Polymorphisms of *Methionine Synthase* and *Methionine Synthase Reductase* and Risk of Squamous Cell Carcinoma of the Head and Neck: A Case-Control Analysis

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Abstract

Although tobacco and alcohol use are the major risk factors, folate deficiency has been implicated in the risk of squamous cell carcinoma of the head and neck (SCCHN). We hypothesized that polymorphisms of methionine synthase (*MTR*) and methionine synthase reductase (*MTRR*) in the folate metabolic pathway are associated with SCCHN risk. In a hospital-based case-control study of 721 SCCHN cases and 1,234 controls of non-Hispanic Whites, frequency matched by age, sex, and smoking status, we genotyped the *MTR* A2756G and *MTRR* G66A polymorphisms. We found that the *MTR* variant AG and AG/GG genotypes were associated with a significantly increased SCCHN risk [adjusted odd ratio (OR) 1.31; 95% confidence interval (95% CI), 1.07-1.60 for AG and OR, 1.28; 95% CI, 1.05-1.56 for AG/GG] compared with the AA genotype. In contrast, the *MTRR* variant AA genotype was associated with a significantly decreased SCCHN risk (OR, 0.68; 95% CI, 0.52-0.90) compared with the 66GG genotype. When the two polymorphisms were evaluated together by the number of risk alleles, the SCCHN risk was significantly increased in a dose-dependent manner (P_{trend} = 0.002). The risk of SCCHN was 1.47 (95% CI, 1.08-1.99) for one risk allele, 1.67 (95% CI, 1.23-2.27) for two risk alleles, and 1.74 (95% CI, 1.18-2.54) for three or four risk alleles compared with the wild-type (0 risk allele) genotype. In conclusion, our data provide evidence that support the association between the *MTR* A2756G and *MTRR* G66A polymorphisms and SCCHN risk and that these two polymorphisms may have a joint effect on risk of SCCHN. (Cancer Epidemiol Biomarkers Prev 2005;14(5):1188–93)

Introduction

Squamous cell carcinoma of the head and neck (SCCHN) includes cancers of the oral cavity, pharynx, and larynx. SCCHN is the sixth most frequent incident cancer and the seventh leading cause of cancer-related death in the world, affecting >500,000 individuals each year worldwide (1). In the United States, it was estimated that there were 38,530 newly diagnosed SCCHN cases and 11,060 related deaths in 2004 (2). Although tobacco and alcohol use are the major risk factors in the etiology of SCCHN (3), only a fraction of exposed individuals develops SCCHN, suggesting that there is variation in individual susceptibility to exposure-related carcinogenesis.

Low dietary intake of fruits and vegetables has been implicated in risk of cancers, including SCCHN (4, 5), and folate, one of the constituents in fruits and vegetables, is thought to be one of the protective nutrients (5). An important biological function of folate is to provide methyl groups, which are essential for intercellular methylation reactions and for *de novo* deoxynucleoside triphosphate synthesis. Therefore, folate deficiency is thought to increase the risk of cancer by causing DNA hypomethylation that may lead to proto-oncogene activation and induction of malignant transformation. Folate deficiency may also induce excessive uracil misincorporation into DNA in place of thymine during DNA synthesis, resulting in catastrophic events that impair DNA repair, and lead to DNA strand breakage and chromosome damage (6, 7). Recently, several studies have implicated folate deficiency in increased risk of SCCHN (8-10). Several enzymes, including methionine synthase (*MTR*; EC2.1.1.13, Genbank accession no. NT_004836) and methionine synthase reductase (*MTRR*; EC2.1.1.135, Genbank accession no: NT_023089), are among the key enzymes involved in folate metabolism (11, 12). Functional polymorphisms of these genes may modulate folate metabolism, which may ultimately effect genetic susceptibility to cancer.

The *MTR* gene is located at 1q43 (13) and the MTR protein is responsible for the remethylation of homocysteine to methionine, which is essential for maintaining adequate intracellular methionine and normal homocysteine concentrations. A common polymorphism in the *MTR* gene, an A-to-G transition at bp 2756 (*MTR* A2756G), causes an amino acid substitution from aspartic acid to glycine at codon 919 (D919G; ref. 14). At least three studies to date have reported that individuals with the 2756GG genotype had lower plasma homocysteine (or higher folate) levels than those with the 2756AA genotype (15-17), but another study reported some conflicting data (18). A significantly increased risk of malignant lymphoma (19) and a slightly lowered risk of colorectal cancer (18) for the GG genotypes have been reported, but these findings were also not confirmed in other studies (20-22).

MTRR is on chromosome 5p15.3-p15.2 and catalyzes the regeneration of methylcobalamin, a cofactor for MTR. This reaction plays an important role in maintaining the MTR enzyme in an active state (23). A common polymorphism in the *MTRR* gene, a G-to-A transition at bp 66 (formerly called *MTRR* A66G, ref. 23; but now called *MTRR* G66A, because the G allele was found to be more common than the A allele based on the new data from >1,000 healthy controls), results in an

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amino acid substitution from methionine to isoleucine at codon 22 (M22I). One study has reported that the 66AA genotype contributes to elevated homocysteine levels compared with the 66GG genotype (24); however, this effect was not observed by others (25, 26). Few studies have investigated the association between the *MTRR* G66A polymorphism and risk of cancer. Recently, Matsuo et al. reported that individuals with the 66GG genotype had an increased risk of colorectal cancer in a Japanese population (27). Similarly, Le Marchand et al. found a borderline increased risk of colorectal cancer among a subgroup of Caucasian subjects with the 66GG genotype (28).

Our previous studies have revealed that suboptimal DNA repair capacity may be a risk factor for SCCHN (29) and that low dietary folate intake was associated with suboptimal DNA repair capacity (30). Because MTR and MTRR are both involved in folate metabolism and folate is required in DNA synthesis and repair, we hypothesized that the *MTR* A2756G and *MTRR* G66A polymorphisms contribute to genetic susceptibility to SCCHN. To test this hypothesis, we genotyped these two polymorphisms in our ongoing hospital-based case-control study of SCCHN.

Materials and Methods

Subjects. The subject recruitment for the ongoing SCCHN study has been described previously (31). Briefly, 721 newly diagnosed SCCHN cases and 1,234 cancer-free controls were recruited into the study between May 1995 and September 2003. All the cases and 631 of the controls were recruited from The University of Texas M.D. Anderson Cancer Center. These controls were recruited from among visitors who were accompanying the patients but unrelated to the cases. There were an additional 603 controls recruited from a multispecialty physician practice, the Kelsey-Seybold Clinic, which has multiple clinics throughout the Houston, TX, metropolitan area. These additional controls provided only older men who were former and current smokers needed for our frequency matching. Because genotype frequencies can vary between ethnic groups and few minority patients were recruited, only non-Hispanic White patients were included in this analysis. Approximately 95% of eligible patients who were contacted chose to participate. Patients with second SCCHN primary tumors, primary tumors of the nasopharynx or sinonasal tract, primary tumors outside the upper aerodigestive tract, cervical metastases of unknown origin, or histopathologic diagnoses other than squamous cell carcinoma were excluded. The 721 non-Hispanic White patients with primary tumors included in the analysis had cancers of the oral cavity (n = 222; 30.8%), pharynx (n = 363; 50.3%), or larynx (n = 136; 18.9%).

For the controls, we first surveyed the potential control subjects by using a short questionnaire to determine their willingness to participate in research studies and to obtain information about their smoking behavior and demographic factors. Among the willing respondents we contacted for recruitment, the response rate was >80%. We interviewed each eligible subject to obtain data on age, sex, smoking status, and alcohol use. We frequency matched the controls to the cases by age (±5 years), sex, and smoking status. After the subjects signed informed consent forms, each subject donated 30 mL of blood collected into heparinized tubes. A leukocyte cell pellet obtained from a buffy coat was used for DNA extraction with a DNA blood Mini Kit (Qiagen, Valencia, CA) according to the manufacturer's instructions. The research protocol was approved by the M.D. Anderson Cancer Center and Kelsey-Seybold Clinic institutional review boards.

Genotyping. The PCR-RFLP assay was used to identify the *MTR* A2756G and *MTRR* G66A polymorphisms. The primers of the *MTR* A2756G were forward 5′-TGTTCCAGACAGTTA-GATGAAAATC-3′ and reverse 5′-GATCCAAAGCCTTTTA-CACTCCTC-3′ (19). The PCR product is digested with *Hae*III (New England Biolabs, Beverly, MA); and the amplified fragment of 211 bp is cut into fragments of 131 and 80 bp in the presence of the G allele. The primers of the *MTRR* G66A were forward 5′-GCAAAGGCCATCGCAGAAGACAT-3′ and reverse 5′-GTGAAGATCTGCAGAAAATCCATGTA-3′ (23). The forward primer contained a mismatch (underlined base C in the primer sequence), which generated an *Nde*I restriction site when the polymorphic allele was present. The expected PCR product of 66 bp is digested into fragments of 44 and 22 bp by *Nde*I (New England Biolabs) in presence of the A allele but remains uncut in the presence of the G allele. More than 10% of the samples were randomly selected for repeat assays, and the results were 100% concordant.

Statistical Analysis. We used the χ^2 test to evaluate differences in the frequency distributions of selected demographic variables, smoking status, alcohol use, and each allele and genotype of the *MTR* A2756G and *MTRR* G66A polymorphisms between the cases and controls. Univariate and multivariate logistic regression analyses were used to obtain the crude and adjusted odds ratios (OR) and 95% confidence intervals (95% CI). The multivariate adjustment included age, sex, smoking status, and alcohol use. The genotype data were further stratified by subgroups of age, sex, smoking status, and alcohol use. Considering the potential gene-gene interaction of the *MTR* A2756G and *MTRR* G66A polymorphisms on SCCHN risk, we evaluated the association between SCCHN risk and the combined genotypes of these two polymorphisms. Logistic regression was also used to assess potential interaction effects by evaluating departures from the models of additive and multiplicative interactions between smoking or alcohol use and the two polymorphisms. A more-than-additive interaction was suggested when $OR_{11} > OR_{10} + OR_{01} - 1$, where OR_{11} = OR when both factors were present, OR_{10} = OR when only factor 1 was present, and OR_{01} = OR when only factor 2 was present. A more-than-multiplicative interaction was suggested when $OR_{11} > OR_{10} \times OR_{01}$. All tests of statistical significance were two sided using SAS software, version 8.2 (SAS Institute, Inc., Cary, NC).

Results

Characteristics of the Study Population. The frequency distributions of selected characteristics of the cases and controls are summarized in Table 1. The mean age was 57.0 years for the cases (±11.9 years; range, 18-90 years) and 57.2 years for the

Table 1. Frequency distributions of selected variables in SCCHN cases and cancer-free controls

Variables	Cases (n = 721), n (%)	Controls (n = 1,234), n (%)	P*
Age (y)			
≤45	105 (14.6)	195 (15.8)	0.277
46-55	227 (31.5)	345 (28.0)	
56-65	220 (30.5)	370 (30.0)	
>65	169 (23.4)	324 (26.2)	
Sex			
Female	181 (25.1)	320 (25.9)	0.686
Male	540 (74.9)	914 (74.1)	
Smoking status			
Never	188 (26.1)	371 (30.1)	<0.001
Former	282 (39.1)	549 (44.5)	
Current	251 (34.8)	314 (25.4)	
Alcohol use			
Never	157 (21.8)	373 (30.2)	<0.001
Former	195 (27.0)	322 (26.1)	
Current	369 (51.2)	539 (43.7)	

*Two-sided χ^2 test.

Table 2. *MTR* A2756G and *MTRR* G66A genotype and allele frequencies among the cases and controls and logistic regression analysis for associations with SCCHN risk

Genotypes	Cases, *n* (%)	Controls*, *n* (%)	*P*†	Crude OR (95% CI)	Adjusted OR (95% CI)‡
Total no. of subjects	721	1,234			
Total no. of alleles	1,442	2,468			
MTR A2756G					
AA	472 (65.4)	876 (71.0)	0.028	1.00 (reference)	1.00 (reference)
AG	232 (32.2)	327 (26.5)		1.32 (1.08-1.61)	1.31 (1.07-1.60)
GG	17 (2.4)	31 (2.5)		1.02 (0.56-1.86)	1.00 (0.55-1.84)
AG/GG	249 (34.5)	358 (29.0)	0.011	1.29 (1.06-1.57)	1.28 (1.05-1.56)
G allele	0.184	0.158	0.155		
MTRR G66A					
GG	231 (32.0)	369 (29.9)	0.002	1.00 (reference)	1.00 (reference)
GA	376 (52.2)	589 (47.7)		1.02 (0.83-1.26)	1.02 (0.82-1.26)
AA	114 (15.8)	276 (22.4)		0.66 (0.50-0.87)	0.68 (0.52-0.90)
GA/AA	490 (68.0)	865 (70.1)	0.323	0.90 (0.74-1.10)	0.91 (0.75-1.11)
A allele	0.418	0.462	0.072		

*The observed genotype frequency among the control subjects was in agreement with Hardy-Weinberg equilibrium ($p^2 + 2pq + q^2 = 1$) ($\chi^2 = 0.00$, $P = 0.941$ for *MTR* A2756G and $\chi^2 = 2.22$, $P = 0.136$ for *MTRR* G66A).
† Two-sided χ^2 test for either genotype distribution or allele frequency.
‡ Adjusted for age, sex, smoking status, and alcohol use in a logistic regression model.

controls (±11.6 years; range, 20-87 years; $P = 0.277$); the male cases (74.9%) and controls (74.1%) were well matched on sex ($P = 0.686$). However, the frequency matching on smoking status was imperfect: there were more current smokers (34.8%) and current drinkers (51.2%) among the cases than among the controls (25.4% and 43.7%, respectively), and these differences were statistically significant ($P < 0.001$ for both tobacco and alcohol use). Thus, these variables were further adjusted in the logistic regression analysis.

Associations between the *MTR* A2756G and *MTRR* G66A Polymorphisms and SCCHN Risk. The genotype and allele frequencies of *MTR* A2756G and *MTRR* G66A polymorphisms and their associations with SCCHN risk are presented in Table 2. The variant *MTR* 2756G and *MTRR* 66A allele frequencies were 0.184 and 0.418, respectively, among the cases and 0.158 and 0.462, respectively, among the controls, but these differences were not statistically significant ($P = 0.155$ for the *2756G* allele; $P = 0.072$ for the *66A* allele). The genotype distributions of the *MTR* A2756G and *MTRR* G66A among the controls were in agreement with the Hardy-Weinberg equilibrium (χ^2 test: $P = 0.941$ for the *MTR* A2756G; $P = 0.136$ for the *MTRR* G66A; Table 2).

As shown in Table 2, the genotype frequencies of the *MTR* A2756G were 65.4%, 32.2%, and 2.4% for the AA, AG, and GG genotypes, respectively, among the cases and 71.0%, 26.5%, and 2.5%, respectively, among the controls, and this difference was statistically significant ($P = 0.028$). Similarly, the genotype frequencies of the *MTRR* G66A were 32.0%, 52.2%, and 15.8% for the GG, GA, and AA genotypes, respectively, among the cases and 29.9%, 47.7%, and 22.4%, respectively, among the controls, and this difference was also statistically significant ($P = 0.002$). For the *MTR* A2756G polymorphism, the AG and the combined genotypes (AG/GG) were associated with significantly increased risk of SCCHN (adjusted OR, 1.31; 95% CI, 1.07-1.60 for AG and OR, 1.28; 95% CI, 1.05-1.56 for AG/GG) compared with the AA genotype. For the *MTRR* G66A polymorphism, the AA genotype was associated with a significantly reduced risk of SCCHN (adjusted OR, 0.68; 95% CI, 0.52-0.90) compared with the GG genotype. However, the GA and the combined genotypes (GA/AA) of the *MTRR* G66A were not associated with risk of SCCHN (adjusted OR, 1.02; 95% CI, 0.82-1.26 for GA and OR, 0.91; 95% CI, 0.75-1.11 for GA/AA; Table 2).

Stratification Analysis of Association between the *MTR* A2756G and *MTRR* G66A Polymorphisms and SCCHN Risk. As shown in Table 3, when we used the *MTR* 2756AA genotype as the reference, we found that the 2756AG genotype was associated with a significantly increased risk of SCCHN among younger subjects (≤55 years; adjusted OR, 1.43; 95% CI, 1.05-1.96), women (OR, 1.70; 95% CI, 1.12-2.58), and former drinkers (OR, 1.53; 95% CI, 1.03-2.27). We also observed a borderline significant association between the 2756AG genotype and SCCHN risk among older subjects (>55 years; adjusted OR, 1.29; 95% CI, 0.98-1.71), men (OR, 1.24; 95% CI, 0.98-1.57), and current drinkers (OR, 1.29; 95% CI, 0.96-1.73). When stratified by tumor site, the 2756AG genotype was associated with a significantly increased risk of SCCHN at the oral cavity site (adjusted OR, 1.58; 95% CI, 1.16-2.16). However, there was no significant association between the 2756GG genotype and SCCHN risk among all subgroups due to the relatively small observations (accounting for only 1.3-3.4% of the subjects) in each stratum (Table 3). In contrast to the *MTR* A2756G, the *MTRR* 66AA, but not the *MTRR* 66GA, genotype was associated with a significantly decreased risk of SCCHN among older subjects (>55 years; adjusted OR, 0.61; 95% CI, 0.41-0.90), men (OR, 0.62; 95% CI, 0.45-0.86), never smokers (OR, 0.57; 95% CI, 0.34-0.96), former smokers (OR, 0.47; 95% CI, 0.30-0.74), and never drinkers (OR, 0.54; 95% CI, 0.32-0.93) compared with the *MTRR* 66GG genotype. The protective association between the *MTRR* 66AA genotype and pharynx site was also statistically significant (adjusted OR, 0.55; 95% CI, 0.38-0.81; Table 3). There was no statistical evidence for any interactions between the genotypes and other risk factors presented in Table 3 except for smoking status ($P < 0.001$ for *MTR* and $P = 0.012$ for *MTRR*). However, when former and current smokers were combined as an ever-smoker group compared with never-smoker group, these interactions disappeared ($P = 0.758$ for *MTR* and $P = 0.743$ for *MTRR*).

Combined Analysis of Association between the *MTR* A2756G and *MTRR* G66A Polymorphisms and SCCHN Risk. Based on the number of risk alleles of the *MTR* 2756G and *MTRR* 66G (because the *MTRR* 66A was protective), we grouped the individuals into four genotype groups: the *MTR* AA and *MTRR* AA (0 risk allele of either gene); the *MTR* AA and *MTRR* GA or *MTR* AG and *MTRR* AA (only one risk allele); *MTR* AA and *MTRR* GG or *MTR* AG and *MTRR* GA or *MTR* GG and *MTRR* AA (two risk alleles); and *MTR* AG and *MTRR* GG or *MTR* GG and *MTRR* GA or *MTR* GG and *MTRR* GG (three or four risk alleles). As shown in Table 4, the frequencies of the combined genotypes were 10.5%, 39.0%, 38.3%, and 12.2% for groups with 0, 1, 2, and 3/4 risk alleles, respectively, among the cases and 16.2%, 39.4%,

Table 3. Associations and stratification analysis between the *MTR* and *MTRR* polymorphisms and SCCHN risk

Variables	*n* (cases/controls)	*MTR* A2756G				
		Percentage (cases/controls)			Adjusted OR (95% CI)*	
		AA	AG	GG	AG	GG
Total	721/1,234	65.4/71.0	32.2/26.5	2.4/2.5		
Age (y)						
≤55	332/540	66.3/72.8	31.3/25.4	2.4/1.8	1.43 (1.05-1.96)	1.72 (0.65-4.55)
>55	389/694	64.8/69.6	32.9/27.4	2.3/3.0	1.29 (0.98-1.71)	0.78 (0.35-1.75)
$P_{interaction}$					0.484	
Sex						
Female	181/320	62.4/72.2	35.4/23.7	2.2/4.1	1.70 (1.12-2.58)	0.64 (0.20-2.06)
Male	540/914	66.5/70.5	31.1/27.5	2.4/2.0	1.24 (0.98-1.57)	1.38 (0.66-2.89)
$P_{interaction}$					0.725	
Smoking status						
Never	188/371	68.6/74.4	29.3/24.3	2.1/1.3	1.33 (0.90-1.99)	1.72 (0.45-6.57)
Former	282/549	66.3/69.6	31.2/27.0	2.5/3.4	1.22 (0.89-1.67)	0.75 (0.31-1.82)
Current	251/314	62.1/69.4	35.5/28.3	2.4/2.3	1.34 (0.92-1.93)	1.16 (0.38-3.56)
$P_{interaction}$					<0.001†	
Drinking status						
Never	157/373	66.9/69.7	31.8/28.2	1.3/2.1	1.20 (0.80-1.81)	0.57 (0.12-2.76)
Former	195/322	63.6/71.4	33.3/24.5	3.1/4.1	1.53 (1.03-2.27)	0.86 (0.32-2.36)
Current	369/539	65.9/71.6	31.7/26.5	2.4/1.9	1.29 (0.96-1.73)	1.52 (0.60-3.84)
$P_{interaction}$					0.259	
Tumor site						
Oral cavity	222 (30.8)	60.8	36.0	3.2	1.58 (1.16-2.16)	1.27 (0.54-2.97)
Pharynx‡	363 (50.3)	67.2	30.0	2.8	1.24 (0.95-1.61)	1.38 (0.66-2.91)
Larynx	136 (18.9)	68.4	31.6	0.0	1.17 (0.79-1.73)	-

*ORs were adjusted for age, sex, smoking status, and alcohol use in a logistic regression model.
† This significance disappeared when comparison was made between groups of never versus ever users (P = 0.758 for *MTR* and P = 0.743 for *MTRR*).
‡ Included both oropharyngeal, and hypopharyngeal cancer cases.

34.0%, and 10.4%, respectively, among the controls, and the difference was statistically significant (P = 0.003). When we used the 0 risk allele as the reference, the group 1, 2, and 3/4 risk alleles were associated with a significantly increased risk of SCCHN (adjusted OR, 1.47; 95% CI, 1.08-1.99 for one risk allele; OR, 1.67; 95% CI, 1.23-2.27 for two risk alleles; and OR, 1.74; 95% CI, 1.18-2.54 for three and four risk alleles) in the multivariate logistic regression analysis (P_{trend} = 0.002). However, there was no statistical evidence for any interactions between these combined genotypes and other risk factors presented in Table 3.

Discussion

Accumulated evidence suggests a role for folate deficiency in the etiology of SCCHN (8-10), and the *MTR* and *MTRR* genes play an important role in folate metabolism (12). Studies have revealed that the polymorphisms of these two genes, *MTR* A2756G and *MTRR* G66A, contributed to alteration of plasma levels of homocysteine and folate. At least three studies showed that individuals with the *MTR* 2756GG genotype had lower levels of plasma homocysteine (or higher levels of folate) than those with the 2756AA genotype (15-17), but one study reported no significant association between the *MTR* A2756G polymorphism and plasma folate level (18). One study suggested that the *MTRR* 66AA genotype contributed to a moderate increased total homocysteine level compared with the 66GG genotype (24), which was not confirmed by other studies (25, 26). Therefore, the association between polymorphisms of these two genes and risk of cancer reported by other studies (18, 19, 27, 28) also needs to be further substantiated in larger studies. To the best of our knowledge, the association between these two polymorphisms and risk of SCCHN has not been reported previously.

In the present study, our genotyping data suggest that the rarer (variant) *MTR 2756G* allele is a risk allele but the rarer (variant) *MTRR* 66A is a protective allele and that both the *MTR* A2756G and *MTRR* G66A polymorphisms had a main effect on SCCHN risk. Further analyses showed that the *MTR* 2756AG but not the *MTR* 2756GG (due to small number of subjects) genotype was associated with a significantly increased risk of SCCHN compared with the 2756AA genotype, and this increased risk was more pronounced among younger subjects, women, former smokers, and cancer of the oral cavity. These findings are consistent with published data on malignant lymphoma (19) but not in agreement with the data on colorectal cancer (18). We also found that the *MTRR* 66AA (but not GA) genotype provided a significant protection against SCCHN (pharyngeal cancer in particular) compared with the 66GG genotype, especially in older men, never or former smokers, and never drinkers. These results were similar to the reported data on colorectal cancer (27, 28).

Le Marchand et al. reported a significant interaction of the *MTR* A2756G and *MTHFR* C677T polymorphisms in protection against colorectal cancer (28), suggesting that there might be a gene-gene interaction involved in folate metabolism. Because the *MTR* and *MTRR* genes are involved in the same pathway of remethylation of homocysteine to methionine, we thought there would be an interaction of these two genes on SCCHN risk. Indeed, our data showed that the SCCHN risk was significantly increased in a dose-response manner as the number of the risk alleles increased, suggesting that the risk alleles of *MTR* A2756G and *MTRR* G66A may have a joint effect in increasing SCCHN risk. Because the interactions among folate-related genes, folate, and related dietary factors in tumorigenesis are complex (12, 32), this possible gene-gene interaction and the mechanisms under which these polymorphisms affect risk of SCCHN warrant further investigations.

To date, studies have shown that the dysfunctional mutations in the *MTR* gene contribute to an elevation of plasma homocysteine levels as a consequence of altered folate metabolism and that defects in MTR activity may be important

Table 3. Associations and stratification analysis between the *MTR* and *MTRR* polymorphisms and SCCHN risk (Cont'd)

MTRR G66A				
Percentage (cases/controls)			Adjusted OR (95% CI)*	
GG	GA	AA	GA	AA
32.0/29.9	52.2/47.7	15.8/22.4		
30.1/29.5	52.7/47.0	17.2/23.5	1.11 (0.80-1.54)	0.70 (0.47-1.06)
33.7/30.2	51.7/48.3	14.6/21.5	0.98 (0.73-1.30)	0.61 (0.41-0.90)
			0.571	
33.1/30.0	49.2/49.7	17.7/20.3	0.90 (0.59-1.39)	0.75 (0.43-1.31)
31.7/29.9	53.1/47.0	15.2/23.1	1.06 (0.83-1.36)	0.62 (0.45-0.86)
			0.534	
33.5/29.9	50.0/43.9	16.5/26.2	1.02 (0.69-1.53)	0.57 (0.34-0.96)
32.6/28.8	54.6/47.3	12.8/23.9	1.02 (0.74-1.42)	0.47 (0.30-0.74)
30.3/31.8	51.0/52.9	18.7/15.3	1.01 (0.69-1.49)	1.24 (0.74-2.07)
			0.012†	
33.1/27.6	49.1/45.6	17.8/26.8	0.88 (0.57-1.36)	0.54 (0.32-0.93)
31.8/28.6	52.8/52.8	15.4/18.6	0.92 (0.61-1.38)	0.75 (0.43-1.29)
31.7/32.3	53.1/46.2	15.2/21.5	1.16 (0.86-1.58)	0.71 (0.48-1.06)
			0.470	
35.1	46.9	18.0	0.83 (0.60-1.15)	0.72 (0.48-1.10)
31.1	55.9	13.0	1.15 (0.88-1.50)	0.55 (0.38-0.81)
29.4	50.7	19.9	1.06 (0.70-1.61)	1.00 (0.59-1.68)

in tumorigenesis (33). Under these circumstances, DNA synthesis may be disturbed by a dysfunctional mutation in the *MTR* and *MTRR* genes by means of affecting transformation of 5-methyltetrahydrofolate to 5,10-methylenetetrahydrofolate, leading to altered DNA repair. Therefore, the *MTR* A2756G and *MTRR* G66A polymorphisms that may cause folate deficiency could increase risk of SCCHN. However, this hypothesis needs to be investigated in future studies.

The major shortcoming of this study is the lack of data on detailed alcohol and dietary intake as well as serum levels of folate and its precursors or metabolites such as homocysteine, because the effect of genetic variations in folate metabolic genes on cancer risk will depend on alcohol and folate intake status (18). Because our study was hospital-based, there are inherent limitations in our study design that could introduce selection bias, compared with population-based studies. However, the genotype distributions in our study population were similar to reported distributions in other studies. For instance, the frequencies of the AA, AG, and GG genotypes of the *MTR* A2756G among our 1,234 Texan non-Hispanic White controls were 71.0%, 26.5%, and 2.5%, respectively, compared with 70.9%, 25.8%, and 3.3%, respectively, for 364 Dutch population-based controls (14) and 67.1%, 29.2%, and 3.7%, respectively, for 767 Physicians' Health Study prospective Caucasian controls (15). The frequencies of the AA, AG, and GG genotypes of the *MTRR* G66A among our 1,234 Texan non-Hispanic White controls were 22.4%, 47.7%, and 29.9%, respectively, compared with 22.9%, 47.5%, and 29.6%, respectively, for 257 Italian Caucasian controls (34) and 21.5%, 50.5%, and 28.0%, respectively, for 205 White Minnesota controls (35). Because the genotype frequencies of the *MTR* A2756G and *MTRR* G66A polymorphisms estimated from the hospital-based controls in our study are very close to those of population-based controls, the selection bias in genotype distribution, if any, is unlikely to be substantial.

Table 4. ORs and 95% CIs for the combined *MTR* and *MTRR* genotypes associated with SCCHN risk

MTR A2756G/*MTRR* G66A combined	No. risk alleles	Cases (n = 721), n (%)	Controls (n = 1,234), n (%)	Crude OR (95% CI)	Adjusted OR (95% CI)*
MTR AA/*MTRR* AA	0	76 (10.5)	200 (16.2)	1.00 (reference)	1.00 (reference)
MTR AA/*MTRR* GA or *MTR* AG/*MTRR* AA	1	281 (39.0)	486 (39.4)	1.52 (1.12-2.06)	1.47 (1.08-1.99)
MTR AA/*MTRR* GG or *MTR* AG/*MTRR* GA or *MTR* GG/*MTRR* AA	2	276 (38.3)	420 (34.0)	1.73 (1.28-2.34)	1.67 (1.23-2.27)
MTR AG/*MTRR* GG or *MTR* GG/*MTRR* GA or *MTR* GG/*MTRR* GG	3 or 4	88 (12.2)	128 (10.4)	1.81 (1.24-2.64)	1.74 (1.18-2.54)
P†			0.003		
P_{trend}‡				0.001	0.002

NOTE: The combined *MTR* AA and *MTRR* AA genotype had zero risk allele of either gene; the *MTR* AA and *MTRR* GA or *MTR* AG/*MTRR* AA genotype had only one risk allele; the *MTR* AA and *MTRR* GG or *MTR* AG and *MTRR* GA or *MTR* GG and *MTRR* AA had two risk alleles; and *MTR* AG and *MTRR* GG or *MTR* GG and *MTRR* GA or *MTR* GG/*MTRR* GG had three or four risk alleles.

*ORs were adjusted for age, sex, smoking status, and alcohol use in a logistic regression model.

† χ^2 test for the distribution of the combined genotypes between the cases and controls.

‡ Obtained in a logistic regression model with adjustments for age, sex, smoking status, and alcohol use. However, there was no evidence for an interaction with other variables listed in Table 3.

In conclusion, we found a statistically significant association between the *MTR* A2756G and *MTRR* G66A polymorphisms and risk of SCCHN in a relatively large hospital-based case-control study, and these two polymorphisms seemed to jointly contribute to genetic susceptibility to SCCHN. However, there was no statistical evidence that these two polymorphisms interacted with other risk factors in this study population.

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