Methylenetetrahydrofolate Reductase Gene Polymorphism and Ischemic Stroke : Sex Difference in Japanese

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【学位論文題目】

Methylenetetrahydrofolate Reductase Gene polymorphism and Ischemic Stroke:Sex Difference in Japanese

（メチルテトラヒドロ葉酸還元酵素遺伝子多型性と虚血性脳血管障害との関係：日本人の性差）

審　査　委　員

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INTRODUCTION

Moderate elevation of homocysteine in the plasma is an established risk factor in the development of coronary heart disease, myocardial infarction, ischemic stroke, and venous thrombosis. 5,10-Methylenetetrahydrofolate reducatase (MTHFR) is a folate-dependent enzyme catalyzing the rate-limiting step in the methylation of homocysteine to methioine. Following the cloning and sequencing of the gene for MTHFR, a C→T transition at 677, which results in the conversion of alanine to valine at amino acid 222, was reported and was associated with a decreased specific MTHFR activity and an elevation of the homocysteine levels in the T/T homozygous state. Subsequent studies have reported that polymorphism of the MTHFR gene is one of the potential genetic risk factors for ischemic stroke, although this is still unclear.

One potential explanation for these inconsistent findings is that previous studies have correlated the MTHFR (C677T) mutation and ischemic stroke but not separating analysis for sex. Sex difference in the association of premature ischemic stroke with factor V Leiden has been found. and gender has also been reported to be independently associated with homocysteine levels. Therefore, we hypothesized that the association with the MTHFR genotype may be different according to gender. To explore this hypothesis, we undertook a case-control study of patients hospitalized with ischemic stroke and examined with a separate analysis for sex specifically whether there may be an association between the C677T MTHFR mutation and ischemic stroke according to gender.

MATERIALS AND METHODS

Subjects

A total of 77 documented ischemic stroke patients (49 men and 28 women; age 61.4±6.8) were selected for the study from May 1998 to February 1999, having been identified by a clinical examination of a CT brain scan or MRI at the Rehabilitation Hospital of Kobe, Japan. The criteria of the National Institute for Neurological Disorders and Stroke were applied in this study. Patients with cerebral hemorrhage were not included.

The control subjects (n=229, 120 men and 109 women, age 59.6±6.9) without any history of ischemic stroke were recruited into the study. They underwent clinical examinations at Hyogo Health Service Association (Kobe, Japan).

DNA analysis

DNA was extracted from the peripheral blood leukocytes with a Puregen DNA Isolation Kit (Gentra systems, Inc., MN, USA). The polymerase chain reactions (PCR) to detect the MTHFR mutation were carried out, the primers and the temperature conditions for the PCR as previously reported. After which the PCR- amplified fragment (198-bp) was digested with the restriction enzyme Hinf I , followed by 3% agarose gel electrophoresis and ethidium bromide staining.

Statistical analysis

All of the analyses were performed using StatView 5.0 (for Macintosh). Baseline differences were examined by means of the χ^2 test or the Fisher's exact test for categorical data and the Student t test for continuous data. Allele and genotype frequencies among the case and control subjects were compared by the χ^2 test with Hardy-Weinberg predictions. The association between MTHFR genotype (independent variable) and ischemic stroke (dependent variable) was examined by means of a logistic regression model. Statistical significance was taken as $P<0.05$.

RESULTS

We found that the patients had a significantly higher prevalence of hypertension, hyperlipidemia, diabetes mellitus, and smoking habit in all the subjects, hypertension and diabetes mellitus in both sexes after a separate account, and hyperlipidemia and smoking habit only in men, which are known as major risk factors for ischemic stroke.

The calculated OR and 95% CI for the T/T genotype were 2.33 (1.05-5.20) compared with the C/C genotype in all, and this trend was significant (P=0.036). The allele frequency of the T-mutation was also significantly higher in the case group than in the control group (0.44/0.35, P=0.046). With a separate analysis for sex, the OR and 95% CI for the C/T genotype were 3.83(1.20-12.23) and 6.71(1.68-26.75) for the T/T genotype compared with the C/C genotype in women. Both of these effects were significant (P=0.017, P=0.003, respectively), and the T allele frequency was also significantly higher in case group than in the control group (0.55/0.34, P=0.004), but the significant trend was not identified in men.

The findings from the univariate analysis were further investigated in a multiple logistic model with adjustment for age (in years), sex, alcohol and smoking habit, and conventional vascular risk factors. Under these circumstances, the conventional vascular risk factors, such as diabetes mellitus, hypertension, smoking habit and hyperlipidemia were identified independently related to ischemic stroke, but the MTHFR T/T mutation was not. A separate analysis for sex was carried out. In men, significant associations were found in hypertension, hyperlipidemia, diabetes mellitus and smoking habit. In women, the statistical relevance of hypertension, diabetes mellitus and MTHFR T/T genotype (adjusted OR, 9.49; 95% CI, 1.75-51.47) with ischemic stroke was found.

DISCUSSION

With the separate analysis for sex, in women a statistical significance in the association between the T/T polymorphism of the MTHFR gene and ischemic stroke was identified. On the other hand, multivariate analysis demonstrated that this association was independent on conventional vascular risk factors related to ischemic stroke.

After stratification for sex, we observed that the MTHFR T/T mutation was independently associated with an increased risk of ischemic stroke in women. Elevating plasma homocysteine concentration being associated with the occurrence of ischemic stroke was reported by many studies. Recently, sex as a non-biochemical variable independently associated with homocysteine has been reported in an Italian study, and there was another consistent report that sex differences exist in levels of homocysteine, which were lower in premenopausal women than in men or postmenopausal women. In our study, most of women subjects were in the postmenopausal state and the relevance of MTHFR T/T mutation was restricted to women. These facts suggest that estrogens may play a role of increasing the resistance to elevated homocysteine levels due to MTHFR T/T mutation. In the postmenopausal state after losing the protective function by these hormones, the potential risk of elevated homocysteine levels for ischemic stroke would manifest although in the present study the small number of women patients and absence of data of homocysteine levels did not allow us to evaluate this hypothesis.

We conclude that the relevance of the MTHFR T/T mutation appears to be restricted to women, suggesting a role of female hormones in the resistance to elevated homocysteine levels due to the MTHFR T/T mutation. The quantity of case samples and the measurement of homocysteine and red cell folate levels should be addressed in further studies.

論文審査の結果の要旨			
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（要旨は１，０００字～２，０００字程度）

メチレンテトラヒドロ葉酸還元酵素(MTHFR) はメチレンテトラヒドロ葉酸をメチルテトラヒドロ葉酸に還元する酵素である。メチルテトラヒドロ葉酸はホモシステインをメチオニンに変換する反応の補因子となる。 MTHFRのC677T変異が生じると、MTHFRタンパク質を構成するアラニンはバリニンになり、そのため酵素の活性が下がり、ホモシステインをメチオニンに変換する反応を阻害し、血中ホモシステイン上昇を介して動脈硬化性、血栓性疾患の遺伝的危険因子になると考えられる。

従来、ホモシステインと虚血性脳血管障害が関連する報告があるが、MTHFR 変異と虚血性脳血管障害との関連性についてまだ明らかになっていなかった。申請者は日本人集団を対象に、case-controlの研究方法を用いて、MTHFR C677T変異と虚血性脳血管障害との関連性について研究を行なった。

対象として虚血性脳血管障害を診断された患者77名、対照は人間ドックを受診した健常人229名、全員のインフォームド・コンセントを得た。MTHFR遺伝子多型性の解析を、MTHFR遺伝子変異部位を含むDNA断片をPCR増幅した後、制限酵素にて切断し、電気泳動により行なった。統計処理は所定の有意差検定以外に虚血性脳血管障害を目的変数として、ロジスティック回帰分析を行った。

属性解析の結果、患者群の高血圧、高脂血症、糖尿病及び喫煙の所見率は健常人より高いと認められた。MTHFR遺伝子型と虚血性脳血管障害の関係では、変異のホモ型であるT/T頻度は、健常人に対して患者群は有意に高い傾向を示した、変異T allele頻度も同じ傾向を示した。男女別に分析した結果では、女性のみT/T頻度及びT allele 頻度の有意差があった。また、ホモの正常型 (C/C) のオッズ比を1とすると、C/T型及びT/T型のオッズ比は各々3.83 (1.20-12.23) 、6.71 (1.68-26.75) であった。

ロジスティック回帰分析の結果では、高血圧、高脂血症、糖尿病及び喫煙は独立した因子であり、特に女性ではMTHFR(T/T)も独立した因子であることが分かった。これは閉経前の女性の血中ホモシステイン濃度は男性及び閉経後の女性より低いという研究があり、女性ホルモンの持つ保護機能が減少すると、MTHFR変異は高ホモシステイン血症を介して虚血性脳血管障害等の血管疾患を引き起こす可能性があると考察した。

本研究は、脳梗塞患者のMTHFR 遺伝子多型性について、その危険因子との関連を研究したものであるが、従来ほとんど行なわれなかった女性患者に変異頻度が高くオッズ比を算出し、重要な知見を得たものとして価値ある集積であると認める。よって、本研究者は、博士（医学）の学位を得る資格があると認める。