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Genetic Mutation of 5,10-Methylenetetrahydrofolate Reductase in the Brain Neoplasms

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Objective : Recent epidermiologic studies suggested that alterations in folate metabolism as a result of polymorphism in the enzyme 5,10-methylenetetrahydrofolate reductase(MTHFR) have been frequently associated with neural tube defects, vascular disease, and some cancers. A common 677C→T polymorphism in the MTHFR gene results in thermolability and reduced MTHFR activity that decreases the pool of 5-methyltetrahydrofolate and increases the pool of 5,10-methylenetetrahydrofolate. A possible cause underlying altered DNA methylation could be an insufficient level of S-adenosylmethionine as a consequence of weaker alleles of MTHFR gene. Therefore, the weak MTHFR activity may underlie susceptibility to brain neoplasms. We now report the associations of MTHFR polymorphisms in three groups of adult brain tumors : gliomas, meningiomas and schwannomas.

Methods : We analyzed DNA of 71 brain tumors and 254 age- and sex-matched controls with a case-control study. MTHFR variant alleles were determined by a PCR-restriction fragment length polymorphism assay.

Results : The incidence of the MTHFR 677TT genotype was higher among 20 schwannoma cases compared with that of 254 controls, conferring a 5-fold increase of the risk of schwannomas(odds ratio, OR=4.75 ; 95% confidence index, CI=1.05 - 21.50). The homozygous mutant group had half the risk of meningioma(OR=0.42 ; 95% CI = 0.11 - 1.58) compared with the homozygous normal or heterozygous genotypes. There was no significant difference in MTHFR 677TT genotype frequency between glioma group(19 cases) and control group(254 cases) (OR = 1.53 ; 95% CI = 0.30 - 7.73).

Conclusion : The data indicate that the homozygous 677TT MTHFR genotype confers the significantly higher risk of schwannoma and the lower risk of meningioma. However, our study had limited a statistical power because of the small sample size, which is reflected in the wide CIs. Hence, these findings need to be confirmed in larger populations.

KEY WORDS : Brain neoplasms · Folic acid · Methylenetetrahydrofolate reductase · Polymorphism · Risk factors.

Introduction

The global and gene-specific anomalies of DNA methylation contribute to the loss of proto-oncogene and tumor suppressor expression. There are some evidences that DNA methylation can be influenced by manipulating the availability of methyl group donors, such as folate[23]. Folate levels are influenced by 2 enzymes : 5,10-methylenetetrahydrofolate reductase(MTHFR) catalyzing the conversion of 5,10-methylenetetrahydrofolate(methylene THF) to 5-methyltetrahydrofolate(methyl THF), and methionine synthase(MTR) catalyzing the transfer of a methyl group from methyl THF to homocysteine. MTHFR is a critical enzyme in folate metabolism[34]. Its product, 5-methyl THF, is the dominant form of folate in plasma, whereas the enzyme substrate, 5,10-methylene THF, is found mainly intracellularly. 5-Methyl THF provides the methyl group for *de novo* methionine synthesis and DNA methylation[33]. Imbalanced DNA methy-

lation, characterized by global genomic hypomethylation[15,18] and the methylation of usually unmethylated CpG sites[20,28], is observed consistently in colonic neoplasia[25]. A decreased 5-methyl THF pool may affect DNA methylation and thereby contribute to carcinogenesis. On the other hand, the substrate for MTHFR, 5,10-methylene THF, is required for conversion of deoxyuridylate to thymidylate, and, thus, the depletion of this form of folate may interfere with the thymidylate biosynthesis and result in development of deoxynucleotide pool imbalance[4]. This leads to the accumulation of deoxyuridylate in DNA pool[4] and the removal of this abnormal base might labilize DNA to strand breaks[2,5,10,14,27,30-32].

A common mutation(677C→T : alanine-to-valine) has been identified in the MTHFR gene[16,19]. This homozygous mutation causes reduced enzyme activity about 30% of the normal activity[16], leading to the reduced levels of circulating folate(5-methyl THF) and to the accumulation of 5,10-methylene THF. Exploration of the association of this mutation with brain tumors therefore permits a further assessment of the role of folate in tumorigenesis and the relative importance of MTHFR genotype with the risk of the several types of brain tumors.

Materials and Methods

Patient selection

Seventy-one unrelated patients(age range, 11 - 75 years ; mean age, 48.4 years ; male, 45.2%) with glial tumor(19 samples), meningioma(32 samples), or schwannoma(20 samples) were studied and compared with 254 Korean control subjects(age range, 21 - 89 ; mean age, 49.3 years ; male, 46.3%) who had not been diagnosed with vascular diseases and cancers. The patients with those brain tumors were enrolled from May, 2000 to April, 2002. Patients with the other pathology of brain tumor were not included. Tissues were collected with the informed consents of patients undergoing tumor resection and the pathological diagnosis of tissues was confirmed by histological analysis. The tissues were snap-frozen in liquid nitrogen and stored at -70℃. We calculated odds ratios(ORs) and 95% confidence indices (CIs) for the association of the MTHFR genotype with brain tumors using unconditional logistic regression.

DNA extraction and mutation analysis

There are three MTHFR genotypes : mutant homozygotes (*val/val* : 677TT), heterozygotes(*val/ala* : 677CT), and wild-type homozygotes(*ala/ala* : 677CC). The genotyping protocol for the detection of the MTHFR 677C→T polymorphism was adopted from the report of Frosst, et al[16]. This C →T base pair substitution creates a *Hin*fI restriction site. In brief, 0.5 - 2.0 μg of human genomic DNA was amplified with 50ng each of forward primer 5′-TGAAGGAGAAGG-TGTCTGCGGGA-3′ and reverse primer 5′-AGGACGG-TGCGGTCA GAGTG-3′. PCR thermal cycling conditions were a 2-min denaturation period at 94℃ and 40 cycles of the following : 94℃ for 30sec, 62℃ for 30sec, and 72℃ for 30sec. This was followed by a 7-min extension at 72℃. The 50 μl PCR mixture contained 10mM Tris.HCl(pH 8.3), 50mM KCl, 2.5mM $MgCl_2$, 0.1mg/ml gelatin, 200 μM each dNTP, and 1.25 units of *Taq* DNA polymerase.

*Hin*fI restriction digestion using 2.5 μl of buffer and 10 units of *Hin*fI restriction enzyme(Promega, CatalysAG, Wallisellen, Switzerland) added to 25 μl of PCR product was incubated at 37℃ for at least 3hr. Digestion products were visualized after electrophoresis on a 3.0% agarose gel with ethidium bromide. Wild-type homozygotes produced single band at 198-bp. Heterozygotes produced 198-, 175-, and 23-bp fragments. Mutant homozygote produced 175- and 23-bp fragments(Fig. 1). Laboratory personnels were blind to the case-control status, and the blinded quality control samples were included.

Statistical analysis

All statistical analyses in this study were performed using

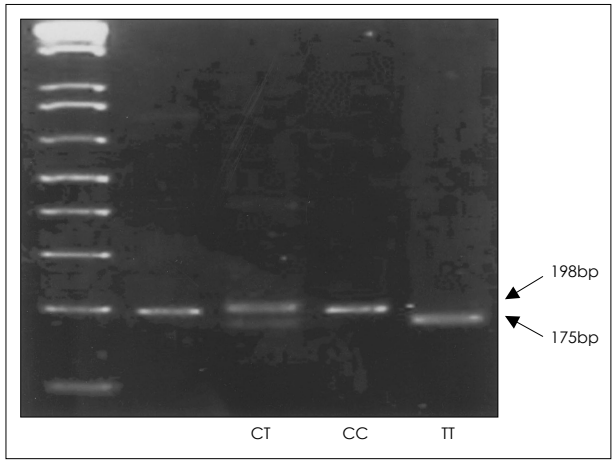

Fig. 1. Methylenetetrahydrofolate reductase genotyping by PCR and restriction analysis. Normal homozygotes lacking the C677T mutation are identified by the presence of single 198-bp fragment, whereas C677T homozygotes show single 175-bp fragment. Heterozygotes for the C677T mutation are characterized by the presence of both the 198-bp and the 175-bp fragment.

SPSS for Windows, version 9.0(SPSS Inc., Chicago, Illinois, USA). Accordance with the Hardy-Weinberg equilibrium was checked for case and control subjects using chi-square test. Odds ratios and 95% CI intervals were adjusted for sex and age using a multivariated logistic regression.

Results

Among the 71 patients diagnosed with brain tumors, the MTHFR C677T mutant allele frequency was 0.4 compared with 0.39 among the 254 control subjects. The frequencies of MTHFR 677CC, 677CT, 677TT genotypes were 35.2%, 49.3%, and 15.5% in the brain tumor cases and 35.8%, 50.8%, and 13.4% in the controls, respectively.

Prevalence of the MTHFR 677 genotypes in the gliomas

Listed in Table 1 are the observed frequencies of the MTHFR C677T polymorphisms among the 19 glioma cases and 254 controls. We found the MTHFR 677CC allele present among 6 glioma cases(31.6%) and 91 controls(35.8%), the 677CT genotype among 10 glioma cases(52.6%) and 129 controls(50.8%), and 677TT allele among 3 glioma cases (15.8%) and 34 controls(13.4%). The effects of the MTHFR 677CT allele and 677TT relative to 677CC are not significant in the risk of glioma(OR=1.35 ; 95% CI=0.46 - 3.99 and OR = 1.53 ; 95% CI = 0.30 - 7.73, respectively).

Table 1. Number of gliomas and controls, adjusted odds ratio and 95% confidence index by MTHFR 677, using 677CC as a reference

Genotype	Case	Control	Odds ratio	95% CI*
CC(reference)	6(31.6)	91(35.8)	1	-
CT	10(52.6)	129(50.8)	1.35	(0.46 - 3.99)
TT	3(15.8)	34(13.4)	1.53	(0.30 - 7.73)

Adjusted for age and sex. Percentages are in parentheses.
*CI : confidence index

Prevalence of the MTHFR 677 genotypes in the meningiomas

Frequencies of the MTHFR 677 polymorphism in the 32 meningioma cases and 254 controls are listed in Table 2. For 677CC, 677CT, and 677TT, we observed 16(50.0%), 13 (40.6%), and 3(9.4%) meningioma cases, respectively. Different proportions were found among their controls ; that is, 91(35.8%) were 677CC, 129(50.8%) were 677CT, and 34 (13.4%) were 677TT. Significant differences were observed for 677CT or 677TT compared with 677CC(OR=0.53 ; 95% CI=0.24 - 1.17 and OR=0.42 ; 95% CI=0.11 - 1.58, respectively).

Table 2. Number of meningiomas and controls, adjusted odds ratio and 95% confidence index by MTHFR 677, using 677CC as a reference

Genotype	Case	Control	Odds ratio	95% CI*
CC(reference)	16(50.0)	91(35.8)	1	-
CT	13(40.6)	129(50.8)	0.53	(0.24 - 1.17)
TT	3(9.4)	34(13.4)	0.42	(0.11 - 1.58)

Adjusted for age and sex. Percentages are in parentheses.
*CI : confidence index

Prevalence of the MTHFR 677 genotypes in the schwannomas

The frequencies of 677CC, 677CT and 677TT genotypes among the controls were 3(15.0%), 12(60.0%) and 5(25.0%), respectively(Table 3). The frequency of 677TT genotype among the schwannoma cases(25.0%) was higher compared with that of the controls(13.4%) ; the age- and sex-adjusted OR for this genotype was 4.75(95% CI=1.05 - 21.50).

Table 3. Number of schwannomas and controls, adjusted odds ratio and 95% confidence index by MTHFR 677, using 677CC as a reference

Genotype	Case	Control	Odds ratio	95% CI*
CC(reference)	3(15.0)	91(35.8)	1	-
CT	12(60.0)	129(50.8)	2.88	(0.78 - 10.56)
TT	5(25.0)	34(13.4)	4.75	(1.05 - 21.50)

Adjusted for age and sex. Percentages are in parentheses.
*CI : confidence index

Discussion

The relationship between folate status and the risk of developing several diseases, including cardiovascular disease, neural tube defects, and tumor has been a topic of intense interest in the field of folate nutrition[21,22,29,38]. In the area of folate and tumorigenesis, several potential mechanisms by which folate status can modulate tumorigenesis have been linked to the disruption of the essential biochemical function of folate[9,22]. The sole biochemical function known for folate is that of mediating the transfer of one-carbon moieties[37]. In this role, folate is critical for the synthesis of S-adenosylmethionine, a compound that serves as the methyl donor for more than 100 biologic methylation reactions, including that of DNA[33]. DNA methylation is an important epigenetic determinant in gene expression, DNA stability and integrity, and mutagenesis[3,40]. Folate is also an essential factor for the

de novo biosynthesis of purines and thymidylate, and hence plays an important role in DNA replication and repair[37]. Aberrant patterns of DNA methylation, unrepaired DNA damage and impaired DNA repair have all been implicated in carcinogenesis[3,9,22,40]. The possible contribution of several enzymes involved in folate metabolism that are essential to folate-mediated carcinogenesis has just begun to be recognized. Recently, common genetic polymorphisms of MTHFR have been observed to modulate the risk of developing cardiovascular disease, neural tube defects, and cancers[1]. MTHFR is a critical enzyme in folate metabolism. MTHFR catalyzes the biologically irreversible reduction of 5,10-methylene THF to 5-methyl THF. A common mutation (677C→T, alanine→valine) has been identified in the MTHFR gene[16,19]. The frequency of the 677C→T polymorphism of MTHFR varies among racial and ethnic groups. The analysis of Caucasian and Asian populations typically shows rates of - 12% for those who are homozygous and up to 50% for those of who are heterozygous[7,16]. Among 677TT individuals, the MTHFR enzyme is less efficient in converting 5,10-methylene THF to 5-methyl THF, thus potentially preventing depletion of 5,10-methylene THF, a cofactor for *de novo* synthesis of nucleotides necessary for DNA synthesis, especially dTMP(Fig. 2). As a result, cells may be less prone to "dTMP stress," which has been shown to promote cancer-associated genetic alterations[21] due to alterations in the pool of nucleotide precursors available for DNA synthesis. Alteration in these precursor pools induced by methyl folate deficiency significantly increases the uracil content and the frequency of chromosome breaks in human leukocyte DNA[5].

With respect cancer risk, the 677C→T polymorphism of MTHFR appears to be protective against the development of colorectal cancer and acute lymphocytic leukemia[8,26,36], whereas it enhances the risk of endometrial[12] and gastric cancers[35]. In various cancers, the MTHFR 677C→T polymorphism modulates carcinogenesis in a site- and stage-specific manner. In the present study, we report an association of the susceptibility to the several types of brain neoplasms with the polymorphism in the gene encoding the enzyme MTHFR. We observed that the 677C→T mutation in MTHFR was associated with the reduced risk of meningiomas and the increased risk of schwannomas. How can we explain these different results in benign brain tumors? First, we suspect that our study had limited statistical power because of the small sample size, which is reflected in the wide CIs. Hence, these findings need to be confirmed in larger populations. Second, we guess that there can be the different mechanisms of sporadic tumorigenesis. The genome of the transformed cell undergoes simultaneously a global genomic hypomethylation and a dense hypermethylation of the CpG islands associated with gene regulatory regions. These dramatic changes may lead to chromosomal instability, activation of endogenous parasitic sequences, loss of imprinting, illegitimate expression, aneuploidy, and mutations, and may contribute to the transcriptional silencing of tumor suppressor genes[13,17]. DNA methylation patterns in hereditary human cancers mimic sporadic tumorigenesis[11]. There are some reports of aberrant expression or mutation of the promotor elements in schwannoma or meningioma[6,24,39]. In this study, unfortunately, we did not measure the level of methylation, homocysteine, folate and MTHFR enzyme activity in the brain tumor tissues. In the future, studies as follow are warranted ; the relationship of DNA methylation with brain tumors, the development of brain tumors in MTHFR knockout mice, and the establishment of a cause-and-effect relationship.

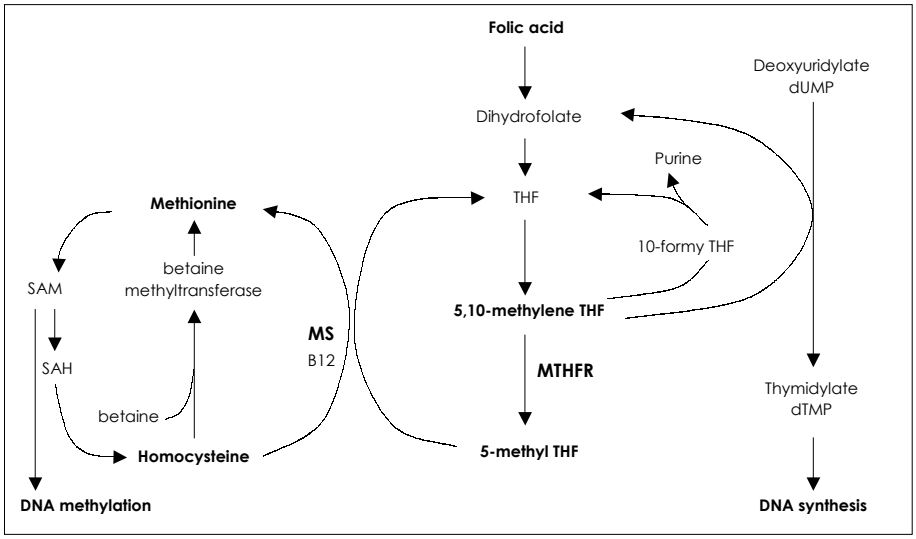

Fig. 2. Simplified scheme of the metabolic role of methylenetetrahydrofolate reductase in folate metabolism involving DNA methylation and DNA synthesis. THF : tetrahydrofolate, MTHFR : methylenetetrahydrofolate reductase, MS : methionine synthetase, B12 : vitamin B_{12}, SAM : S-adenosylmethionine, SAH : S-adenosyl homocysteine.

Conclusion

Our data suggest that the MTHFR enzyme activity plays an important role in schwannoma and meningioma. Brain tumor is a relatively rare disease, making it difficult to perform a large epidemiological study, but studies in multicenters should be undertaken to confirm the findings presented here.

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