5,10-Methylenetetrahydrofolate Reductase Polymorphisms and the Risk of Pancreatic Cancer

Donghui Li,[1] Maha Ahmed,[1] Yanan Li,[1] Li Jiao,[1] Ta-Hsu Chou,[1] Robert A. Wolff,[1] Renato Lenzi,[1] Douglas B. Evans,[2] Melissa L. Bondy,[3] Peter W. Pisters,[2] James L. Abbruzzese,[1] and Manal M. Hassan[1]

Departments of [1]Gastrointestinal Medical Oncology, [2]Surgical Oncology, and [3]Epidemiology, The University of Texas M.D. Anderson Cancer Center, Houston, Texas

Abstract

To test the hypothesis that 5,10-methylenetetrahydrofolate reductase (MTHFR) polymorphisms modify the risk of pancreatic cancer, we conducted a hospital-based, case-control study involving 347 patients with newly diagnosed pancreatic adenocarcinoma and 348 healthy controls, frequency matched by age, sex, and race. *MTHFR* polymorphisms were determined using the PCR-RFLP method. Association of these polymorphisms with the risk of pancreatic cancer was estimated by unconditional logistic regression analysis. We found that the C667T (but not the A1298C) polymorphism had a significant main effect on the risk of pancreatic cancer. The frequencies of the *MTHFR* 667CC, 667CT, and 667TT genotypes were 49.5%, 38.6%, and 11.9%, respectively, among cases compared with 48.5%, 45.0%, and 6.5%, respectively, among controls. Individuals with the 667TT genotype displayed a 2-fold increased risk for pancreatic cancer compared with those with the CC/CT genotypes [adjusted odds ratio (OR), 2.14; 95% confidence interval (95% CI), 1.14-4.01]. Multivariate analyses found that the effect of the 677TT genotype on the risk of pancreatic cancer was present among ever smokers (OR, 5.53; 95% CI, 2.0-15.3) and ever alcohol drinkers (OR, 3.16; 95% CI, 1.30-7.69) but not in never smokers (OR, 0.82; 95% CI, 0.33-2.06) and never drinkers (OR, 1.42; 95% CI, 0.56-3.62). Furthermore, a positive interaction between the *MTHFR* TT genotype and heavy smoking or heavy alcohol consumption was detected. The OR (95% CI) of pancreatic cancer was 6.83 (1.91-24.38) for heavy smokers among the TT carriers compared with never smokers with the CC/CT genotypes and 4.23 (0.88-20.3) for heavy drinkers with the TT genotype compared with non-drinkers with the CC/CT genotypes. These observations support a role for folate metabolism in pancreatic cancer, especially among smokers and heavy drinkers. (Cancer Epidemiol Biomarkers Prev 2005;14(6):1470–6)

Introduction

Pancreatic cancer is the fourth leading cause of cancer death in this country, and each year, ~30,000 Americans die of the disease (1). In this deadly disease, the mortality rate approximately equals the incidence rate. The etiology of pancreatic cancer is poorly understood (2). High-risk populations include those with a family history of pancreatic cancer (3). Several familial syndromes with known genetic defects have been implicated, but they account for <5% of the total cases (4). The risk factors most consistently established by epidemiologic studies are age and cigarette smoking (2, 5). The protective role of a diet high in fruits and vegetables, vitamin C, and fiber has been shown in pancreatic cancer (2, 5). However, it remains unknown whether (or which) specific nutrients in fruits and vegetables account for this association.

There is growing evidence that mild folate deficiency (a low normal level) is associated with an increased risk of developing certain cancers, including in particular, colorectal cancer (6). However, the association between folate status and risk for pancreatic cancer is not clear. A cohort study of male Finnish smokers has shown a significant inverse association between the risk of pancreatic cancer and the dietary folate intake and serum folate level (7, 8). In other case-control studies, the results are equivocal, one study showed an inverse association between folate intake and pancreatic cancer (9) and the other one found no association (10). A recent report from the Nurses' Health Study and the Health Professionals Follow-up Study also failed to show a strong association between energy-adjusted dietary folate intake and risk for pancreatic cancer (11).

Evidence from both experimental and epidemiologic studies support the hypothesis that folate maintains DNA stability and prevents cancer (12). There are two mechanisms by which folate deficiency (caused either by low intake or abnormal metabolism) could affect the stability of DNA and increase the risk of malignancy (12). The first mechanism is through altered DNA methylation. The 5-methyl tetrahydrofolate (5-methyl THF) serves as a methyl donor in the remethylation of homocysteine to methionine, which in turn is converted into *S*-adenosylmethionine. *S*-adenosylmethionine is a universal methyl donor in the methylation of DNA, RNA, and protein. As a consequence of folate deficiency, 5-methyl THF and *S*-adenosylmethionine are depleted, which in turn induces DNA hypomethylation and oncogene activation. The second mechanism through which folate affects DNA stability is alteration of DNA synthesis and DNA repair. 5,10-Methylenetetrahydrofolate (5,10-methylene THF) serves as a methyl donor for the conversion of uracil to thymine, which is required for DNA synthesis and repair. In folate deficiency, uracil may misincorporate into DNA, which may lead to gene mutation or DNA strand breaks (13).

Folate status is determined by both dietary folate intake and folate metabolism. Defects in folate metabolism have been linked to risk of a wide range of adverse health conditions (e.g., birth defects, cardiovascular disease, and cancer; ref. 14). Numerous genes involved in the folate metabolism pathway and 5,10-methylenetetrahydrofolate reductase (*MTHFR*) is the most extensively studied gene among all. MTHFR acts at a critical juncture in folate metabolism by catalyzing the irreversible conversion of 5,10-methylene THF to 5-methyl

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Requests for reprints: Donghui Li, Department of Gastrointestinal Medical Oncology, Unit 426, The University of Texas M.D. Anderson Cancer Center, 1515 Holcombe Boulevard, Houston, TX 77030. Phone: 713-792-7493; Fax: 713-563-1195. E-mail: dli@mdanderson.org

THF, thereby directing folate metabolites towards the DNA methylation pathway and away from the DNA synthesis pathway. Two significant functional polymorphisms of the *MTHFR* gene, C677T and A1298C, have been identified. The 677C-to-T transition causes an alanine-to-valine substitution in the MTHFR protein, with subsequent reduction in the enzyme activity and increase in its thermolability (15). Relative to the specific activity of MTHFR in the normal CC genotype, that of the homozygous TT genotype is reduced by ~70%, and that of the heterozygous CT genotype is reduced by ~35% (15). Enzyme activity is also decreased in *MTHFR* 1298CC homozygotes and, to a lesser extent, in the AC heterozygotes and in compound C677T and A1298C heterozygotes (16). The TT genotype has been observed to increase plasma homocysteine levels in association with low folate intake and low level of serum folate (17, 18).

The potential influence of MTHFR activity on the metabolism of methyl groups, DNA methylation, and the availability of uridylates and thymidylates for DNA synthesis and repair makes *MTHFR* attractive as a candidate cancer-modifying gene. Molecular epidemiologic studies have revealed that *MTHFR* polymorphisms are associated with an increased risk of esophageal cancer (19), gastric cancer (20), breast cancer (21), hepatocellular carcinoma (22), cervical neoplasia (23), bladder cancer (24), and indolent prostate cancer (25). Conversely, *MTHFR* polymorphisms have also been associated with a reduced risk of colon cancer (26-28), leukemia (29), lymphoma (30), and highly aggressive prostate cancer (25). In addition, no association has been found between the C677T variant and colon adenoma (31) and lung cancer (32). The organ site-specific effects of MTHFR on risk of cancer may be related to the tissue specific distribution of folate and different etiologic factors involved in various types of cancers. For example, the effects of *MTHFR* genotype seem to depend on nutrient status in colon cancer. The 677TT genotype was associated with a reduced risk of colon cancer among individuals with adequate folate intake or serum folate levels but an increased risk of colon cancer in those with low folate intake or serum folate levels (26, 27, 33). In certain types of cancers (e.g., bladder, breast, and liver cancer), the increased risk by *MTHFR* genotype was associated with cigarette smoking and alcohol consumption (21, 22, 24).

To the best of our knowledge, no study has evaluated the role of MTHFR in pancreatic cancer in a U.S. population. We hypothesized that polymorphisms of the *MTHFR* gene that result in reduced enzyme activity may modify the risk of pancreatic cancer. We tested this hypothesis in a case-control study. The results showed an increased risk of pancreatic cancer in individuals with the *MTHFR* 667TT genotype in general and in heavy smokers and heavy alcohol drinkers in particular.

Materials and Methods

Study Subjects. This hospital-based, case-control study enrolled 347 patients with newly diagnosed, pathologically confirmed pancreatic adenocarcinoma and 348 healthy controls at the University of Texas M.D. Anderson Cancer Center from 2000 to 2004. The controls were healthy, genetically unrelated family members, spouses, and friends of patients who had various types of cancers other than pancreatic. Cases were recruited from the Gastrointestinal Clinic and controls were recruited from the Diagnostic Radiology Clinic. The cases and controls were frequency-matched by age (±5 years), sex, and race. The study was approved by the University of Texas M.D. Anderson Cancer Center Institutional Review Board. An informed consent was obtained from each study participant for an interview and a blood sample. Each eligible subject was interviewed to collect demographic data and information on smoking history, alcohol (ethanol) consumption, exposure history, medical history, and other risk factors. Those who had smoked >100 cigarettes in their lifetime were defined as ever smokers. Those who had consumed six alcoholic drinks per month for 6 months during their lifetime were defined as ever drinkers. The frequency of each alcoholic beverage consumed and the alcohol content of the specified portion size was used to estimate the weekly ethanol intake (g). A 12-oz can or bottle of beer, a 4-oz glass of wine, and a standard drink of liquor were considered equivalent to ~12.0 g of ethanol (34). A blood sample was collected from each study participant.

DNA Isolation and Genotyping. DNA was extracted from peripheral mononuclear cells using the FlexiGen DNA kit (Qiagen, Valencia CA). The DNA was kept at −70°C until used for genotyping.

For *MTHFR* C677T and A1298C polymorphism analyses, we used the PCR-RFLP method as previously described (15).

For quality control purposes, the laboratory personnel were blinded to the case-control status of the study participants. About 10% of the samples were randomly selected for duplicate analysis, and concordant results were observed for all duplicates.

Statistical Analysis. STATA 8.0 (Stata Corp., College Station, TX) and SPSS 11.5 (SPSS, Inc., Chicago, IL) programs were used in statistical analysis. χ^2 tests were used to compare the distribution of categorical variables and genotype frequencies between cases and controls. The observed genotype frequencies were compared with those calculated from the Hardy-Weinberg disequilibrium theory ($p^2 + 2pq + q^2 = 1$, where p is the frequency of the variant allele and $q = 1 - p$). Unconditional logistic regression analysis was used to calculate odds ratios (OR) and their 95% confidence intervals (95% CI). ORs were also calculated by multivariate analysis, including variables of age (categorized by quartiles of the controls), smoking (ever versus never), drinking (ever versus never), history of pancreatitis (yes or no), type II diabetes (yes or no), and family history of pancreatic cancer (yes or no). The accumulated exposure to smoking, defined as the number of pack-years smoked, and the weekly consumption of ethanol (g) were dichotomized using the median values of controls as the cutoff points. *MTHFR* C667T and A1298A polymorphisms were analyzed as dichotomized variables using either the wild type or the combined wild type and heterozygous genotype as the reference category. Stratified analyses by smoking and alcohol consumption status were used to explore potential gene-environment interaction. The synergisms between genetic and environmental factors were estimated by evaluating departure from additivity. Multiple logistic regression models were used to evaluate the combined effect of genotypes and exposure (cigarette smoking, alcohol consumption, heavy smoking, and heavy alcohol consumption). Heavy smokers were defined as those who smoked >20 pack-years and heavy drinkers were defined as those who consumed >86 g ethanol/wk (median value of the controls). Variables for combined effects were coded using a common referent group (e.g., never smokers with the *MTHFR* CC and CT genotypes). Haplotype frequencies were inferred from the genotype data by using the expectation maximization algorithm, and the haplotype effect on risk of pancreatic cancer was estimated by using the CHAPLIN computer software (35). All statistical tests were two tailed, and $P < 0.05$ indicated statistical significance. Exact test was done when appropriate.

Results

Characteristics of the Study Subjects. The demographics and potential risk factors of 347 patients with pancreatic cancer and 348 cancer-free controls, are summarized in Table 1. The cases and controls were properly matched on sex and race but

Table 1. Selected variables of cases and controls

Variable	Cases, n = 347 (%)	Controls, n = 348 (%)	P*
Age (y)			
≤52	64 (18)	95 (27)	0.04
53-62	107 (31)	101 (29)	
63-69	85 (24)	78 (22)	
≥70	91 (26)	74 (21)	
Sex			
Male	200 (58)	202 (58)	0.91
Female	147 (42)	146 (42)	
Race			
White	304 (88)	311 (89)	0.89
Hispanic	21 (6)	17 (5)	
African American	17 (5)	16 (5)	
Others	5 (1)	4 (1)	
Smoking			
Never	134 (39)	155 (47)	0.03
Ever	213 (61)	177 (53)	
Pack-years of smoking†			
0-20	219 (64)	272 (76)	<0.001
>20	125 (36)	86 (24)	
Alcohol†			
Never	160 (46)	137 (41)	0.19
Ever	166 (54)	195 (59)	
Weekly ethanol intake (g/wk)			
0-86	221 (68)	231 (70)	0.43
>86	105 (32)	96 (30)	
Family history of cancer (first-degree relatives)			
No	95 (28)	101 (30)	0.51
Yes	243 (72)	231 (70)	
Family history of pancreatic cancer (first-degree relatives)			
No	329 (95)	335 (97)	0.13
Yes	18 (5)	10 (3)	
Pancreatitis			
No	313 (90)	341 (99)	<0.001
Yes	34 (10)	4 (1)	
Diabetes (y)			
No	284 (83)	308 (94)	<0.001
≤3	21 (6)	9 (3)	
>3	36 (11)	12 (4)	

*χ^2 analysis.
†Due to missing information, some cells do not add up to the number of study subjects.

the controls were over represented in the youngest quartile age group (Table 1). The mean age ± SD was 62.1 ± 10.3 years for cases and 59.9 ± 11.5 years for controls (P = 0.01). About 88% of the cases and 89% of the controls were non-Hispanic whites, whereas Hispanics, African Americans, and other ethnic groups accounted for 12% of the cases and 11% of the controls. Ever smokers comprised 61% of the cases and 53% of the controls, which translates into a 1.5-fold increased risk of pancreatic cancer among ever smokers (95% CI: 1.1-2.0). About 36% of the cases and 24% of the controls smoked >20 pack-years. Ever drinkers comprised 54% of the cases and 59% of the controls, and the difference was not statistically significant. Using the median value of ever drinkers among controls as the cutoff point, 32% of the cases and 30% of the controls consumed >86 g ethanol/wk. Other factors that were significantly associated with an increased risk of pancreatic cancer included history of pancreatitis and history of diabetes (Table 1). Because some diabetes could be a manifestation of pancreatic cancer, we separated those who had a recent diagnosis of diabetes from those who had a >3-year history of the disease, and both groups showed significantly increased risk for pancreatic cancer (Table 1).

***MTHFR* Genotypes and Risk of Pancreatic Cancer.** The frequencies of the *MTHFR* polymorphisms varied among different racial groups, but none deviated significantly from the Hardy-Weinberg equilibrium (Table 2). Because only a small number of study participants came from minority groups and because of the known racial variation in frequencies of the *MTHFR* polymorphisms, the association between *MTHFR* genotypes and risk of pancreatic cancer was analyzed only among 304 non-Hispanic white patients and 311 race-matched controls.

For the 677 locus, the frequencies of the CC, CT, and TT genotypes were 48.5%, 45.0%, and 6.5%, respectively, among the control subjects (Table 2). The corresponding frequencies among the cases were 49.5%, 38.6%, and 11.9%, respectively. The T allele frequency was 0.31 for cases and 0.29 for controls. The TT genotype was associated with a 2-fold increased risk of pancreatic cancer when compared with the CC genotype after adjustment for age, smoking, and history of pancreatitis and diabetes (Table 3). The distribution of *MTHFR* A1298C genotypes did not vary significantly between cases and controls. The prevalence of the AA, AC, and CC genotypes was 42.9%, 44.2%, and 12.9%, respectively, among the controls and 42.6%, 47.9%, and 9.6%, respectively, among the cases (Table 2). The C allele frequency was 0.34 for cases and 0.35 for controls. This polymorphism showed no significant main effect on the risk of pancreatic cancer (Table 3).

Interaction of *MTHFR* Genotypes with Smoking and Alcohol Consumption. Stratified analyses revealed that the 677TT genotype increased the risk for pancreatic cancer in ever smokers and ever drinkers but not in never smokers and never drinkers (Table 4). The adjusted OR (95% CI) of pancreatic cancer for TT carriers compared with that of CC carriers was 5.53 (2.00-15.3) for ever smokers versus 0.82 (0.33-2.06) for never smokers. The adjusted OR (95% CI) of pancreatic cancer was 3.16 (1.30-7.69) for ever drinkers versus 1.42 (0.56-3.62) for never drinkers. The 1298CC genotype was associated with a significantly reduced risk for pancreatic cancer among ever smokers (OR, 0.40; 95% CI, 0.19-0.85) but with a nonsignificant increased risk among never smokers (OR, 1.89; 95% CI, 0.80-4.48).

A positive interaction was observed between the 677TT genotype and cigarette smoking with respect to the risk of pancreatic cancer. When using never smokers with the CC and

Table 2. Racial distribution of *MTHFR* gene polymorphisms

Genotype	Case, n (%)	Control, n (%)	Variant allele frequency		P*	χ^2 (HWE)
			Case	Control		
C677T						
White						
CC	150 (49.5)	149 (48.5)				
CT	117 (38.6)	138 (45.0)				
TT	36 11.9	20 (6.5)	0.31	0.29	0.04	2.59
Hispanic						
CC	10 (47.6)	5 (29.4)				
CT	10 (47.6)	5 (52.9)				
TT	1 (4.8)	3 (17.6)	0.29	0.44	0.31	0.09
African American						
CC	11 (64.7)	12 (75.0)				
CT	6 (35.3)	4 (25.0)				
TT	0 (0)	0 (0)	0.18	0.13	0.52	0.33
A1298C						
White						
AA	129 (42.6)	133 (42.9)				
AC	145 (47.9)	137 (44.2)				
CC	29 (9.6)	40 (12.9)	0.34	0.35	0.47	0.26
Hispanic						
AA	13 (61.9)	10 (58.8)				
AC	7 (33.3)	6 (35.3)				
CC	1 (4.8)	1 (5.9)	0.21	0.24	0.98	0.01
African American						
AA	12 (70.6)	9 (56.3)				
AC	5 (29.4)	6 (37.5)				
CC	0	1 (6.3)	0.15	0.25	0.48	0.00

Abbreviation: HWE, Hardy-Weinberg equilibrium in controls.
*χ^2 analysis of frequencies between cases and controls.

Table 3. *MTHFR* polymorphisms and risk of pancreatic cancer

Genotype	Case/control (*n*/*n*)	Crude OR (95% CI)	*P*	Adjusted OR* (95% CI)	*P*
C677T					
CC	150/149	1.0		1.0	
CT	117/138	0.84 (0.60-1.18)	0.32	0.90 (0.63-1.27)	0.54
TT	36/20	1.79 (1.00-3.23)	0.05	2.14 (1.14-4.01)	0.02
A1298C					
AA	129/133	1.0		1.0	
AC	145/137	1.09 (0.78-1.53)	0.61	1.12 (0.79-1.60)	0.52
CC	29/40	0.75 (0.44-1.28)	0.29	0.77 (0.44-1.34)	0.35

*OR was adjusted for age, smoking (ever or never), history of pancreatitis (yes or no), and history of diabetes (yes or no).

CT genotypes as the referent, we found that light smokers (≤20 pack-years) with the TT genotype had a much higher risk of pancreatic cancer (OR, 2.82; 95% CI, 0.82-9.67) compared with never smokers with the TT genotype (OR, 1.08; 95% CI, 0.45-2.59) or light smokers with the CC or CT genotype (OR, 0.94; 95% CI, 0.62-1.44). This interaction was even more prominent among heavy smokers. The OR (95% CI) was 6.83 (1.91-24.38) in heavy smokers (>20 pack-years) with the TT genotype and 1.67 (1.11-2.50) in heavy smokers with the CC and CT genotypes (Table 5). A similar effect was observed for heavy drinkers (weekly ethanol consumption greater than the median value for controls) with the TT genotype. Light drinkers and heavy drinkers with the TT genotype had an OR (95% CI) of 1.59 (0.59-4.27) and 4.23 (0.88-20.3), respectively, compared with never drinkers with the CC/CT genotype. A significantly reduced risk of pancreatic cancer was observed among light drinkers with the CC/CT genotypes (OR, 0.47; 95% CI, 0.30-0.73; Table 5).

Linkage Disequilibrium and Haplotype Analysis. Among non-Hispanic whites, linkage test showed that the two loci of *MTHFR* 677 and 1298 were in linkage disequilibrium. The χ^2 test of linkage disequilibrium gave a test statistical value of 92.2 ($D' = 0.56$) for the whole study population, 15.3 ($D' = 0.34$) for cases, and 103.5 ($D' = 0.80$) for controls ($P < 0.001$). The frequencies of combined genotypes and their associations with the risk of pancreatic cancer are presented in Table 6. The only significant association, which is an inverse association, was observed for the CC-CC genotype (OR, 0.44; 95% CI, 0.23-0.87). The frequency of the 677T-1298C haplotype was 6% for the cases and 3% for controls, and this haplotype was significantly associated with an increased risk of pancreatic cancer ($P = 0.01$, Wald test; Table 6). Using the 677C-1298A haplotype as a referent, the 677T-1298C haplotype exerted a 3-fold increased risk of pancreatic cancer (OR, 3.26; 95% CI, 1.38-7.7, univariate logistic regression).

Discussion

This hospital-based case-control study has shown a significant association between the *MTHFR* C677T polymorphism and risk of pancreatic cancer in a U.S. population. Individuals carrying the homozygous TT variant genotype conferring low enzyme activity had a 2-fold increased risk of developing pancreatic cancer compared with individuals with the CC wild-type or the heterozygous CT genotype. Furthermore, interactions between the TT genotype and heavy smoking and heavy alcohol use were detected. Smokers, especially those who smoke heavily, and heavy alcohol users with the TT genotype showed a 4- to 6-fold increased risk of pancreatic cancer compared with those who had either exposure alone or the variant genotype alone. These observations suggest that cigarette smoking, heavy alcohol consumption, and folate deficiency may share some common pathways contributing to pancreatic carcinogenesis. Considering the previously reported associations between the low levels of folate intake and serum folate and the risk of pancreatic cancer (7, 8), our observations support an important role of folate in the etiology of this disease.

Table 4. Stratified analysis by smoking and alcohol

Genotype	Case *n* (%)	Control *n* (%)	Adjusted OR* (95% CI)	Case *n* (%)	Control *n* (%)	Adjusted OR* (95% CI)
	Never smokers			Ever smokers		
C677T						
CC	63 (54)	51 (37)	1.0	87 (47)	91 (60)	1.0
CT	42 (36)	75 (54)	0.48 (0.28-0.84)	75 (40)	58 (36)	1.39 (0.88-2.21)
TT	11 (10)	13 (9)	0.82 (0.33-2.06)	25 (13)	5 (4)	5.53 (2.00-15.3)
A1298C						
AA	46 (40)	65 (46)	1.0	83 (44)	62 (40)	1.0
AC	55 (47)	62 (44)	1.26 (0.72-2.19)	90 (48)	68 (44)	1.03 (0.64-1.64)
CC	15 (13)	13 (10)	1.89 (0.80-4.48)	14 (8)	26 (17)	0.40 (0.19-0.85)
	Never drinkers			Ever drinkers		
C677T						
CC	60 (43)	52 (43)	1.0	89 (55)	90 (53)	1.0
CT	65 (46)	60 (49)	1.03 (0.60-1.77)	52 (32)	73 (43)	0.77 (0.48-1.24)
TT	16 (11)	10 (8)	1.42 (0.56-3.62)	20 (13)	8 (5)	3.16 (1.30-7.69)
A1298C						
AA	61 (43)	53 (43)	1.0	68 (42)	74 (43)	1.0
AC	67 (48)	50 (41)	1.18 (0.68-2.06)	78 (48)	80 (46)	1.08 (0.68-1.72)
CC	12 (9)	20 (16)	0.60 (0.26-1.39)	16 (10)	19 (11)	0.91 (0.42-1.97)

*OR with adjustment for age, diabetes, pancreatitis, and smoking or alcohol.

Table 5. Interaction of *MTHFR* C677T polymorphisms with smoking and alcohol

C677T genotype	Exposure	Case/control (*n*/*n*)	Adjusted OR (95% CI)*	*P*
CC/CT	Never	123/141	1.00 (reference)	
TT	Never	11/13	1.08 (0.45-2.59)	0.86
CC/CT	≤20 pack-years	76/90	0.94 (0.62-1.44)	0.79
TT	≤20 pack-years	9/4	2.82 (0.82-9.67)	0.10
CC/CT	>20 pack-years	105/77	1.67 (1.11-2.50)	0.01
TT	>20 pack-years	18/3	6.83 (1.91-24.38)	0.003
CC/CT	Never	144/126	1.00 (reference)	
TT	Never	11/8	1.02 (0.38-2.77)	0.96
CC/CT	≤86 g/wk	51/86	0.47 (0.30-0.73)	0.001
TT	≤86 g/wk	11/7	1.59 (0.59-4.27)	0.36
CC/CT	>86 g/wk	95/93	0.77 (0.51-1.16)	0.22
TT	>86 g/wk	9/2	4.23 (0.88-20.3)	0.07

*Adjusted OR was adjusted for age, smoking, pancreatitis, and diabetes.

Evidence from experimental studies support an association between folate and pancreatic disease. The pancreas contains high folate levels, second only to the liver (36). Glycine *N*-methyltransferase, which requires folate coenzymes in regulating the ratio of *S*-adenosylmethionine to *S*-adenosylhomocysteine, is abundant in the liver and pancreas of rats (37). Rats fed a folate deficient diet had a significantly reduced ratio of *S*-adenosylmethionine to *S*-adenosylhomocysteine, which indicates methyl donor depletion (38). The pancreas of rats fed a folate-deficient diet contained more immature secretory granules and reduced amylase secretion than the pancreas of controls (39). Some studies have suggested that the reduced pancreatic exocrine function was a result of disturbed methyl metabolism secondary to dietary folate deficiency (40, 41). A methyl (methionine and choline)–deficient diet indeed causes abnormal cellular differentiation, reduced exocrine function of the pancreas, and increased sensitivity to toxic injury and carcinogenicity in rats (42). In addition, animals treated with ethionine, an inhibitor of cellular methylation reactions, develop acute hemorrhagic pancreatitis (43) as a consequence of autolytic destruction of the pancreas (44), and chronic pancreatitis has been associated with increased pancreatic cancer risk (5).

DNA methylation is an important epigenetic determinant in gene expression, maintenance of DNA integrity and stability, chromosomal modifications, and development of mutations. Aberrant DNA methylation is observed in a nonrandom, tumor type–specific manner. In particular, certain types of tumors show regional hypermethylation of CpG islands associated with the promoter regions of tumor suppressor genes, such as *RB*, *VHL*, $p16^{INAK4A}$, and *hMLH1* (45, 46). Furthermore, the regional hypermethylation is often associated with the inactivation of the tumor suppressor genes and hypomethylation is associated with activation of the oncogenes. Folate and methyl donor deficiency have been implicated in global DNA hypomethylation but their association with specific gene hypermethylation is not clear.

Genetic polymorphisms that decrease MTHFR activity result in the depletion of 5-methyl THF for homocysteine remethylation and the accumulation of 5,10-methylene THF, the precursor for thymidylate and purine synthesis. Although there are several studies reporting significant associations between *MTHFR* genotypes and gene hypomethylation in peripheral lymphocytes and in tumor tissues (47-49), other studies either found no association (50) or observed this association only in the presence of folate deficiency (51, 52). Pancreatic adenocarcinoma is a highly aggressive cancer with multiple genetic and epigenetic alterations. A previous study has shown that gene hypomethylation is a frequent epigenetic event in pancreatic cancer and is commonly associated with overexpression of the affected genes (53). Whether the detrimental effect of the homozygous *MTHFR* TT genotype (with much-reduced enzyme activity) on the risk of developing pancreatic cancer acts through the DNA hypomethylation mechanism needs further investigation.

We observed a clear dose-response relationship in the interaction between cigarette smoking and *MTHFR* 677 TT genotype in this study population. The interaction between the *MTHFR* polymorphism and smoking with respect to disease risk has been observed in many previous investigations. For example, cigarette smoking interacts with the *MTHFR* polymorphisms, resulting in an increased risk of cardiovascular disease (54, 55) and cancer of the colon (56), stomach (57), and urinary bladder (22). Smoking is a known risk factor for pancreatic cancer, and we observed this risk association in the current study population. The strong interaction between heavy smoking and the *MTHFR* 677TT genotype observed in this study indicates that folate plays a critical role in the common causal pathway of this disease. The interaction between smoking and *MTHFR* polymorphism in increased risk of pancreatic cancer could be explained by two possible mechanisms: (a) Smokers may tend to have lower intake of dietary folate and lower levels of serum or tissue folate (58) and smoking is also known to interfere with the metabolism of

Table 6. Diplotype distribution and risk of pancreatic cancer

Genotype	Reported frequency*	All subjects frequency	*n*	Cases frequency	*n*	Controls frequency	OR (95% CI)
CC/AA	0.15	0.17	54	0.18	48	0.16	1.0
CC/AC	0.22	0.23	77	0.26	63	0.21	1.09 (0.65-1.81)
CC/CC	0.085	0.09	19	0.06	38	0.12	0.44 (0.23-0.87)
CT/AA	0.22	0.20	53	0.18	67	0.22	0.70 (0.41-1.19)
CT/AC	0.20	0.20	56	0.19	68	0.22	0.73 (0.43-1.23)
CT/CC	0.0025	0.01	7	0.02	2	0.01	3.11 (0.62-15.7)
TT/AA	0.11	0.06	22	0.07	15	0.05	1.30 (0.60-2.79)
TT/AC	0.0046	0.03	11	0.04	5	0.02	1.96 (0.60-6.03)
TT/CC	0.00032	0.005	3	0.01	0	0	Infinite

*Reported frequency in 12,647 controls from a meta-analysis of 16 studies.

the methyl donors (59); thus, smokers are more susceptible to the interactive effects of folate deficiency and reduced MTHFR activity, which deplete the 5-methyl THF and result in DNA hypomethylation. (b) Cigarette smoke contains many chemical carcinogens that cause DNA damage and folate deficiency has been associated with a reduced capacity for repairing DNA (60), which makes an individual more susceptible to smoking-induced DNA damage and gene mutation.

Alcohol has been found to interact with *MTHFR* polymorphisms in modifying the risk of colon cancer (61), breast cancer (62), and hepatocellular carcinoma (22). Although heavy users of alcohol may tend to have a diet low in fruits and vegetables, which may lead to a suboptimal level of serum folate, alcohol itself is known to decrease folate absorption, alter its metabolism, increase its excretion, and therefore deplete 5-methyl THF (63-66). In this study population, light alcohol consumption (≤86 ethanol/wk, median value of control drinkers) showed a protective effect on the risk of pancreatic cancer, whereas heavy alcohol consumption did not show any significant effect on the risk of pancreatic cancer. However, a positive interaction between alcohol and *MTHFR* polymorphisms was observed in the current study. Among light drinkers, the *MTHFR* 677CC/CT genotype was associated with a significantly decreased risk of pancreatic cancer (OR, 0.47; 95% CI, 0.30-0.73) and, in contrast, the TT genotype was associated with a nonsignificant higher risk of pancreatic cancer (OR, 1.59; 95% CI, 0.59-4.27). The increased risk of pancreatic cancer among the TT genotype carriers was more prominent in heavy drinkers (OR, 4.23; 95% CI, 0.88-20.3). It is possible that heavy alcohol consumption may lead to folate deficiency and in the presence of the reduced MTHFR activity associated with the polymorphic gene would increase the risk of cancer by causing DNA hypomethylation.

We observed a differential effect of *MTHFR* 677TT and *MTHFR* 1298CC variants on the risk of pancreatic cancer in the current study, although both variants have been related to reduced MTHFR enzyme activities *in vitro*. The 677TT variant was positively associated with risk, whereas the 1298CC variant was inversely associated with risk for pancreatic cancer among smokers. This phenomenon can be explained by the observation that the *MTHFR 677C* allele is in linkage disequilibrium with the *1298C* allele ($D' = 0.56$) in this study population. Among the 69 individuals with the 1298CC genotype, 57 carried the protective *677C* allele. The detrimental effect of the 677TT variant was much stronger than the protective effect of the 1298CC variant in our study. The protective effect of the 1298CC disappeared in presence of the *677T* allele, whereas the effect of the 677TT variant was not affected by its combination with the *1298C* allele (Table 6). The different effects of the two minor genetic variants has previously been reported in a study of prostate cancer, where the 677T variant was associated with a reduced risk and the 1298C variant was associated with an increased risk of more aggressive disease (25). As reviewed in a meta-analysis (67), the association between the *677C* allele and the *1298C* allele has been frequently observed in the general population. Compared with the reported frequencies of the combined genotypes in the general population by a meta-analysis (67), our study population had higher frequencies of the CC-CC, CT-CC, and TT-AC genotypes but a lower frequency of the TT-AA genotype. Furthermore, we also observed three male cancer patients with the TT-CC double homozygote, which is extremely rare in the general population. Although haplotype analysis showed a significantly increased risk of pancreatic cancer for the 677T-1298C haplotype (Table 7), the R_h^2 value (a measure of uncertainty) was relatively small for this haplotype compared with the R_h^2 value for the other haplotypes. Thus, these results should be interpreted with caution.

Our study has two major limitations, one is the inherent limitation of the hospital-based case control design and the other is the lack of information on folate status. The relatively lower frequency of the 677TT genotype (6.5%) in our control group compared with the frequencies reported in other U.S. study populations (9-15%) may reflect a selection bias. Our controls were recruited from cancer patient companions who are not genetically related to the cancer patients. Compared with the general U.S. population, this control group has a higher prevalence of cigarette smoking and family history of cancer (Table 1). Nevertheless, the strong interaction between this genotype with heavy smoking and alcohol consumption is less likely to be explained by selection bias. Therefore, the association between the *MTHFR* polymorphisms and the risk of pancreatic cancer needs to be confirmed in other study populations. We have recently begun collecting dietary data using a food frequency questionnaire in our ongoing case control study. In the future, we will be able to assess the interactions among folate intake, smoking, alcohol, and *MTHFR* genotype.

Table 7. Haplotype analysis

Haplotype	Frequency		*P* (Wald)*	$R_h^{2\dagger}$	OR (95% CI)
	Cases	Controls			
677C-1298A	0.41	0.39	0.89	0.93	1.00 (reference)
677C-1298C	0.28	0.32	0.28	0.92	0.81 (0.62-1.06)
677T-1298A	0.25	0.26	0.96	0.91	0.92 (0.69-1.21)
677T-1298C	0.06	0.03	0.01	0.66	3.26 (1.38-7.70)

*Wald, test for the haplotype effect on disease.
†R_h^2 is a measure of haplotype uncertainty.

In conclusion, our study has shown the *MTHFR* 677TT genotype has a significant effect on the risk of pancreatic cancer. A strong synergistic interaction of this genotype with cigarette smoking and heavy alcohol consumption was detected. Considering the facts that the previously reported association of folate intake/serum folate level and risk of pancreatic cancer was in male smokers (7, 8) and this association was not observed in studies of the general U.S. population (10, 11), additional large studies that include information on the *MTHFR* genotype and folate status in relation to smoking and alcohol consumption would be helpful in understanding the complex interactions of these genetic and environmental factors in pancreatic cancer. Such knowledge would have important implications for the primary prevention of pancreatic cancer.

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