydrofolate, the main circulating form of folate, which provides methyl groups for synthesis of methionine subsequently used for DNA methylation. The reduced availability of thymidylate results in misincorporation of uracil into DNA, the repair of which can result in dsDNA breaks. The reduced enzyme activity associated with the T allele results in increased availability of 5,10-methylenetetrahydrofolate for DNA synthesis and thus may reduce the likelihood of uracil misincorporation in DNA and the consequent dsDNA breaks during its repair. This effect in the background of impaired MMR can be hypothesized to be even more deleterious in Lynch syndrome than in subjects with normal MMR and could explain the increased risk associated with the higher *MTHFR* activity in carriers of the 677CC genotype. In addition, reduced capacity for DNA methylation may also mediate the protective effect associated with the 677T allele because hypermethylation of the promoter region of certain genes (including *MLH1*, and tumor suppressor genes like *p16*) result in their silencing and potential carcinogenesis. It has been estimated that ~10% of sporadic colorectal cancer cases have *MLH1*-deficient MSI-high tumors, as a result of gene silencing of *MLHI* by hypermethylation of its promoter region (45). Promoter hypermethylation may also be involved in Lynch syndrome where it may act by silencing the second allele of the inherited germline mutation (46). Our results showing the predominant contribution of MLH1 mutation carriers to the difference in age at onset for colorectal cancer by the C677T genotype lends support to the argument that the protective mechanism may be driven at least in part by the reduced methylation capacity associated with the variant 677T allele resulting in decreased or delayed promoter hypermethylation of MLH1.

Our findings did not indicate any association between the *MTHFR* A1298C polymorphism and age at onset of colorectal cancer in the study subjects. The influence of this SNP seems to be weaker than that of C677T in modulating risk of colorectal cancer, although most of the studies that did find an association described a decreased risk associated with the 1298C allele (13, 16, 17, 20, 26). The hypothesis that decreased risk is associated with the 1298C allele was supported by a recent meta-analysis (44). Plaschke et al. (26) found an increased frequency of 1298AA+AC genotypes among 60 cases of suspected or verified hereditary disease (subjects that met the Bethesda guidelines but were not confirmed MMR gene mutation carriers) compared with 346 controls but only 3 cases had the 1298CC genotype and the odds ratios were not statistically significant. Our study population was larger ($n = 195$) and consisted of confirmed carriers of inherited MMR gene mutations and
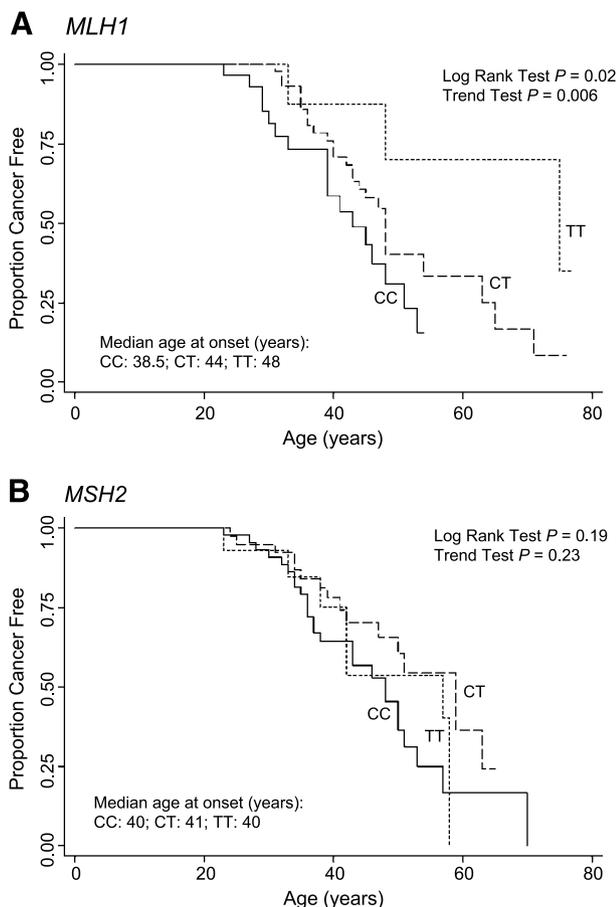
Figure 2. Kaplan-Meier survival plots comparing time to onset of colorectal cancer according to the three *MTHFR* C677T genotypes CC, CT, and TT stratified by the MMR gene mutated: *MLH1* (**A**); *MSH2* (**B**).

Table 3. MTHFR genotype and colorectal cancer risk in MMR gene mutation carriers

MTHFR		HR (95% CI) univariate	P	HR (95% CI) adjusted*	P
Polymorphism	Genotype [†]				
C677 T	CC	1.0 (Referent)		1.0 (Referent)	
	CT	0.61 (0.40-0.94)	0.024	0.59 (0.38-0.91)	0.018
	TT	0.42 (0.21-0.87)	0.019	0.44 (0.22-0.88)	0.021
(Any variant, T) CT+TT		0.57 (0.37-0.87)	0.009	0.55 (0.36-0.85)	0.007
A1298C	AA	1.0 (Referent)		1.0 (Referent)	
	AC	1.27 (0.88-1.83)	0.20	1.26 (0.87-1.82)	0.22
	CC	1.44 (0.76-2.70)	0.26	1.41 (0.78-2.56)	0.25
(Any variant, C) AC+CC		1.30 (0.92-1.85)	0.14	1.29 (0.92-1.81)	0.14
Haplotypes					
n = 146	677C-1298A	1.0 (Referent)		1.0 (Referent)	
103	677C-1298C	0.97 (0.72-1.31)	0.86	0.97 (0.72-1.30)	0.84
140	677T-1298A	0.64 (0.46-0.89)	0.008	0.65 (0.47-0.90)	0.009
1	677T-1298C	2.31 (1.66-3.20)	0.000	2.1 (1.43-3.0)	0.000

NOTE: HRs are corrected for any familial correlation in ages of onset by applying robust variance correction.
*Adjusted for gender, MMR gene mutated, and MMR mutation type.
[†]Genotypes are listed in the following order: homozygous WT, heterozygous, and homozygous polymorphic.

did not suggest any differences by A1298C genotype between the colorectal cancer-affected and unaffected groups.

In combined analysis of the two SNPs and the haplotype analysis, the alteration in age-associated risk seems to be mediated largely by the 677T allele of the C677T polymorphism, as evidenced by the decreased risk for time to colorectal cancer onset associated with the 677 polymorphic 1298 WT haplotype, 677T-1298A (HR, 0.64; 95% CI, 0.46-0.89; P = 0.008), but no difference in risk for the 677 WT-1298 polymorphic haplotype, 677C-1298C (HR, 0.97; 95% CI, 0.72-1.31; P = 0.86), compared with the WT haplotype for both SNPs, 677C-1298A.

A potential limitation of our study is that it is clinic based, as the probands in the HNPCC Registry were recruited through the patients seen in the Gastrointestinal Medicine and Nutrition Clinic at M. D. Anderson Cancer Center and therefore may not be truly representative of the general population of MMR gene mutation carriers as would be seen with a population-based study. However, because the subjects were recruited without any knowledge of their SNP genotypes, therefore, no selection bias has occurred with regards to the exposure variables that are the focus of our analysis. In addition, to address any possible ascertainment bias due to the hospital identified probands, we assessed genotypic effects in the family members alone, excluding the probands and found that the median ages of onset were 37 and 41.5 years for the CC and CT+TT genotypes, which is consistent with the later age at onset associated with the CT+TT genotypes for the group overall. We restricted our analyses to Caucasians to avoid heterogeneity due to race and because a majority (83%) of the individuals in the M. D. Anderson HNPCC Registry are non-Hispanic whites. It is possible that results may differ in other race/ethnicities.

Imbalances in folate and methyl group metabolism may be attributable to dietary causes as well as genetic predisposition; recent studies have shown that the influence of the *MTHFR* polymorphism may differ by the levels of folate or B vitamins or by alcohol intake (14, 16-19, 25, 29, 31-35). Folate levels in particular have been consistently inversely associated with colorectal cancer risk. This has been postulated to be due to the development of genomic global hypomethylation as a result of folate deficiency (47). Therefore, another limitation of our study is that we could not examine folate status and other dietary and lifestyle exposures. However, as additional data for the study participants becomes available through expanded data collection, these issues could be addressed in future studies.

In conclusion, this is the first report, to our knowledge, to provide evidence that the *MTHFR* C677T polymorphism, widely known to influence risk of sporadic colorectal cancer, also modifies risk of a hereditary form of cancer (i.e., colorectal cancer in Lynch syndrome). Future studies will be important to validate the findings of our studies. As the evidence increases for the role of polymorphisms that may influence the age-associated risk of colorectal cancer in these high-risk individuals, it may be possible to develop a genetic signature (of markers) to help identify individuals likely to develop cancer at a younger age. Further, an understanding of the underlying biology of the mechanism of the genetic variation along with the role of dietary, lifestyle, and environmental factors may aid in individualized risk prediction for cancer susceptibility, which has implications for tailored prevention in the evolving era of personalized medicine.

References

1. Lynch HT, de la Chapelle A. Hereditary colorectal cancer. N Engl J Med 2003;348:919–32.
2. Boland CR. Evolution of the nomenclature for the hereditary colorectal cancer syndromes. Fam Cancer 2005;4:211–8.
3. Lindor NM, Petersen GM, Hadley DW, et al. Recommendations for the care of individuals with an inherited predisposition to Lynch syndrome: a systematic review. JAMA 2006;296:1507–17.
4. Mecklin JP, Jarvinen HJ. Tumor spectrum in cancer family syndrome (hereditary nonpolyposis colorectal cancer). Cancer 1991;68:1109–12.
5. Peltomaki P. Deficient DNA mismatch repair: a common etiologic factor for colon cancer. Hum Mol Genet 2001;10:735–40.

6. Aaltonen LA, Salovaara R, Kristo P, et al. Incidence of hereditary nonpolyposis colorectal cancer and the feasibility of molecular screening for the disease. N Engl J Med 1998;338:1481–7.
7. Samowitz WS, Curtin K, Lin HH, et al. The colon cancer burden of genetically defined hereditary nonpolyposis colon cancer. Gastroenterology 2001;121:830–8.
8. Choi SW, Mason JB. Folate status: effects on pathways of colorectal carcinogenesis. J Nutr 2002;132:2413–8S.
9. Blount BC, Mack MM, Wehr CM, et al. Folate deficiency causes uracil misincorporation into human DNA and chromosome breakage: implications for cancer and neuronal damage. Proc Natl Acad Sci USA 1997;94:3290–5.
10. Vogelstein B, Fearon ER, Kern SE, et al. Allelotype of colorectal carcinomas. Science 1989;244:207–11.
11. 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.
12. 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.
13. Chen J, Ma J, Stampfer MJ, Palomeque C, Selhub J, Hunter DJ. Linkage disequilibrium between the 677C>T and 1298A>C polymorphisms in human methylenetetrahydrofolate reductase gene and their contributions to risk of colorectal cancer. Pharmacogenetics 2002;12:339–42.
14. Chen J, Giovannucci E, Kelsey K, et al. A methylenetetrahydrofolate reductase polymorphism and the risk of colorectal cancer. Cancer Res 1996;56:4862–4.
15. Slattery ML, Potter JD, Samowitz W, Schaffer D, Leppert M. Methylenetetrahydrofolate reductase, diet, and risk of colon cancer. Cancer Epidemiol Biomarkers Prev 1999;8:513–8.
16. Keku T, Millikan R, Worley K, et al. 5,10-Methylenetetrahydrofolate reductase codon 677 and 1298 polymorphisms and colon cancer in African Americans and whites. Cancer Epidemiol Biomarkers Prev 2002;11:1611–21.
17. Curtin K, Bigler J, Slattery ML, Caan B, Potter JD, Ulrich CM. MTHFR C677T and A1298C polymorphisms: diet, estrogen, and risk of colon cancer. Cancer Epidemiol Biomarkers Prev 2004;13:285–92.
18. Le Marchand L, Wilkens LR, Kolonel LN, Henderson BE. The MTHFR C677T polymorphism and colorectal cancer: the multiethnic cohort study. Cancer Epidemiol Biomarkers Prev 2005;14:1198–203.
19. Koushik A, Kraft P, Fuchs CS, et al. Nonsynonymous polymorphisms in genes in the one-carbon metabolism pathway and associations with colorectal cancer. Cancer Epidemiol Biomarkers Prev 2006;15:2408–17.
20. Le Marchand L, Donlon T, Hankin JH, Kolonel LN, Wilkens LR, Seifried A. B-vitamin intake, metabolic genes, and colorectal cancer risk (United States). Cancer Causes Control 2002;13:239–48.
21. Park KS, Mok JW, Kim JC. The 677C > T mutation in 5,10-methylenetetrahydrofolate reductase and colorectal cancer risk. Genet Test 1999;3:233–6.
22. Ryan BM, Molloy AM, McManus R, et al. The methylenetetrahydrofolate reductase (MTHFR) gene in colorectal cancer: role in tumor development and significance of allelic loss in tumor progression. Int J Gastrointest Cancer 2001;30:105–11.
23. Sachse C, Smith G, Wilkie MJ, et al. A pharmacogenetic study to investigate the role of dietary carcinogens in the etiology of colorectal cancer. Carcinogenesis 2002;23:1839–49.
24. Shannon B, Gnanasampanthan S, Beilby J, Iacopetta B. A polymorphism in the methylenetetrahydrofolate reductase gene predisposes to colorectal cancers with microsatellite instability. Gut 2002;50:520–4.
25. Heijmans BT, Boer JM, Suchiman HE, et al. A common variant of the methylenetetrahydrofolate reductase gene (1p36) is associated with an increased risk of cancer. Cancer Res 2003;63:1249–53.
26. Plaschke J, Schwanebeck U, Pistorius S, Saeger HD, Schackert HK. Methylenetetrahydrofolate reductase polymorphisms and risk of sporadic and hereditary colorectal cancer with or without microsatellite instability. Cancer Lett 2003;191:179–85.
27. Pufulete M, Al Ghnaniem R, Leather AJ, et al. Folate status, genomic DNA hypomethylation, and risk of colorectal adenoma and cancer: a case control study. Gastroenterology 2003;124:1240–8.
28. Toffoli G, Gafa R, Russo A, et al. Methylenetetrahydrofolate reductase 677 C→T polymorphism and risk of proximal colon cancer in north Italy. Clin Cancer Res 2003;9:743–8.
29. Kim DH, Ahn YO, Lee BH, Tsuji E, Kiyohara C, Kono S. Methylenetetrahydrofolate reductase polymorphism, alcohol intake, and risks of colon and rectal cancers in Korea. Cancer Lett 2004;216:199–205.
30. Ulvik A, Vollset SE, Hansen S, Gislefoss R, Jellum E, Ueland PM. Colorectal cancer and the methylenetetrahydrofolate reductase 677C→ T and methionine synthase 2756A→ G polymorphisms: a study of 2,168 case-control pairs from the JANUS cohort. Cancer Epidemiol Biomarkers Prev 2004;13:2175–80.
31. Yin G, Kono S, Toyomura K, et al. Methylenetetrahydrofolate reductase C677T and A1298C polymorphisms and colorectal cancer: the Fukuoka Colorectal Cancer Study. Cancer Sci 2004;95:908–13.
32. Jiang Q, Chen K, Ma X, et al. Diets, polymorphisms of methylenetetrahydrofolate reductase, and the susceptibility of colon cancer and rectal cancer. Cancer Detect Prev 2005;29:146–54.
33. Matsuo K, Ito H, Wakai K, et al. One-carbon metabolism related gene polymorphisms interact with alcohol drinking to influence the risk of colorectal cancer in Japan. Carcinogenesis 2005;26:2164–71.
34. Otani T, Iwasaki M, Hanaoka T, et al. Folate, vitamin B6, vitamin B12, and vitamin B2 intake, genetic polymorphisms of related enzymes, and risk of colorectal cancer in a hospital-based case-control study in Japan. Nutr Cancer 2005;53:42–50.
35. Wang J, Gajalakshmi V, Jiang J, et al. Associations between 5,10-methylenetetrahydrofolate reductase codon 677 and 1298 genetic polymorphisms and environmental factors with reference to susceptibility to colorectal cancer: a case-control study in an Indian population. Int J Cancer 2006;118:991–7.
36. Slattery ML, Edwards SL, Samowitz W, Potter J. Associations between family history of cancer and genes coding for metabolizing enzymes (United States). Cancer Causes Control 2000;11:799–803.
37. Keku TO, Millikan RC, Martin C, Rahkra-Burris TK, Sandler RS. Family history of colon cancer: what does it mean and how is it useful? Am J Prev Med 2003;24:170–6.
38. Frazier ML, O'Donnell FT, Kong S, et al. Age-associated risk of cancer among individuals with N-acetyltransferase 2 (NAT2) mutations and mutations in DNA mismatch repair genes. Cancer Res 2001;61:1269–71.
39. Chen J, Sen S, Amos CI, et al. Association between Aurora-A kinase polymorphisms and age of onset of hereditary nonpolyposis colorectal cancer in a Caucasian population. Mol Carcinog 2007.
40. Zhao JH. 2LD, GENECOUNTING, and HAP: computer programs for linkage disequilibrium analysis. Bioinformatics 2004;20:1325–6.
41. SNP500Cancer [database on the Internet]. Bethesda (MD): National Cancer Institute Cancer Genome Anatomy Project. [cited 2007 May 30] Available from: http://snp500cancer.nci.nih.gov/.
42. Database of single nucleotide polymorphisms (dbSNP) [database on the Internet]. Bethesda (MD): National Center for Biotechnology Information, National Library of Medicine. (dbSNP Build ID: {build ID}). [cited 2007 May 30]. Available from: http://www.ncbi.nlm.nih.gov/SNP/.
43. Hubner RA, Houlston RS. MTHFR C677T and colorectal cancer risk: a meta-analysis of 25 populations. Int J Cancer 2007;120:1027–35.
44. Huang Y, Han S, Li Y, Mao Y, Xie Y. Different roles of MTHFR C677T and A1298C polymorphisms in colorectal adenoma and colorectal cancer: a meta-analysis. J Hum Genet 2007;52:73–85.
45. Cunningham JM, Christensen ER, Tester DJ, et al. Hypermethylation of the hMLH1 promoter in colon cancer with microsatellite instability. Cancer Res 1998;58:3455–60.
46. Herman JG, Umar A, Polyak K, et al. Incidence and functional consequences of hMLH1 promoter hypermethylation in colorectal carcinoma. Proc Natl Acad Sci USA 1998;95:6870–5.
47. Sharp L, Little J. Polymorphisms in genes involved in folate metabolism and colorectal neoplasia: a HuGE review. Am J Epidemiol 2004;159:423–43.
48. InSiGHT mutation database [database on the Internet]: International Society for Hereditary Gastrointestinal Tumours [cited 2007 May 30]. Available from: http://www.insight-group.org/.
49. Raevaara TE, Korhonen MK, Lohi H, et al. Functional significance and clinical phenotype of nontruncating mismatch repair variants of MLH1. Gastroenterology 2005;129:537–49.
50. Lagerstedt RK, Liu T, Vandrovcova J, et al. Lynch syndrome (hereditary nonpolyposis colorectal cancer) diagnostics. J Natl Cancer Inst 2007;99:291–9.

Corrections

In an article in the September 2007 issue (1), an error occurred in the reporting of results in one sentence of the abstract.

The fifth sentence should read as follows.

''The median ages at onset were <u>39</u> years for the CC genotype and <u>43</u> years for the combined <u>CT</u> and <u>TT</u> genotypes and the <u>CT+TT</u> genotypes were associated with a reduced age-associated risk for developing colorectal cancer (hazard ratio, 0.55; 95% confidence interval, 0.36-0.85).''

The changes are underlined.

Reference

1. Pande M, et al. Influence of *Methylenetetrahydrofolate Reductase* gene polymorphisms C677T and A1298C on age-associated risk for colorectal cancer in a Caucasian Lynch Syndrome population. Cancer Epidemiol Biomarkers Prev 2007;16:1753–9.

Influence of *Methylenetetrahydrofolate Reductase* Gene Polymorphisms C677T and A1298C on Age-Associated Risk for Colorectal Cancer in a Caucasian Lynch Syndrome Population

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