Polymorphisms of 5,10-methylenetetrahydrofolate reductase and thymidylate synthase in squamous cell carcinoma and basal cell carcinoma of the skin

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Abstract. Genetic instability resulting from mutations in repair genes, defects in folic acid metabolism or DNA synthesis has been reported to contribute significantly to the development of skin cancer. The enzymes 5,10-methylenetetrahydrofolate reductase (MTHFR) and thymidylate synthase (TS) are essential participants in folic acid metabolism and DNA synthesis. Thus, the present case-control study was conducted to determine whether an association exists between the *MTHFR*/*TS* polymorphisms and squamous cell carcinoma (SCC) and/or basal cell carcinoma (BCC) among Korean individuals. The study subjects comprised 95 patients with SCC, 100 patients with BCC and 207 controls with no evidence of malignancy or pre-malignant lesions. Patients with skin cancer and control samples were analyzed for polymorphisms of the *MTHFR* or *TS* genes by means of polymerase chain reaction-restriction fragment length polymorphism. The *MTHFR* 677C>T and *MTHFR* 1298A>C polymorphisms showed no significance with regard to the development of SCC and BCC. However, within the 6 bp insertion (ins)/deletion (del) polymorphism in the 3'-untranslated region (3'-UTR) of the *TS* gene, the BCC group showed statistical significance with a 2.8-fold increased risk of cancer development [adjusted odds ratio (AOR)=2.821] in heterozygous mutations (0 bp/6 bp), 7.5-fold (AOR=7.539) in homozygous mutations (6 bp/6 bp) and 3-fold (AOR=3.079) upon combination of heterozygous mutations and homozygous mutations (0 bp/6 bp + 6 bp/6 bp). We thus conclude that the 6 bp ins/del polymorphism in the 3'-UTR is associated with increased risk of the development of skin cancer among Korean individuals with BCC.

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Introduction

Skin cancers such as basal cell carcinoma (BCC) and squamous cell carcinoma (SCC) are reported to occur as a consequence of genetic instability that includes mutations in repair genes, defects in folic acid synthesis or DNA synthesis. Mutations resulting in such genetic instability modify protein synthesis and function, as well as the capacity for repairing DNA damage, thereby inducing a carcinogenic effect (1).

5,10-methylenetetrahydrofolate reductase (*MTHFR*), located on chromosome 1p36.3, is involved in the metabolic process of methionine synthesis by means of homocysteine after converting 5,10-methylenetetrahydrofolate into 5-methylenetetrahydrofolate, and either suppresses or increases the likelihood of carcinogenesis. Hypomethylation of DNA resulting from the functional decline of MTHFR gives rise to oncogenes such as *c*-myc and *c*-N-ras, and leads to defects in DNA synthesis and repair (2). It also potentiates DNA synthesis and repair through a folate co-factor, thus preventing uracil misincorporation and double-strand breaks (3). The processes of methylation as well as DNA synthesis and repair are secured with the assistance of such mutual complementary interactions.

Mutations of the *MTHFR* gene include polymorphisms of 677C>T with an alanine (A) to valine (V) substitution and 1298A>C with a glutamate (G) to alanine (A) substitution (4). The 677TT homozygous variants have only 30% the activity of the normal enzyme, while the heterozygous variants retain 65% of normal activity (5,6). On the other hand, a homozygous variant, 1298CC, retains only 40% of its enzymatic activity compared to 1298AA (7). As a result, the accumulation of homocysteine that is not converted to methionine leads to defects in methionine synthesis, DNA methylation and dTMP synthesis (8,9). This consequently increases the risk of the development of various vascular diseases (10), stomach cancer (11) and esophageal cancer (12). By contrast, such defects have

been reported to act against the development of hepatocellular carcinoma, colon cancer (13) and acute lymphoblastic leukemia (14).

Thymidylate synthase (TS), located on the short arm of chromosome 18, is an enzyme responsible for the transformation of dUMP into dTMP during the process of converting 5,10-methylene-tetrahydrofolate into 5-methylenetetrahydrofolate. It therefore acts as a co-factor in folic acid metabolism as well as DNA synthesis (15-18). The untranslated region (UTR) of the *TS* gene is known to play an important role in transcription and translocation (19-22).

TS is characterized by 3 polymorphisms: its 5'- and 3'-UTR, which are a variable number of tandem repeats, a G/C SNP in the TS enhance region (TSER) and a 3'-UTR 1494 6 bp insertion (ins)/deletion (del) mutation. All three polymorphisms pertain to the expression of the *TS* gene (23-25). The 3'-UTR 1494 6 bp ins/del mutation in particular is related to the long-term stability of mRNA. Mutations in the *TS* gene lead to an accumulation of homocysteine, a decrease in methionine synthesis and a subsequent decrease in TS levels due to a defect in dTMP synthesis. This results in a single or double-strand break depletion through uracil misincorporation (26). Some researchers have reported that dysfunctional TS increases the risk of colon cancer, non-Hodgkin's lymphoma and breast cancer (27-29), and found that *TS* gene expression in malignant melanoma was considerably increased compared to normal controls.

In view of the key roles that MTHFR and TS play in the occurrence of skin cancer, we performed an association study between polymorphisms of the *MTHFR* and *TS* genes and skin cancer.

Materials and methods

Study subject. The case group comprised 195 patients (mean age ± SD 69.13±12.6 years; range 36-94) with non-melanoma skin cancer recruited from the Department of Plastic and Reconstructive Surgery of Yonsei Wonju Christian Hospital and the Department of Plastic and Reconstructive Surgery of Bundang CHA Medical Center between January 1998 and March 2006. Of these patients, 100 were pathologically diagnosed with BCC and the other 95 with SCC. The control group consisted of 207 individuals (mean age ± SD 46.42±16.6 years; range 21-85) without any history of pre-malignant skin lesions or other malignant disorders randomly selected from the visitors to the Bundnag CHA Medical Center. The institutional review board of Bundang CHA Medical Center approved this genetic study. All the patients and controls were Korean and gave their informed consent prior to enrollment in the study.

Genetic analysis. Genomic DNA was extracted from anti-coagulated peripheral blood using the G-DEX blood extraction kit (Intron, Seongnam, Korea). Nucleotide changes in the *MTHFR* 677C>T and 1298A>C genotypes were determined by polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) analysis using isolated genomic DNA as a template. The polymorphisms were identified following the digestion of amplified DNA with the endonucleases *Hin*fI and *Fnu*4HI for the *MTHFR* 677C>T and 1298A>C polymorphisms. The ins/del polymorphism in the 3'-UTR of the *TS* gene was classified as 6 bp or 0 bp according to a 6 bp insertion or deletion after *Dra*I digestion. Primers and PCR conditions for each polymorphism analysis were as described previously (5,30).

Statistical analysis. The χ^2 test was used to compare allele and genotype frequencies between the case and control groups and to test for Hardy-Weinberg equilibrium. To measure the strength of the association between genotype frequencies and the case group, odds ratios (ORs) and 95% confidence intervals (CIs) were used. For multivariate analysis, logistic regression was used, adjusted for the effects for possible confounders (age, gender, number of cigarettes smoked per day, occupation and duration of sun exposure per day). Statistical significance was accepted at the $p<0.05$ level. Analysis was performed using SPSS for Windows version 11.0 (SPSS Inc., Chicago, IL, USA) and SNPAlyze™ version 5.10 (Dynacom Co., Ltd., Yokohama, Japan).

Results

MTHFR 677C>T and 1298A>C polymorphisms. No significant association was found between the risk of the development of BCC or SCC and the *MTHFR* 677C>T and 1298A>C polymorphisms in comparison to the control group. The adjusted odds ratio (AOR) values, adjusted to age, gender, smoking history, occupation and duration of sun exposure, showed no statistical significance (Tables I and II). However, the *MTHFR* 677C>T mutation had the tendency to suppress the development of SCC, while *MTHFR* 1298A>C mutation tended to promote the occurrence of SCC. Heterozygous mutation of *MTHFR* 677C>T, in comparison to its homozygous mutation, served to suppress SCC carcinogenesis by 70% (AOR=0.304, 95% CI 0.07-1.25), while the overall (CT+TT) genotype showed an overall suppression of carcinogenesis by 50% (AOR=0.516, 95% CI 0.21-1.25).

A combination analysis of the *MTHFR* 677C>T and *MTHFR* 1298A>C polymorphisms did not reveal statistically significant differences in either SCC or BCC (Tables I and II). However, in SCC, combined genotypes of 677CC+1298AC, 677CT+1298AA and 677TT+1298AA (AOR=0.538, 0.281 and 0.146; 95% CI 0.12-2.34, 0.07-1.19 and 0.02-1.00, respectively) suppressed carcinogenesis with marginal significance. The combined genotype of 677TT+1298AA had a suppressive effect against SCC (Table I). Although no statistically significant difference was found in BCC, the combined genotypes of 677CC+1298AC, 677CT+1298AA and 677TT+1298AA (AOR=0.206, 0.399 and 0.350; 95% CI 0.03-1.23, 0.14-1.11 and 0.09-1.32, respectively) tended to reduce the risk of cancer by >60% in comparison to the genotype 677CC+1298AA (Table II).

6 bp ins/del polymorphism in the 3'-UTR of the TS gene. Although the *TS* 3'-UTR 6 bp ins/del polymorphism in SCC showed no statistical significance compared to the control group (Table III), it significantly increased the risk of BCC by 2.8-fold (AOR=2.821) in heterozygous variants, 7-fold (AOR=7.539) in homozygous variants and 3-fold (AOR=3.079) with the combination of the variants (0 bp/6 bp + 6 bp/6 bp).

Table I. Polymorphisms of *MTHFR* 677C>T and *MTHFR* 1298A>C in squamous cell carcinoma.

Genotype	Controls (%)	Cases (%)	OR (95% CI)	AOR (95% CI)[a]
677C>T				
CC	71 (34.3)	40 (42.1)	-	-
CT	106 (51.2)	44 (46.3)	0.737 (0.44-1.24)	0.590 (0.24-1.47)
TT	30 (14.5)	11 (11.6)	0.651 (0.30-1.44)	0.304 (0.07-1.25)
CT+TT	136 (65.7)	55 (57.9)	0.718 (0.44-1.18)	0.516 (0.21-1.25)
1298A>C				
AA	149 (72.0)	60 (63.2)	-	-
AC	54 (26.1)	35 (36.8)	1.610 (0.96-2.71)	1.737 (0.69-4.38)
CC	4 (1.9)	0 (0.0)	0.275 (0.02-5.18)	-
AC+CC	58 (28.0)	35 (36.8)	1.499 (0.90-2.51)	1.585 (0.64-3.93)
677C>T/1298A>C				
CC/AA	38 (18.4)	23 (24.2)	-	-
CC/AC	29 (14.0)	17 (17.9)	0.969 (0.44-2.14)	0.538 (0.12-2.34)
CC/CC	4 (1.9)	0 (0.0)	0.182 (0.01-3.54)	-
CT/AA	81 (39.1)	26 (27.4)	0.530 (0.27-1.05)	0.281 (0.07-1.19)
CT/AC	25 (12.1)	18 (18.9)	1.190 (0.54-2.64)	0.726 (0.17-3.07)
TT/AA	30 (14.5)	11 (11.6)	0.606 (0.26-1.44)	0.146 (0.02-1.00)
Total	207 (100.0)	95 (100.0)		

[a]Adjusted for age, gender, number of cigarettes smoked per day, occupation (outdoor or indoor) and duration of sun exposure per day. OR, odds ratio; CI, confidence interval; AOR, adjusted odds ratio.

Table II. Polymorphisms of *MTHFR* 677C>T and *MTHFR* 1298A>C in basal cell carcinoma.

Genotype	Controls (%)	Cases (%)	OR (95% CI)	AOR (95% CI)[a]
677C>T				
CC	71 (34.3)	35 (35.0)	-	-
CT	106 (51.2)	49 (49.0)	0.938 (0.55-1.59)	0.676 (0.30-1.55)
TT	30 (14.5)	16 (16.0)	1.082 (0.52-2.24)	0.566 (0.17-1.84)
CT+TT	136 (65.7)	65 (65.0)	0.970 (0.59-1.60)	0.675 (0.31-1.45)
1298A>C				
AA	149 (72.0)	79 (79.0)	-	-
AC	54 (26.1)	20 (20.0)	0.699 (0.39-1.25)	0.480 (0.18-1.26)
CC	4 (1.9)	1 (1.0)	0.472 (0.05-4.29)	0.232 (0.01-6.11)
AC+CC	58 (28.0)	21 (21.0)	0.683 (0.39-1.21)	0.450 (0.18-1.16)
677C>T/1298A>C				
CC/AA	38 (18.4)	24 (24.0)	-	-
CC/AC	29 (14.0)	10 (10.0)	0.546 (0.23-1.32)	0.206 (0.03-1.23)
CC/CC	4 (1.9)	1 (1.0)	0.396 (0.04-3.76)	-
CT/AA	81 (39.1)	39 (39.0)	0.762 (0.40-1.44)	0.399 (0.14-1.11)
CT/AC	25 (12.1)	10 (10.0)	0.633 (0.26-1.55)	0.295 (0.05-1.61)
TT/AA	30 (14.5)	16 (16.0)	0.844 (0.38-1.87)	0.350 (0.09-1.32)
Total	207 (100.0)	100 (100.0)		

[a]Adjusted for age, gender, no. of cigarettes per day, occupation and duration of sun exposure per day. OR, odds ratio; CI, confidence interval; AOR, adjusted odds ratio.

Table III. Polymorphisms of the *TS* 3'-UTR 1494 6 bp insertion/deletion in squamous cell carcinoma and basal cell carcinoma.

Genotype	Controls (%)	Cases (%)	OR (95% CI)	AOR (95% CI)[a]
SCC				
0 bp/0 bp	96 (46.6)	39 (41.9)	-	-
0 bp/6 bp	99 (48.1)	53 (57.0)	1.318 (0.80-2.17)	1.970 (0.50-2.87)
6 bp/6 bp	11 (5.3)	1 (1.1)	0.224 (0.03-1.79)	2.058 (0.17-25.40)
0 bp/6 bp + 6 bp/6 bp	110 (53.4)	54 (58.1)	1.208 (0.74-1.98)	1.234 (0.52-2.95)
BCC				
0 bp/0 bp	96 (46.6)	28 (28.0)	-	-
0 bp/6 bp	99 (48.1)	65 (65.0)	2.251 (1.33-3.80)	2.821 (1.21-6.58)
6 bp/6 bp	11 (5.3)	7 (7.0)	2.182 (0.77-6.16)	7.539 (1.10-51.66)
0 bp/6 bp + 6 bp/6 bp	110 (53.4)	72 (72.0)	2.244 (1.34-3.76)	3.079 (1.33-7.12)
Total	206 (100.0)	100 (100.0)		

[a]Adjusted for age, gender, number of cigarettes smoked per day, occupation and duration of sun exposure per day. SCC, squamous cell carcinoma; BCC, basal cell carcinoma; OR, odds ratio; CI, confidence interval; AOR, adjusted odds ratio.

Table IV. Combinations of *MTHFR* 677C>T, 1298A>C and the *TS* 3'-UTR 1494 6 bp insertion/deletion in basal cell carcinoma.

Genotype	Controls (%)	Cases (%)	OR (95% CI)	AOR (95% CI)[c]
MTHFR 677C>T/*TS* 6 bp ins/del				
CC/6 bp(-)[a]	34 (16.5)	8 (8.0)	-	-
CC/ 6 bp(+)[b]	37 (18.0)	27 (27.0)	3.101 (1.24-7.75)	4.192 (0.75-23.48)
CT/6 bp(-)	51 (24.8)	14 (14.0)	1.167 (0.44-3.08)	0.374 (0.06-2.46)
CT/6 bp(+)	54 (26.2)	35 (35.0)	2.755 (1.14-6.64)	2.809 (0.68-11.57)
TT/6 bp(-)	11 (5.3)	6 (6.0)	2.318 (0.66-8.16)	54.750 (0.40-7.46)
TT/6 bp(+)	19 (9.2)	10 (10.0)	2.237 (0.76-6.63)	1.204 (0.20-7.40)
MTHFR 1298A>C/*TS* 6 bp ins/del				
AA/6 bp(-)	71 (34.5)	23 (23.0)	-	-
AA/6 bp(+)	77 (37.4)	56 (56.0)	2.245 (1.25-4.02)	3.288 (1.24-8.73)
AC/6 bp(-)	23 (11.2)	4 (4.0)	0.537 (0.17-1.72)	0.509 (0.08-3.20)
AC/6 bp(+)	31 (15.0)	16 (16.0)	1.593 (0.74-3.42)	1.703 (0.38-7.69)
CC/6 bp(-)	2 (1.0)	1 (1.0)	1.543 (0.13-17.83)	-
CC/6 bp(+)	2 (1.0)	0 (0.0)	0.609 (0.03-13.14)	-
Total	206 (100.0)	100 (100.0)		

[a]6 bp(-) represents 0 bp/0 bp. [b]6 bp(+) represents 0 bp/6 bp and 6 bp/6 bp. [c]Adjusted for age, gender, number of cigarettes smoked per day, occupation and duration of sun exposure per day. OR, odds ratio; CI, confidence interval; AOR, adjusted odds ratio.

However, the combined 677CC/6 bp(+) and 677CT/6 bp(+) genotypes of the *MTHFR* 677C>T and *TS* 6 bp ins/del polymorphisms were found to increase the risk for BCC when adjusted for age, gender, smoking history, occupation and duration of sun exposure (Table IV). When the *MTHFR* 677C>T and *TS* 6 bp ins/del polymorphisms were combined, the 1298AA/6 bp(+) combined genotype was responsible for elevating the risk of cancer development by >3-fold (AOR=3.288, 95% CI 1.24-8.73). However, the combined genotypes of the *MTHFR* 677C>T and *TS* 6 bp ins/del polymorphisms did not show any association with SCC.

Discussion

In the present study, the *MTHFR* 677C>T polymorphism did not significantly contribute to the development of SCC when adjusted to conventional factors such as age, gender, smoking history, occupation and duration of sun exposure. However, the 677CT and 677TT genotypes tended to suppress the development of SCC. When only age was taken into consideration (data not shown), both a homozygous mutation (677TT) and an overall genotype (677CT+TT) of the *MTHFR* 677C>T polymorphism showed a statistically significant difference

(OR=0.334, 95% CI 0.12-0.92; OR=0.343, 95% CI 0.13-0.94, respectively). The *MTHFR* 677TT genotype (OR=0.343, 95% CI 0.13-0.94) upon adjustment for age and gender as well as the 677CT+TT genotype (OR=0.437, 95% CI 0.20-0.94) upon adjustment for age, gender and smoking history were associated with a significantly reduced risk of cancer. This suggests that the *MTHFR* 677C>T polymorphism has a very low probability of inducing carcinogenesis. Concerning the *MTHFR* 1298A>C polymorphism in SCC of the skin, there was a 2-fold increased risk of cancer development associated with the 1298AC genotype (OR=2.208, 95% CI 1.09-4.48) upon adjustment for age, and with the 1298AC genotype and overall genotype of 1298AC+CC (OR=2.267, 95% CI 1.11-4.63; OR=2.034, 95% CI 1.01-4.09) upon adjustment for age and gender, and for age, gender and smoking history (OR=2.381, 95% CI 1.71-5.26).

Due to the lack of studies on the *MTHFR* 677C>T and *MTHFR* 1298A>C polymorphisms in SCC of the skin, it is not possible to make direct comparisons. There is, however, a study conducted among Hispanic Americans with regards to SCC of the head and neck (31). This study documented that the 1298AC and 1298CC genotypes and the overall genotype (1298AC+CC) significantly suppressed the occurrence of cancer (AOR=0.69, 0.28 and 0.65; 95% CI 0.5-0.9, 0.1-0.6 and 0.5-0.8, respectively) upon adjustment for age, gender and smoking and drinking history.

When studying crude OR, the 677CT genotype of the *MTHFR* 677C>T polymorphism appeared to increase the risk of cancer development, though without statistical significance. Upon categorizing subjects with less than one mutation and those with two or more mutations, the possibility of cancer development increased by 2- to 3-fold in each of the cases where there was an increase in age, smoking and drinking, while all the conventional risk factors combined showed no statistical significance. Overall, the *MTHFR* 677C>T polymorphism had a tendency to inhibit carcinogenesis in SCC of the skin, in contrast to the *MTHFR* 1298A>C polymorphism, which tended to promote carcinogenesis. It is necessary to consider the difference between the location of cancer occurrence as well as the ethnicity of the patients.

Through studies of single nucleotide polymorphisms (SNPs), enzymes that are involved in the process of base repair, such as X-ray repair cross-complementing groups 1 (*XRCC1*), have been reported to be associated with the development of BCC (32-36).

With regards to BCC, the *MTHFR* 677C>T and *MTHFR* 1298A>C polymorphisms did not show any statistical significance in this study, but both had the tendency to supress the occurrence of BCC by 35 and 60%, respectively. The 1298CC genotype of the *MTHFR* 1298A>C polymorphism in particular provided more than 70% inhibition compared to the 1298AA normal genotype. Taken together, the results of the present study reflect the suppressive effects of the *C* allele of the *MTHFR* 1298A>C polymorphism on cancer development. Overall, the 1298AC+CC genotype of the *MTHFR* 1298A>C polymorphism, upon adjustment for duration of sun exposure, showed a significance of 50% (OR=0.48, 95% CI 0.234-0.985) in suppressing carcinogenesis compared to the 1298AA genotype.

In a study performed on the correlation between the *MTHFR* 677C>T polymorphism and the development of BCC among a Swedish population, the 677TT genotype conferred an increased risk of BCC development compared to the 677CC genotype (OR=1.67, 95% CI 1.13-2.47). This indicated that the *T* allele was associated with an elevated risk of cancer development. The *C* allele of the *MTHFR* 1298A>C polymorphism was associated with the occurrence of cancer, though without statistical significance (37). Investigation into the *MTHFR* 677C>T and *MTHFR* 1298A>C polymorphisms in cases with BCC of the skin among Swedish and Finnish populations revealed no statistical significance (36). However, a combination analysis of the 677TT genotype of the *MTHFR* 677C>T polymorphism and of the AA genotype of the *MTHFR* 1298A>C polymorphism indicated that the mutations (677TT/1298AA) increased the likelihood of cancer development (OR=1.94, 95% CI 0.96-3.89, $p<0.07$).

In the present study, statistical significance was not obtained in the combination analysis of the *MTHFR* 677C>T and *MTHFR* 1298A>C polymorphisms, but the combined genotypes 677CC+1298AC, 677CT+1298AA and 677TT+1298AA (AOR=0.206, 0.399 and 0.350; 95% CI 0.03-1.23, 0.14-1.11 and 0.09-1.32, respectively) tended to diminish the risk of cancer development by more than 60% as compared to the 677CC+1298AA genotype. The combination of the *MTHFR* 677C>T and *MTHFR* 1298A>C mutations exerted a stronger suppressive effect; thus, coexistence of the two mutations is postulated to suppress cancer development to a greater degree. The *MTHFR* 1298A>C mutation demonstrated a greater suppressive effect; hence, it can be expected that the occurrence of cancer will be increasingly inhibited in individuals with both mutations. Furthermore, it can be presumed that the *MTHFR* 1298A allele exerted a stronger suppressive effect; hence, a homozygous variant would act as a stronger inhibitor of carcinogenesis. The same data is obtained in terms of OR. However, these results are contradictory to those obtained in Swedish and Finnish populations, indicating that the *C* allele of the *MTHFR* 1298A>C polymorphism exerts the opposite effect in Koreans.

Studies performed on the link between hepatocellular carcinoma and the *MTHFR* 677C>T, *MTHFR* 1298A>C and *TS* 3'-UTR 6 bp ins/del polymorphisms among individuals from Los Angeles, California and Guangxi, China did not exhibit statistical significance. However, the 677TT and 1298CC genotypes exerted 30-50% suppressive effects compared to their normal counterparts. Homozygous variants (677TT and 1298CC) had a tendency to suppress carcinogenesis, contrary to a case with only one mutation, which did not show any difference in the risk of cancer development. Despite a discrepancy in the type of cancer, the above results correlate entirely to those drawn in the present study.

This study is the first to report the link between TS and the development of skin cancer. Thymidylate, as a rate-limiting nucleotide, is involved in the process of DNA synthesis and repair, and thus must be present in sufficient quantities to minimize the misincorporation of uracil into DNA, chromosome breakage and fragile site induction. The 0 bp/0 bp genotype has been reported in several studies to diminish the risk of the development of spina bifida, lung cancer, non-Hodgkin's lymphoma, breast cancer and malignant melanoma.

In the present study, the 6 bp/6 bp genotype of the *TS* 3'-UTR 6 bp ins/del polymorphism failed to produce statis-

tically significant differences as compared to the control group, although it showed an inclination to raise the risk of the development of SCC. With regards to BCC, the mutations were associated with an elevated risk of cancer. However, we were unable to produce statistically meaningful data due to the small number of homozygous variants within the BCC and control groups.

TS 3'-UTR 6 bp ins/del polymorphism analyses performed on a group of healthy individuals and a group of hepatocellular carcinoma patients (among them inhabitants of Los Angeles, California and Guangxi, China) showed that the 0 bp/6 bp and 0 bp/6 bp genotypes suppressed carcinogenesis as compared to the 6 bp/6 bp genotype. These results demonstrate that the 6 bp insertion contributed towards an increased risk of cancer development. Our data show that the 6 bp genotype was associated with a 2- to 3-fold increased risk of cancer. The Han population (Asians) was also found to be liable to an increased risk of cancer development. The 0 bp genotype of *TS* produced an enzyme with diminished functional capacity, leading to the accumulation of 5,10-methylenetetrahydrofolate. This consequently activated the synthesis of thymidylate. Such a phenomenon secondarily provides protective effects by minimizing the misincorporation of uracil and double-strand breaks (26).

In the present study, a combination analysis of the *MTHFR* and *TS* 3'-UTR polymorphisms did not provide any statistically significant results. Regarding SCC, however, the combined genotypes 677TT/6 bp of *TS* 3'-UTR afforded a 70% suppressive effect (AOR=1.776, 95% CI 0.41-7.63) compared to the genotype 677TT/0 bp. The 677TT genotype of the *MTHFR* 677C>T polymorphism is assumed to exert a stronger suppressive effect than the 6 bp insertion of the *TS* 3'-UTR polymorphism. On the other hand, the combination of 1298AC/6 bp had a tendency to produce a 2-fold increase in carcinogenesis (AOR=2.646, 95% CI 0.71-9.83) compared to a homozygous variant or 0 bp, presumably owing to both the mutations acting towards increasing the risk.

The combined genotypes 677CC/6 bp and 677CT/6 bp in BCC had a statistically significant tendency to increase the risk of cancer development. The combination of the homozygous variant (677TT) and 6 bp, when juxtaposed with the combination of the heterozygous variant (677CT) and 6 bp(-), displayed a 3-fold increase in the likelihood of carcinogenesis. We therefore postulated that the alleles *677T* and *6 bp* exerted a synergistic effect. The combined genotype 1298AA/6 bp(+) of the *MTHFR* 1298A>C and 3'-UTR ins/del polymorphisms had an AOR of 3.288 with a 95% CI of 1.24-8.73, thus increasing the risk of cancer development by 3-fold (Table IV). It is assumed that the combination genotypes, in the absence of the suppressive action of the *1298C* allele, exerted a synergistic effect, increasing the risk of cancer by 6-fold in the case of the combined genotype 1298AC and 6 bp(-). The suppressive action of the *C* allele and promoting action of the 6 bp insertion were also assumed to be relatively stronger.

Further studies taking into account the variable number of tandem repeats and the G/C SNP of the *TS* gene are required. Environmental factors that were excluded in the present study, such as folic acid intake, also warrant examination in relation to carcinogenesis of the skin. Such studies may form the basis on which may be built the possibility of accurate identification of groups manifesting risk factors for the early diagnosis of cancer.

Conclusively, although there were no associations between the *MTHFR* and *TS* polymorphisms and patients with skin cancer among Korean individuals, a relationship between the type of skin cancer and the combined genotypes was demonstrated to exist. To the best of our knowledge, this is to date the first study on the significant association between the *TS* polymorphism and skin cancer patients. To obtain more evidence on the association between *TS* polymorphisms and skin cancer, population studies conducted among other ethnicities are required. Furthermore, the biochemical mechanisms of the *MTHFR* and *TS* polymorphisms require analysis.

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