

## C677T Gene Polymorphism of Methylenetetrahydrofolate Reductase (MTHFR) in Meningiomas and High-grade Gliomas

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**Abstract.** Background: Methylenetetrahydrofolate reductase (MTHFR) plays a role in DNA biosynthesis, methylation and repair in actively dividing cells by acting on folate metabolism. A common C677T polymorphism in the gene for MTHFR leads to an enzyme with decreased activity. MTHFR polymorphisms have been studied in various cancers but not in primary brain tumors. The purpose of this case-control study was to explore a possible association between MTHFR C677T polymorphism and primary brain tumors. Materials and Methods: The MTHFR C677T genotype was determined in 74 patients with histologically-verified primary brain tumors and 98 cancer-free control subjects. Results: The MTHFR 677T variant genotype was observed in 49% of cases and 46% of controls. Although the difference was not significant ( $p=0.194$ ), the homozygous TT genotype was found at a higher frequency in high-grade glioma (HGG) patients compared to controls (15.4% and 7.1%, respectively). The MTHFR genotype was not associated with meningioma patients. Defining patients with the CC genotype as reference, the relative risk of HGG for subjects with the T allele (CT+TT genotype) was 1.17. Conclusion: In spite of the established effect of the MTHFR 677 TT genotype on DNA hypomethylation with concomitant inadequate folate levels, the MTHFR 677 TT genotype is not associated with individual susceptibility to HGG.

The flavin adenine dinucleotide (FAD)-dependent enzyme methylenetetrahydrofolate reductase (MTHFR) catalyzes the irreversible conversion of 5,10-methylenetetrahydrofolate

to 5-methyltetrahydrofolate. MTHFR directs folate species either to DNA synthesis or to homocysteine (Hcy) remethylation. A common thermolabile mutation in the MTHFR gene, consisting of a cytosine (C) to thymidine (T) substitution at nucleotide position 677, leads to the exchange of a highly conserved alanine to valine (677C→T, alanine →valine), resulting in reduced activity of this enzyme and, hence, folate distribution. The MTHFR 677 TT genotype led to elevated homocysteine levels and DNA hypomethylation in folate-depleted subjects. (1, 2). Low serum folate levels are known to cause several cancers (3-6) by influencing DNA methylation (7, 8).

In folate-replete subjects, the TT genotype affords 50% risk reduction, whereas in subjects with low folate status, the TT genotype confers no protection or probable risk enhancement in colon cancer (9, 10). Published data on the MTHFR C677T polymorphism and cancer risk indicate that the T allele protected against cancer in folate-replete subjects, but increased the risk under conditions of impaired folate status. The protection might be related to abundant purines and pyrimidines available for DNA synthesis, leading to efficient DNA repair and essentially no uracil incorporation into the DNA (2). Friso *et al.* determined that only the MTHFR 677 TT genotype with low levels of folate accounted for the diminished DNA methylation (1) known to be involved in carcinogenesis.

The relationships between MTHFR C677T polymorphism and cervical, colorectal and breast cancers were investigated (7, 9, 11). In another study, however, no association was found between the MTHFR polymorphism and breast cancer (12). In the course of brain tumor development, gene silencing by DNA methylation is an early and important mechanism by which tumor-suppressor genes are inactivated (13).

In this study, the association between primary brain tumors and MTHFR C677T polymorphism was analyzed in order to evaluate whether inherited polymorphisms regarding DNA methylation place an individual at increased risk of primary brain tumor development.

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Table I. Data for patients with high-grade gliomas, meningiomas and controls.

	High-grade glioma	Meningioma	Control
Female/Male	16/23	23/12	51/47
Age	47±12.7	48.1±12.6	46.9±9.8
Smoking history	28 (71%)	17 (48%)	58 (59%)
Symptoms of elevated ICP	29 (74%)	15 (42%)	–
Epilepsy	13 (33%)	7 (20%)	–
Duration of symptoms (months)	2.5±6.7	14.2±15.4	–
Edema on MRI	28 (71%)	14 (40%)	–
Necrosis on MRI	26 (59%)	6 (17%)	–

ICP=intracranial pressure.

## Materials and Methods

**Study population.** The hospital-based prospective case-control study included 74 patients (39 females and 35 males) with primary brain tumors. Eligible cases were newly-diagnosed high-grade glioma (HGG) (WHO classification grades III and IV ) and meningioma (grade I ) patients treated at the Istanbul University, Cerrahpasa Medical Faculty, Department of Neurosurgery, Turkey, from May 2004 to February 2005. Patients who had had previous radiotherapy and chemotherapy were not included in the study. All the patients underwent surgery and histopathological confirmations of the brain tumors were determined according to the World Health Organization Classification of Tumors (14). Among the 74 cases analyzed in this study, 39 were HGG and 35 were meningiomas. The clinical data of the patients were recorded and followed-up prospectively.

A total of 98 healthy persons (51 females, 47 males; mean age 46.9±9.81), with no previous cancer diagnosis or radio- or chemotherapy, were selected for the control group.

**DNA extraction and MTHFR C677T mutation.** Blood samples were drawn from the cases and controls and collected in tubes containing EDTA. The DNA samples were extracted from whole blood by a salting-out procedure (15).

The DNA samples were analyzed for the C677T missense mutation by using a polymerase chain reaction with locus-specific primers and subsequent analysis of a restriction fragment length polymorphism created by the mutation, as described elsewhere (16). The 677C→T substitution creates a *HinfI* recognition sequence, which digests the initial polymerase chain recognition product of 198 base pairs (bp) into 175- and 23-bp fragments. The presence of the mutation was determined by digestion of the initial polymerase chain reaction product with *HinfI* at 37°C for 24 h. The digested DNAs were separated on 3% agarose gel in 1x Tris borate EDTA buffer, followed by staining with ethidium bromide solution and the MTHFR C677T genotypes were typed by visualization under ultraviolet light (16).

**Statistical analyses.** The data are presented as means±SD. Statistical analyses, using SPSS version 10.0, included the  $\chi^2$  test for genotype and allele frequency comparison. Odds ratios and 95% confidence

Table II. Prevalence of the MTHFR C677T genotypes in patients and control subjects.

	Control (n=98)	HGG (n=39)	Meningioma (n=35)
MTHFR C677T			
CC	53 (54.1%)	18 (46.2%)	20 (57.1%)
TT	7 (7.1%)	6 (15.4%)	3 (8.6%)
CT	38 (38.8%)	15 (38.4%)	12 (34.3%)

\* $\chi^2=2.686$ ;  $p=0.612$ .

HGG=high-grade glioma.

Table III. Association between CC and CT + TT genotypes in tumor patients with control subjects after defining patients with CC genotype as reference.

	Control	HGG	Meningioma
CC	53 (54%)	18 (46%)	20 (57%)
CT + TT	45 (46%)	21 (54%)*	15 (43%)

Chi-square: 0.702,  $p=0.402$ ; OR: 1.17, 95% CI: 0.82-1.68.

HGG=high-grade glioma.

intervals were calculated as a measure of the relationships between primary brain tumors and MTHFR genotypes. The clinical characteristics were compared by the Student's *t*-test and C677T allele frequencies were estimated by gene counting methods. A *p*-value of less than 0.05 was regarded as being statistically significant.

## Results

The patient and control subject baseline characteristics are shown in Table I. The mean age was 47.5±12.67 years for the controls and 46.9±9.81 years for patients with primary brain tumors. The age of all the patients ranged from 23 to 74 years. There were no significant differences in age and sex between the patient and the control groups.

The MTHFR genotype was determined in 172 subjects (patients and controls). The distribution of each MTHFR genotype in HGG, meningiomas and controls is shown in Table II. Homozygosity (TT) of the MTHFR C677T gene mutation was more prevalent in HGG (15.4%) than in meningiomas (8.6%) or control subjects (7%), but this difference was not statistically significant. The relative risk of HGG in patients who had homozygotic (TT) MTHFR C677T mutations was twice that of the control group (OR=2.15; 95% CI: 0.77-6.0). This difference was not significant statistically (Chi-square= 2.206;  $p=0.137$ ).

Defining patients with the CC genotype as reference, the relative risk of HGG for subjects with the T allele (CT+TT genotype) was 1.17 (95% CI : 0.82 – 1.68,  $p=0.402$ ). There was no association between either (CC and non-CC

Table IV. Association between primary brain tumors and control subjects in terms of C and T alleles.

Alleles	All tumor patients	Meningioma	HGG *	Controls
C (+)	75 (100%)	35 (47% )	39 (53%)	98
p value	65 (87.8%)	32 (91.4%)	33 (84.6%)	91 (92.9%)
Odds ratio (95%CI)	0.262	0.783	0.137	–
T (+)	0.56 (0.19-1.56)	1.22 (0.30-5.00)	2.36 (0.74-7.54)	1.00
p value	36 (48.6%)	15 (42.9%)	21 (53.8%)	45 (45.9%)
	0.722	0.755	0.402	–
Odds ratio (95%CI)	1.12 (0.61-2.04)	1.13 (0.53-2.46)	0.72 (0.35-1.53)	1.00

\*HGG=high-grade glioma.

genotype) group regarding meningiomas. No differences were observed in the distribution of MTHFR CT+TT genotype frequencies in meningiomas *versus* controls (Chi-square: 0.098,  $p=0.752$ ) (Table III).

The association between specific types of brain tumor and control subjects regarding the C and T alleles of the MTHFR gene was further investigated. Although there was no statistically significant association between certain alleles and tumor types, the T allele was more frequent among all brain tumor groups and especially in HGG (53.8%), compared to controls (45.9%) and meningiomas (42.9%) (Table IV).

## Discussion

The MTHFR C667T polymorphism and the effect of the T allele and TT genotype, which in low serum folate levels may play a role in carcinogenesis through DNA hypomethylation, have not been investigated in primary brain tumors. In this study, the association between primary brain tumors and MTHFR C667T polymorphism was investigated to evaluate whether inherited polymorphisms regarding DNA methylation place an individual at increased risk of primary brain tumor development.

The DNA methylation patterns are maintained by DNA methyltransferases (17). Hypomethylation has frequently been observed in DNA repeats in diverse cancers. Global genomic hypomethylation has been reported in many types of human cancer, such as prostate (18), leukemia (19) and cervical cancer (20). Recently, it was demonstrated that the 677 C→T transition in the MTHFR gene affected genomic DNA methylation. These studies revealed that the MTHFR 677 TT genotype was associated with significantly decreased DNA methylation status, with concomitant inadequate folate levels, when compared with subjects bearing the wild-type genotype (CC) (1, 21).

In the present prospective case-control study, the primary brain tumors were divided into two groups, namely tumors with a malignant course (HGGs) and tumors with a benign course (meningiomas). Further, the difference in MTHFR

C677T genotypes between Grade 1 meningiomas and HGG were evaluated. No association between MTHFR C677T polymorphism and primary brain tumors was observed.

The etiology of brain tumors still remains obscure. The role of DNA hypermethylation in gliomagenesis has been widely investigated (22). Recently, it was demonstrated that an aberrant hypermethylation profile was associated with atypical and anaplastic meningiomas (23). Previous results showed that the glioma subtypes had characteristic methylation profiles and, with the exception of pilocytic astrocytomas, showed both locus specific hyper- as well as hypomethylation (24). The effect of the MTHFR C677T polymorphism on the leucocyte genomic DNA methylation status, which may have revealed more specific data regarding the role of hypomethylation in brain tumors, could not be assessed. However, as mentioned above, studies have shown that the MTHFR 677TT genotype was associated with significantly decreased DNA methylation status with concomitant inadequate folate levels (1, 21). The combination of low folate and the TT genotype impairs Hcy remethylation to methionine; this could cause DNA hypomethylation, known to be involved in carcinogenesis (2).

Although the association between MTHFR C677T mutation and several cancer types is well established (3, 5, 7, 9-11, 25), this polymorphism has not been investigated in primary brain tumors. In our study, the TT genotype was rare in both the control and patient groups. However, it was shown that in HGG patients homozygous 677TT MTHFR was more common compared to the control subjects (15.4% and 7.1%, respectively), although the difference was not statistically significant.

Grade I meningioma patients had TT genotype frequencies similar to the control group (8.6% and 7.1%, respectively), as expected since they have a relatively benign course compared to HGG. It is well established that abnormal DNA methylation patterns are associated with carcinogenesis and that the MTHFR 677 TT genotype is a predictor of decreased DNA methylation status (21). The higher frequency of the TT genotype among HGG cases

compared to control subjects and meningiomas suggests that DNA hypomethylation may play a role in the development of HGG through genomic hypomethylation, but not in Grade I meningiomas. The MTHFR enzyme resides at a metabolic branch point directing the folate pool towards Hcy remethylation at the expense of DNA and RNA biosynthesis (2). The MTHFR 677 TT genotype results in decreased enzyme activity and may reduce the availability of *de novo* (folate-derived) methyl groups that are destined for CpG methylation, which may be important in oncogenesis because a deficiency of methyl groups could modify proto-oncogene expression (26). Hypomethylation may induce DNA strand breaks and subsequent mutations (27).

Luckock *et al.* speculated that the protective effect of the TT genotype or T allele under folate-rich conditions is reversed if there is inadequate folate. In folate-depleted subjects with the MTHFR 677TT genotype, this single nucleotide polymorphism may be linked to cancer promotion through its influence on the role of folate at fragile chromosome sites (27). This process is likely to be exacerbated by both reduced DNA-excision repair, an event that is linked to folate depletion (28) and impaired mismatch repair, which also helps to maintain genomic integrity. Cells that are proficient in mismatch repair are highly sensitive to folate deficiency compared to cells that are defective in mismatch.

The geographical variability of the MTHFR C677T polymorphism is also a well established phenomenon (29, 30). The natural abundance of folate can explain the diverse global distribution of the C677T MTHFR polymorphism. In Europe, a MTHFR 677T gradient that runs from north to south might depend on the higher folate content of the Mediterranean diet. In the Americas, a similar tendency could be detected (27). To further highlight the possible relationship between the abundance of foods that are rich in folate and the frequency of the 677T MTHFR allele, it has been suggested that the atypically low prevalence of the recessive TT genotype in Africans might also be related to a deficiency in nutritional sources of folate in countries that are subject to malnourishment and disease (31). Another interesting finding is that the frequency of the 677T allele seems to have increased in African-Americans: its frequency of 6% in sub-Saharan Africans has risen to 12-35% in Americans of African descent (30, 31). Luckock and Yates speculated that this might have arisen as a result of improved folate nutrition among African-Americans, although population admixture is also a probable factor (27).

Based on the report by Gulec *et al.* (32), the frequencies of the CC, CT and TT genotypes were 60%, 47% and 9%, respectively, in the Turkish population. In our study, the CC genotype was more common than the CT+TT genotypes both in brain tumors and controls. The distributions of the CC, CT and TT genotypes in the control subjects were 54%,

39% and 7%, respectively. These findings were consistent with other reports.

Since our investigation included a relatively small number of primary brain tumor patients, these results need to be tested in larger patient cohorts. Additional studies may reveal the relationship between MTHFR C677T polymorphism and primary brain tumors, especially in HGG, lending further support to the idea that risk factor-gene interaction allows the determination of specific predictive information regarding brain tumor development.

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