

Homozygous V/V (677C to T) and D/D (2756G to A) variants in the methylenetetrahydrofolate and methionine synthase genes in a case of hyperhomocysteinemia with stroke at young age

Kyung Soon Song^{1,2}, Jae Woo Song¹,
Jong Rak Choi¹, Hyun Kyung Kim¹,
Jung Sik Shin¹ and Jeong Ho Kim¹

¹Department of Clinical Pathology, Yonsei University College of Medicine, CPO Box 1217, Seoul, Korea

²Corresponding author: Tel, +82-2-3497-3531;
Fax, +82-2-3462-9483; E-mail, kssong@yumc.yonsei.ac.kr

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Abstract

Hyperhomocysteinemia is known to be associated with an increased risk of myocardial infarction, stroke, peripheral arterial disease, and venous thrombosis. Gene polymorphisms in methylenetetrahydrofolate reductase (MTHFR) and methionine synthase (MS) may account for reduced enzyme activity and hyperhomocysteinemia. A recent study has documented evidence of polygenic regulation of plasma homocysteine. We report here on a case of occlusive stroke at young age and hyperhomocysteinemia with homozygous V/V (677C to T) variant in the MTHFR gene as well as homozygous D/D (2756G to A) variant in the MS gene.

Keywords: methylenetetrahydrofolate reductase, methionine synthase, homocysteine, stroke

Introduction

Hyperhomocysteinemia is known to be associated with risk or severity of cardiovascular diseases and cerebrovascular diseases (Wilcken and Wilcken, 1976; Stampfer *et al.*, 1992; Boushey *et al.*, 1995; Perry *et al.*, 1995). Biochemically, homocysteine is a naturally occurring sulfhydryl amino acid and its metabolism is regulated by two major pathways, re-methylation to methionine and trans-sulfuration to cysteine (Harmon *et al.*, 1996). The conversion of homocysteine to methionine occurs through re-methylation step with N⁵-methyl-tetrahydrofolate, which is formed from N⁵,N¹⁰-methylene-tetrahydrofolate in a reaction catalyzed by methylene tetrahydrofolate reductase (MTHFR) (Makris, 2000). In addition, methionine

synthase (MS) catalyzes the re-methylation of homocysteine to methionine in a methyl-cobalamine dependent reaction (Harmon *et al.*, 1999). In the trans-sulphuration pathway, homocysteine condenses with serine to form cystathionine in a reaction catalyzed by cystathionine β -synthase (CBS) (Sacco *et al.*, 1998).

In 1988, Kang *et al.* described a thermolabile variant of MTHFR that is associated with decreased enzyme activity and mildly elevated plasma homocysteine levels. The responsible mutation in the MTHFR gene, a C \rightarrow T substitution at the base pair 677 leading to the exchange of an alanine to a valine, was identified by Frosst *et al.* in 1995.

In 1996, Leclerc *et al.* identified a missense mutation of MS gene, D919G, which is common in the general population and inferred that it might lead to mild hyperhomocysteinemia with a consequent effect on vascular disease.

In the present study, we describe a case of hyperhomocysteinemia with stroke at young age with identification of homozygous V/V (677C to T) and D/D (2756G to A) variants in the MTHFR and MS genes, respectively.

Materials and Methods

Case

A 21 years old man (non-smoker) suffered from recurrent paresthesia and weakness of left arm and leg for several days. There was neither previous nor family history of thrombosis or hypertension. Brain MRI study revealed an infarct on the right basal ganglia and caudate nucleus area. Extensive search for an underlying predisposition to thrombosis was done in parallel with routine complete blood count and biochemical tests. All the results including serum cholesterol level were normal except for elevated Lp(a) (57 mg/dl; normal reference range, < 30 mg/dl) and total homocysteine (22.2 nM/ml; normal reference range, 6-15 nM/ml) levels. Pre-therapeutic serum vitamin B₁₂ level was also normal (240 pg/ml; normal reference range, 190-914 pg/ml) and folate level was 2.7 ng/ml, which was slightly decreased, compared with normal reference range (> 3.0 ng/ml). DNA study was undertaken to observe the genotypes of CBS, MTHFR and MS genes. The patients' parents and one sister were also included for the genetic DNA study. Oral anticoagulant therapy was performed.

Homocysteine assay

Homocysteine was measured 3 months after the stroke as total homocysteine (*i.e.*, free and protein-bound forms) in fasting plasma collected in an ethylenediaminetetraacetic acid (EDTA) tube. Homocysteine was reduced by tri-n-butylphosphine and was determined by means of high-performance liquid chromatographic analysis as previously described (Araki and Sakoy, 1987).

DNA study

Genomic DNA was extracted from peripheral blood samples drawn into EDTA tubes using a commercially available DNA isolation kit (Easy-DNA, Invitrogen, USA). Polymerase chain reaction (PCR) amplification of genomic DNA was performed using specific oligonucleotide primers for MS D/G genotype, MTHFR A/V genotype, and CBS 844ins68 genotype as previously described (Morita *et al.*, 1998; Morita *et al.*, 1999; Tsai *et al.*, 2000). We used digestion with *Hae*III and *Hin*I restriction enzymes for the detection of A2756G variant of the MS gene and C677T variant of the MTHFR genes, respectively.

Results

As shown in Figures 1 and 2, the proband was found to have V/V genotype (A222V) in the MTHFR gene polymorphism (C677T) and D/D genotype (D919G) in the MS gene polymorphism (A2756G). However, as

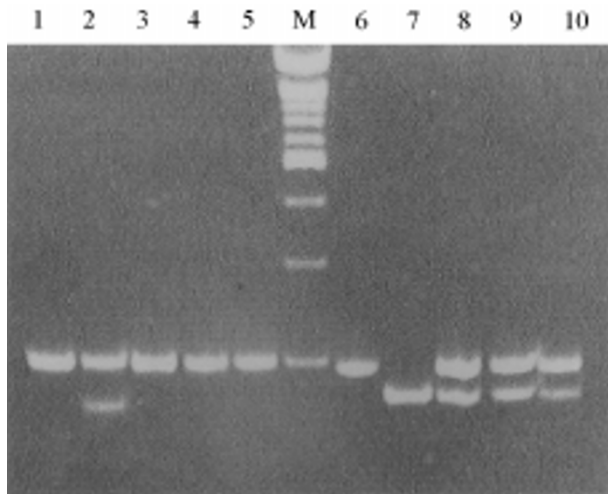


Figure 1. Restriction enzyme analysis of MS and MTHFR genes in the proband and his family. The A2756G (D919G) substitution of MS gene creates a *Hae*III recognition sequence which digests the 189 bp fragment into 159 and 30 bp fragments (Lane 1: proband, D/D; Lane 2: father, D/G; Lane 3: mother, D/D; Lane 4: sister, D/D). The C677T (A222V) substitution of MTHFR gene creates a *Hin*I recognition sequence which digests the 198 bp fragment into 177 and 21 bp fragments (Lane 7: proband, V/V; Lane 8: father, V/A; Lane 9: mother, V/A; Lane 10: sister, V/A). Lane 5 and 6: intact PCR products of proband before digestion. Lane M: DNA size marker, 100 bp ladder.

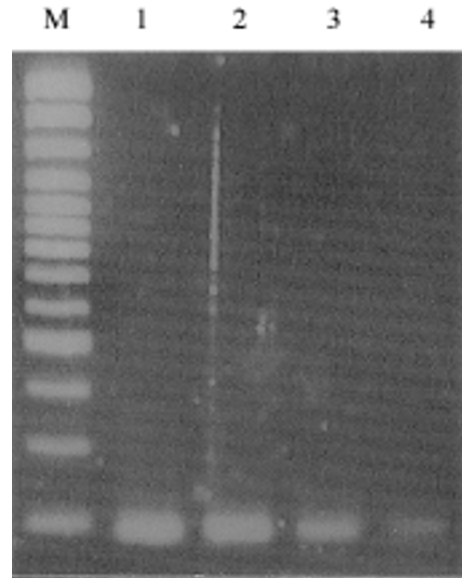


Figure 2. Polymerase chain reactions of CBS gene in the proband and his family. The 68 bp insertion (844 ins68) of CBS gene creates 252 bp instead of 184 bp without 68 bp insertion (Lane 1: proband; Lane 2: father; Lane 3: mother; Lane 4: sister). All the products showed no insertions. Lane M: DNA size marker, 100 bp ladder.

Table 1. MTHFR, MS, and CBS genotypes

| Genotype | MTHFR (A222V) | MS (D919G) | CBS (844ins68) |
|----------|---------------|------------|----------------|
| Proband | V/V | D/D | Ins68 -- |
| Father | V/A | D/G | Ins68 -- |
| Mother | V/A | D/D | Ins68 -- |
| Sister | V/A | D/D | Ins68 -- |

MTHFR, methylene tetrahydrofolate reductase; MS, methionine synthase; CBS, cystathionine β-synthase.

shown in the Figure 2, the proband was not a carrier of 844ins68 allele of CBS gene. Genotypes of the proband as well as his family members were summarized in Table 1.

Discussion

Strokes remain as the leading cause of death in Korean population with high mortality (80/100000), in which cerebral infarction is predominantly involved (Yoo *et al.*, 1998). The conventional risk factors for cerebral infarction in Koreans include hypertension, smoking, hypercholesterolemia, diabetes, and aging (The Korean Neurological Association, 1993). Routine laboratory tests to detect hereditary or acquired thrombotic disorders are not warranted in older patients with stroke due to the presence of atherosclerosis of the carotid or cerebral arteries. However, in younger adults in whom there is no obvious reason for stroke, a search for an underlying predispo-

sition to thrombosis can be fruitful and could alter therapy (Hathaway and Goodnight, 1993). As a result of clinical investigation to search any other risk factors in addition to dyslipidemia, we present a patient with an elevated plasma homocysteine level and variations in the MTHFR and MS genes, which code for key enzymes in the homocysteine metabolic pathways.

The association between homocysteinemia and stroke have been reported in previous studies (Brattstrom *et al.*, 1990; Coull *et al.*, 1990; Verhoef *et al.*, 1994), and multivariate adjusted odds ratio for the highest quartile versus the lowest was 2.5 among whites (Giles *et al.*, 1998). This association may not differ by race and the odds ratio of the highest 5% of homocysteine levels in control group in Koreans was reported to be 1.70 after an adjustment for known risk factors (Yoo *et al.*, 1998).

A common missense mutation, A222V, has been identified in the MTHFR gene, where substitution of alanine (A) by valine (V) results in a thermolabile variant with 50% reduced enzymatic activity at 37°C and complete loss of activity at 46°C (Kang *et al.*, 1988; Frosst *et al.*, 1995). This thermolabile variant has been consistently associated with mild elevations of plasma homocystein concentration (Brattstrom *et al.*, 1998). In fact, individuals homozygous for the V222 allele were reported to have 1.6 nM/ml higher homocysteine compared to individuals with the other genotypes, and the effect of the thermolabile variant was particularly profound in the presence of low folate levels (Dekou *et al.*, 2001). A significant decrease of blood folate concentrations in a subgroup of stroke patients who had increased plasma homocysteine concentrations has also been described (Hultberg *et al.*, 1997). Thus the increase in plasma homocysteine concentration in the present case may partly be caused by a marginal folate deficiency. Therefore, folate supplement could have a therapeutic importance to abolish the V/V genotype effect in the present case.

Leclerc *et al.* (1996) described the D919 polymorphism in the MS gene and the results from the study of healthy men have demonstrated that the D919G polymorphism has a modest effect on homocysteine levels, with carriers of the G919 allele having levels 0.6 nM/ml lower than homozygotes for the common D allele.

It has also been reported that carriers of the 68 bp allele in the CBS gene had a mean homocysteine 0.8 nM/ml lower than those lacking the allele, and in the group of men homozygous for the common D/D allele, those lacking the CBS 68 bp allele had a median homocysteine 1.0 nM/ml higher than those also carrying the 68bp allele in a genetic interaction analysis (Dekou *et al.*, 2001). Taken together, these findings suggest that there is a biological "cross-talk" between the re-methylation and the trans-sulfuration pathway in determining plasma homocysteine levels (Dekou *et al.*, 2001). In subjects with MS D/D genotype, the odds ratio

for comparing the MTHFR A/A genotype with A/V or V/V genotype increased to 1.99 in a Japanese study to assess the association between late onset vascular disease including ischemic stroke and the A/V polymorphism of MTHFR (Morita *et al.*, 1998). A recent study have documented the evidence of polygenic regulation of plasma homocysteine, thus providing new insights of the importance of genetic influences in carriers of common polymorphic traits, which predisposed them to either higher or lower homocysteine concentration (Tsai *et al.*, 2000). Therefore, our case study suggests that the interactions of the CBS 68 bp allele with MTHFR V/V homozygosity and with MS D/D homozygosity in determining plasma levels of homocysteine could be additive and of clinical importance, especially in the thrombotic patients at young ages. However, there appear to be no prospective data on the association of these polygenic effects on homocysteine levels with incidence or onset of ischemic stroke.

The limitation of this study is that we could not measure plasma homocysteine levels in the probands family members, which have made us impossible to observe the cosegregation of genotypes and phenotypes in this family. Anyway, the summary odds ratios of all the previous studies for patients with hyperhomocysteinemia were reported to be 2.5 for cerebrovascular disease (Markis, 2000). Therefore, additional prospective studies are needed to clarify these potential polygenic risk factors and clinical significance for stroke. In conclusion, disturbances of re-methylation enzymes (MTHFR, MS), or deficiencies of the trans-sulfuration enzyme (CBS) as well as role of genetic factors of these genes should be considered in association with hyperhomocysteinemia, particularly in thrombotic patients with no other acquired or hereditary causes (Fodinger *et al.*, 1999; Markis, 2000).

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