Genetic Polymorphisms of 5,10-Methylenetetrahydrofolate reductase (MTHFR C677T and A1298C) in Brain Neoplasms

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Directed by Professor Kyu Sung Lee

The Master's thesis submitted to the Department of Medicine, the Graduate School of Yonsei University in partial fulfillment of the requirements for the degree of Master of Medicine

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This certifies that the Master's Thesis of

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감사의 글

연구계획서를 쓰기 시작한다고 뛰어다닌 것이 초여름이었는데 이제 어느덧 겨울의 문턱에 들어섰습니다. 부족하기만 한 지식으로 논문을 쓰면서 한없이 깊고 넓기만 한 학문 앞에 제 자신을 겸허히 바라볼 수 있는 기회가 되었던 것이 무엇보다 소중한 경험이었습니다.

힘든 신경외과 전공의 시절부터 항상 지켜봐 주셨으며, 항상 조언과 관심을 아낌없이 주신 이규성 교수님께 무한한 존경과 감사를 드립니다. 연구와 진료로 항상 바쁘신 와중에도 제 부족한 논문의 심사를 기꺼이 맡아주신 신경과 김승민 교수님, 실험에서부터 논문의 세세한 부분까지 지도해주신 안정용 교수님께 다시 한번 고개 숙여 감사의 말씀을 올립니다.

언제나 변함 없는 사랑으로 제게 힘이 되어준 가족들과 오늘의 저를 있게 해주셨으며 힘들 때마다 저를 지킬 수 있는 정신적 지주가 되어주신 부모님께 사랑과 함께 이 논문을 바칩니다.

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ABSTRACT

Genetic Polymorphisms of 5,10-Methyleneteterahydrofolate reductase (MTHFR C677T and A1298C) in Brain Neoplasms

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Previous 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 vascular occlusive disease and some cancers. A common C677T and A1298C polymorphism in the MTHFR gene results in thermolability and reduced MTHFR activity. Elevated plasma homocysteine levels can result from defective remethylation of MTHFR and imbalanced DNA methylation may related with carcinogenesis. We investigated the associations of MTHFR polymorphisms in three groups of brain tumors : glioma, meningioma and schwannoma. We analyzed DNA of 99 brain tumors and 122 controls. However, there were no statistical differences in allelic frequency for both C677T and A1298C mutations. But, they show some potential association, which is reflected in the narrow CIs. The incidence of 677TT allele among 3 glioma cases(12.0%) and 17 controls(13.9%) (OR=0.797 ; 95%CI=0.161 - 3.095). The frequencies of CT/AA allele among 12 meningiomas(25.6%) and 51 controls(41.8%)(OR=0.795 ; 95%CI=0.574 - 1.102). CT/AC allele among 8 meningiomas(17.0%) and 14 controls(11.5%)(OR=1.038 ; 95%CI=0.776 - 1.390). Our data did not demonstrate that the polymorphism of MTHFR plays a role in brain neoplasm, but further studies in large population are required.

KEY WORDS : Brain neoplasm, Folic acid, Metylenetetrahydrofolate reductase, Polymorphism

Genetic Polymorphisms of 5,10-Methyleneteterahydrofolate reductase(MTHFR C677T and A1298C) in Brain Neoplasms

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Ⅰ. Introduction

Previous studies suggested that 5,10-methylenetetrahydrofolate reductase(MTHFR) is the critical enzyme in folate metabolism[22] and its common mutation(C677T) results in hyperhomocysteinemia. It is known that hyperhomocysteinemia and low folate levels are related with the vascular occlusive disease and carcinogenesis[1,2,16,18,20]. MTHFR catalyzes the conversion of 5,10-methylenetetralydrofolate, required for purine and thymidine synthesis, to 5-methyltetrahydrofolate, the predominant circulating form of folate act as the methyl donor for remethylation of homocysteine to methionine[21]. The MTHFR gene was mapped to chromosomal region 1p36.3. A common C to T transition at nucleotide 677(C677T) of the MTHFR gene-coding sequence, leading to the substitution of alanine to valine residue at position 226 in the protein[11]. This common mutation increases MTHFR thermolability and reduces the enzyme activity and subsequent depletion of 5-methyl THF. Decreased 5-methyl THF level causes the imbalanced DNA methylation and may related with the carcinogenesis[1]. It was shown in most studies that homozygous(TT) mutant

subjects had significantly elevated plasma total homocysteine concentrations, whereas the total homocysteine concentration in subjects without the mutation(CC) and in heterozygous(CT) subjects was indistinguishable[7,11,13,14]. Recently, a second common mutation in the same gene was described[25,27]. In this new mutation, an A to C transition at nucleotide 128(A1298C) leads to a glutamate to alanine substitution in the MTHFR protein. The A1298C mutation, like the C677T mutation, results in a decrease in MTHFR activity that is more pronounced in the homozygous(CC) than in the heterozygous(AC) or normal (AA) states.

The aim of this study was to investigate the association between the polymorphism of the MTHFR gene(C677T and A1298C) and brain neoplasm.

Ⅱ. Methods

1. Subjects

99 patients(age range, 2 - 75 ; mean age, 49.0 ; male, 47) with glial tumor(25 samples), meningioma(47 samples) and schwannoma(27 samples) were investigated and compared with 122 korean control group(age range,27 - 85 ; mean age, 56.6 ; male, 66)who did not been diagnosed with the vascular disease and cancers. The patients with the brain tumors were selected from May, 2001 to April, 2003. Other pathology of brain tumor were excluded. Tissues were collected with 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.

A. DNA extraction and mutation analysis for C677T

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 adapted from the report of Frosst, et al. This C→T base pair substitution creates a *Hin*fI restriction site. In brief, 0.5 - 2.0 ㎍ of human genomic DNA was amplified with 50ng each of forward primer 5′-TGAAGGAGAAGGTGTCTGCGGGA-3′ and reverse primer 5′-AGGACGGTGCG GTCA 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㎕ PCR mixture contained 10mM Tris. HCl(pH8.3), 50mM KCl, 2.5 units of Taq DNA polymerase. *Hin*fI restriction digestion using 2.5㎕ of buffer and 10 units of *Hin*fI restriction enzyme(Promega, CatalysAG, Walisellen, Switzerland) added to 25㎕ of PCR product was incubated at 37℃ for at least 3hrs.

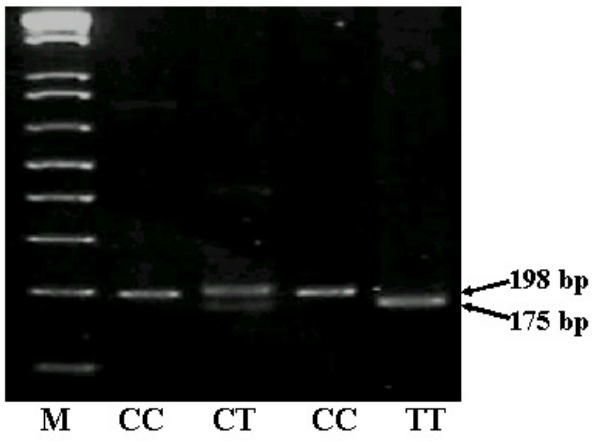

FIg 1. Methyenetetrahydrofolate reductase genotyping by PCR and restriction analysis for C677T

Digestion products were visualized after electrophoresis on a 3.0% agarose gel with ethidum 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.

B. DNA extraction and mutation analysis for A1298C

The second A1298C mutation was analyzed by PCR by using the following primer pairs: 5′-CTTT GGGGAGCTGAA GGACTACTAC-3′ and 5′-CACTTTGTGACCATTCCG GTTTG-3′. The reaction mixture was the same as for the C677T mutation. Conditions were as follows. Initial denaturation-annealing-extension at 95℃ for 5min, 55℃ for 2min, and 72℃ for 2min, followed by 35 cycles of denaturation at 95℃ for 75sec, annealing at 5 5℃ for 75sec, extension at 72℃ for 90sec, and a final extension time of 6min at 72℃. Digestion of 163-bp fragment of the 1298AA genotype gives five fragments of 56,31,30,28 and 18 bp, whereas the 1298CC genotype results in four fragments of 84,31,30, and 18bp.

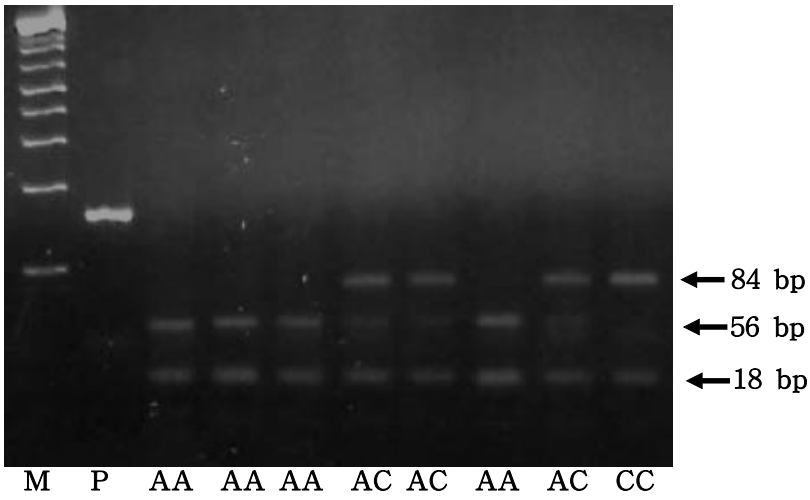

FIg 2. Methyenetetrahydrofolate reductase genotyping by PCR and restriction analysis for A1298C

2. Statistical analysis

All statistical analyses in this study were performed using SPSS for Windows, version 11.0(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 (confidence intervals) were adjusted for sex and age using a multivariated logistic regression.

Ⅲ. Results

1. MTHFR 677 and 1298 genotypes in the glioma

Listed in Table 1 are the observed frequencies of the MTHFR C677T polymorphism among the 25 glioma cases and 122 control subjects. We found 677CC allele present among 9 glioma cases(36.0%) and 40 controls(32.8%), the 677CT genotype among 13 glioma cases (52.0%) and 65 controls(53.3%), and 677TT allele among 3 glioma cases(12.0%) and 17 controls(13.9%). The effect of MTHFR 677CT and 677TT are not significant in the risk of glioma(OR=1.089 ; 95%CI=0.395 - 3.004 and OR=0.797 ; 95%CI=0.161 - 3.095, 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)	9(36)	40(32.8)	1	-
CT	13(52)	65(53.3)	1.089	0.395 - 3.004
TT	3(12)	17(13.9)	0.797	0.161 - 3.095

Listed in Table 2. are the frequencies of the MTHFR A1298C polymorphism among the 25 glioma cases and 122 controls. MTHFR 1298AA allele present 17 cases(68.0%) and 94 controls(77.0%), the 1298AC genotype among 5 gliomas(20.0%) and 25 controls(20.5%) and 1298CC allele among 3 glioma cases(12.0%) and 3 controls(2.5%). The effects of the MTHFR 1298AC and 1298CC are not significant in the risk of glioma(OR=1.261 ; 95%CI=0.381 - 4.180 and OR=4.464 ; 95%CI=0.712 - 27.985, respectively).

Table 2. Number of gliomas and controls, adjusted odds ratio and 95% confidence index by MTHFR 1298, using 1298AA as a reference

Genotype	Case	Control	Odds ratio	95% CI
AA	17(68)	94(77.0)	1	-
AC	5(20)	25(20.5)	1.261	0.381 - 4.180
CC	3(12)	3(2.5)	4.464	0.712 - 27.985

Combined MTHFR C677T and A1298C polymorphism of glioma and control subjects are listed in Table 3. MTHFR 677CC and 1298AA genotype among 4 gliomas(16.0%) and 26 controls(21.3%). The frequencies of genotype CC/AC; 2 gliomas(8.0%) and 11 controls(9.0%), CC/CC; 3 gliomas(12.0%) and 3 controls(2.5%), CT/AA; 10 gliomas(40.0%) and 51 controls(41.8%), CT/AC; 3 gliomas(12.0%) and 14 controls(11.5%) and TT/AA; 3 gliomas(12.0%) and 17 controls(13.9%), respectively. They show no significant statistical values.

Table 3 Number of gliomas and controls, adjusted odds ratio and 95% confidence index by MTHFR 677 and 1298, using 677CC and 1298AA as a reference

Genotype	Case	Control	Odds ratio	95% CI
CC/AA	4(16)	26(21.3)	1	-
CC/AC	2(8)	11(9.0)	1.553	0.214 - 11.250
CC/CC	3(12)	3(2.5)	2 .453	0.853 - 7.054
CC/CC	10(40)	51(41.8)	1.179	0.732 - 1.898
CT/AC	3(12)	14(11.5)	1.139	0.743 - 1.746
TT/AA	3(12)	17(13.9)	1.030	0.759 - 1.398

2. MTHFR 677 and 1298 genotypes in the meningioma

Listed in Table 4 are the observed frequencies of the MTHFR C677T polymorphism among the 47 meningioma cases and 122 control subjects. We found 677CC allele present among 21 meningioma cases(44.7%) and 40 controls(32.8%), the 677CT genotype among 20 meningioma cases (42.6%) and 65 controls(53.3%), and 677TT allele among 6 meningioma cases(12.8%) and 17 controls(13.9%). The effect of MTHFR 677CT and 677TT are not significant in the risk of meningioma(OR=0.650 ; 95%CI=0.299 - 1.417 and OR=0.726 ; 95%CI=0.237 - 2.224, respectively).

Table 4. 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	21(44.7)	40(32.8)	1	-
CT	20(42.6)	65(53.3)	0.650	0.299 - 1.417
TT	6(12.8)	17(13.9)	0.726	0.237 - 2.224

Listed in Table 5 are the frequencies of the MTHFR A1298C polymorphism among the 47 meningioma cases and 122 controls. MTHFR 1298AA allele present 30 cases(63.8%) and 94 controls(77.0%), the 1298AC genotype among 15 meningioma(31.9%) and 25 controls(20.5%) and 1298CC allele among 2 meningioma cases(4.3%) and 3 controls(2.5%). The effects of the MTHFR 1298AC and 1298CC are not significant in the risk of meningioma(OR=1.672 ; 95%CI=0.751 - 3.722 and OR=1.015 ; 95%CI=0.125 - 8.257, respectively).

Table 5 Number of meningiomas and controls, adjusted odds ratio and 95% confidence index by MTHFR 1298, using 1298AA as a reference

Genotype	Case	Control	Odds ratio	95% CI
AA	30(63.8)	94(77.0)	1	-
AC	15(31.9)	25(20.5)	1.672	0.751 - 3.722
CC	2(4.3)	3(2.5)	1.015	0.125 - 8.257

Combined MTHFR C677T and A1298C polymorphism of meningioma and control subjects are listed in Table 6. MTHFR 677CC and 1298AA genotype among 12 meningioma(25.6%) and 26 controls(21.3%). The frequencies of genotype CC/AC; 7 meningioma(14.9%) and 11 controls(9.0%), CC/CC; 2 meningioma(4.3%) and 3 controls(2.5%), CT/AA; 12 meningioma(25.6%) and 51 controls(41.8%), CT/AC; 8 meningioma(17.0%) and 14 controls(11.5%) and TT/AA; 6 meningioma(12.8%) and 17 controls(13.9%), respectively. They show no significant statistical values.

Table 6 Number of meningiomas and controls, adjusted odds ratio and 95% confidence index by MTHFR 677 and 1298, using 677CC and 1298AA as a reference

Genotype	Case	Control	Odds ratio	95% CI
CC/AA	12(25.6)	26(21.3)	1	-
CC/AC	7(14.9)	11(9.0)	1.183	0.351 - 3.988
CC/CC	2(4.3)	3(2.5)	0.854	0.268 - 2.722
CT/AA	12(25.6)	51(41.8)	0.795	0.574 - 1.102
CT/AC	8(17.0)	14(11.5)	1.038	0.776 - 1.390
TT/AA	6(12.8)	17(13.9)	0.952	0.780 - 1.162

3. MTHFR 677 and 1298 genotypes in the schwannoma

Listed in Table 7 are the observed frequencies of the MTHFR C677T polymorphism among the 27 schwannoma cases and 122 control subjects. We found 677CC allele present among 5 schwannoma cases(18.5%) and 40

controls(32.8%), the 677CT genotype among 16 schwannoma cases (59.3%) and 65 controls(53.3%), and 677TT allele among 6 schwannoma cases(22.2%) and 17 controls(13.9%). The effect of MTHFR 677CT and 677TT are not significant in the risk of schwannoma(OR=2.265 ; 95%CI=0.752 -6.829 and OR=2.467 ; 95%CI=0.576 - 10.560, respectively).

Table 7 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	5(18.5)	40(32.8)	1	-
CT	16(59.3)	65(53.3)	2.265	0.752 - 6.829
TT	6(22.2)	17(13.9)	2.467	0.576 - 10.560

Listed in Table 8 are the frequencies of the MTHFR A1298C polymorphism among the 27 schwannoma cases and 122 controls. MTHFR 1298AA allele present 19 cases(70.4%) and 94 controls(77.0%), the 1298AC genotype among 8 schwannoma(29.6%) and 25 controls(20.5%) and 1298CC allele among no schwannoma cases(0%) and 3 controls(2.5%). The effects of the MTHFR 1298AC and 1298CC are not significant in the risk of schwannoma(OR=1.476 ; 95%CI=0.552 - 3.947 and OR<0.001 ; 95%CI<0.001 >999.999, respectively).

Table 8 Number of meningiomas and controls, adjusted odds ratio and 95% confidence index by MTHFR 1298 using 1298AA as a reference

Genotype	Case	Control	Odds ratio	95% CI
AA	19(70.4)	94(77.0)	1	-
AC	8(29.6)	25(20.5)	1.467	0.552 - 3.947
CC	-	3(2.5)	<0.001	<0.001 >999.999

Combined MTHFR C677T and A1298C polymorphism of schwannoma and control subjects are listed in Table 9. MTHFR 677CC and 1298AA genotype among 3 schwannoma(11.1%) and 26 controls(21.3%). The frequencies of genotype CC/AC; 2 schwannoma(7.4%) and 11 controls(9.0%), CC/CC; 0 schwannoma(0%) and 3 controls(2.5%), CT/AA; 10 schwannoma(37.0%) and 51 controls(41.8%), CT/AC; 6 schwannoma(22.2%) and 14 controls(11.5%) and TT/AA; 6 schwannoma(22.2%) and 17 controls(13.9%), respectively. They show no significant statistical values.

Table 9 Number of schwannomas and controls, adjusted odds ratio and 95% confidence index by MTHFR 677 and 1298, using 677CC and 1298AA as a reference

Genotype	Case	Control	Odds ratio	95% CI
CC/AA	3(11.1)	26(21.3)	1	-
CC/AC	2(7.4)	11(9.0)	1.411	0.181 - 11.013
CC/CC	-	3(2.5)	0.002	<0.001 >999.999
CT/AA	10(37.0)	51(41.8)	1.223	0.744 - 1.962
CT/AC	6(22.2)	14(11.5)	1.398	0.936 - 2.087
TT/AA	6(22.2)	17(13.9)	1.157	0.877 - 1.525

Ⅳ. Discussion

Some previous studies revealed that a common mutation in the MTHFR gene C677T is related to homocysteinemia and might increase the risk for several disease and cancer[2,6,20]. In the current study, we determined the newly described mutation in the MTHFR gene A1298C. MTHFR is the is one of the critical

regulatory enzymes of homocysteine metabolism and closely related to folate status[22]. 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[8,16]. The biochemical function known for folate is that of mediating the transfer of one-carbon moieties[26]. 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[26]. Aberrant patterns of DNA methylation, unrepaired DNA damage and impaired DNA repair have all been implicated in carcinogenesis[1,8,16,30]. If the MTHFR enzyme is less efficient I converting 5,10-methylene THF to 5-methyl THF, it prevent a potential depletion of 5,10-methylene THF, a cofactor de novo synthesis of nucleotides necessary for DNA synthesis, especially dTMP(Fig 3).

As a result, cells may be less prone to "dTMP stress" which has been shown to promote cancer associated genetic alterations[15] due to alterations in the pool of nucleotide precursors available for DNA synthesis.

Combined heterozygosity of 677T and 1298C allleles is associated not only with a reduced MTHFR activity, but also with higher plasma homocysteine levels, comparable with the observations among patients that are homozygous for the 677T allele[25], which might be resultant from diverse effects of both mutations on homocysteine levels. The C677T mutation is the region encoding the N-terminal catalytic domain, as opposed to the A1298C substitution located in the C-terminal regulatory domain[28]. Guenther et al. reported that enzyme stabilization and improvement of catalytic enzyme function may mediate the protective effect of folate. With the respect cancer risk, the polymorphism of MTHFR appears to be protective against the development of colorectal cancer and acute lymphocytic leukemia[4,19,24], whereas it enhances the risk of endometrial[9] and gastric cancers[23]. In various cancers, the MTHFR polymorphism modulates carcinogenesis in a site and stage specific manner.

In this study, we investigated whether a second common polymorphism of the gene, MTHFR A1298C is a independent risk factor for brain tumor, and also

examined whether the combined with the C677T confer the risk. We observed no statistically significant value, but analysis of combined MTHFR genotype frequencies for our brain tumor and control groups showed some deviation from Hardy-Weinberg equilibrium in the patient group(P= 0.0959). We conclude as follows, first, 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 larger population. Second, we guess that there can be the different mechanisms of sporadic tumorigenesis.

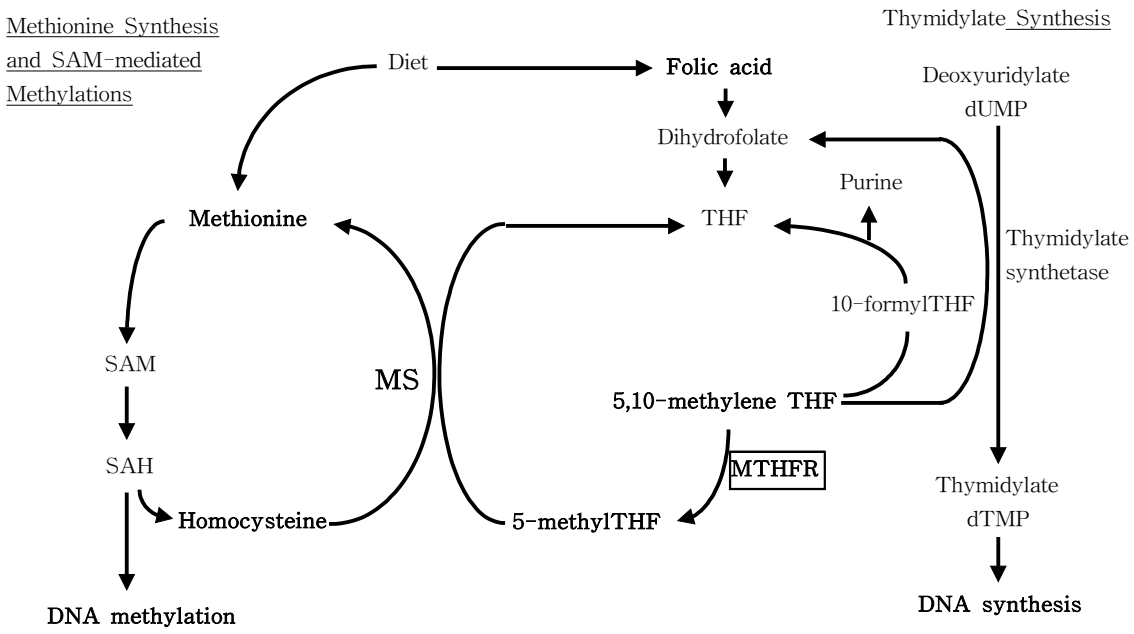

Fig 3. simplified scheme of the metabolic role of methylenetetrahydrofolate reductase in folate metabolism involving DNA methylation and DNA synthesis. THF : tetrahydrofolate, MTHFR : methylenetetrahydrofolate reductase, MS : methionine synthetase, SAM : S-adenosylmethionine, SAH : S-adenosyl homocysteine.

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, anuploidy, and mutations, and may contribute to the transcriptional silencing of tumor suppressor genes[10,12]. DNA methylation patterns hereditary human cancers mimic sporadic tumorigenesis[8]. There are some reports of aberrant expression or mutation of the promotor elements in schwannoma or meningioma[3,17,29]. However, In this study we did not measure the level of homocysteine, folate, and MTHFR enzyme activity in the brain tumor tissue.

Ⅴ. Conclusion

Our data did not demonstrate that the polymorphism of MTHFR plays a role in brain neoplasm. There were no statistical differences in allelic frequency for both C677T and A1298C mutations. However, they suggest some potential association but had limited a statistical value because of the small sample size. Further studies in large population are required to determine the role of the C677T/A1298C genotype and its association with brain neoplasm.

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국문요약

뇌종양에서 5,10-methylenetetrahydrofolate reductase(MTHFR C677T 과 A1298C) 의 유전적 다형태성

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정진영

5,10-methylenetetrahydrofolate reductase(MTHFR) 의 유전자 돌연변이의 결과로 엽산대사에 변화가 생길 수 있고 이와 관련된 혈관질환 및 종양의 발생에 대한 연구가 보고되고 있다. MTHFR C677T site 돌연변이와 최근 보고되고 있는 A1289C site 의 돌연변이는 는 비교적 흔하게 발생하며 이의 결과로 효소의 활성이 감소하게 된다. MTHFR 돌연변이로 인한 활성 감소로 인해 호모시스테인이 메티오닌으로 전환되지 못하여 과량의 호모시스테인이 체내에 축적되게 되며 DNA methylation, dTMP 합성 등에 이상을 초래하여 암의 원인으로 작용할 수 있다. 본 실험은 99명의 뇌종양(신경교종, 뇌수막종, 신경초종) 환자 군과 122명의 정상 대조군의 DNA를 분석하여 MTHFR 유전자 돌연변이와 뇌종양과의 상관관계 및 종양생성(tumorigenesis) 에 한 비교분석을 시도하였다. 신경교종 환자 와 정상대조군에서 MTHFR 677TT type 의 빈도는 각각 3(12.0%), 17(13.9%) (OR=0.797 ; 95%CI=0.776 - 1.390)으로 나타났으며, 뇌수막종 환자에서는 정상 군과 비교하여 CT/AA type이 각각12(25.6%), 51(41.8%) (OR=0.795 ; 95%CI=0.574 - 1.102) 그리고 CT/AC type 이 8(17.0%), 14(11.5%) (OR=1.038 ; 95%CI=0.776 - 1.390) 으로 나타났다. 이는 모두 통계학적 의의를 갖지 못하였으나 유의한 가능성을 보여주는 결과로 생각되며 추후 좀더 큰 실험 군에 대한 지속적인 연구가 필요하다고 하겠다.

핵심되는말 : 뇌종양, 엽산, methylenetetrahydrofolate reductase, 돌연변이