

## Research on the relationship between the polymorphisms of Methionine synthase (MS A2756G ) gene and ischemic cerebrovascular disease

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### Abstract

**Objective:** To explore the relationship between the polymorphisms of Methionine synthase (MS A2756G ) gene and ischemic cerebrovascular disease. **Methods:** The genotypes of MS A2756G were determined by PCR-RFLP, in 512 patients with ischemic cerebrovascular disease and 500 healthy controls in Henan Han population. **Result:** The distribution of genotype and allele in MS A2756G had no significant difference between the patient and the control groups, while the frequencies of MS A2756G mutations had significant differences between the Henan Han population and other races. **Conclusion:** Gene mutations as MS A2756G may not be independent risk factors for ischemic cardiovascular in Northern Chinese Han population. The prevalence of MS A2756G may vary among different ethnic groups or geographic regions. [Life Science Journal. 2009; 6(3): 27 – 31] (ISSN: 1097 – 8135)

**Key words:** Ischemic cerebrovascular disease, Methionine synthase, Polymorphism

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### 1 Introduction

CVD, which has high incidence and mortality and disability, is a common and dangerous disease and now has become one of three most fatal diseases. Among them, ICVD is up to 80%. The etiology of ICVD is complicated and not understood completely until now. New researches have discovered that ICVD is related with both hereditary and environmental factors.

Recently, many researchers focus on the predisposing genes of the related risk factors of cerebral infarction. Several recent studies have shown that Hyperhomocysteinemia (HHcy) is increasingly one of the more important and independent risk factors for thrombotic vascular or arteriosclerotic diseases<sup>[1,2,3]</sup>. Therefore, the metabolism-relative enzyme of Hcy has become one of candidate genes. But the correlation between the mutations of MS A2756G and ischemic cerebrovascular disease remains controversial. The aim

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of the present study was to investigate the association between the polymorphism of MS A2756G gene and ischemic stroke in a case-control study in the Han population in Chinese of Henan.

### 2 Methods

#### 2.1 Subjects

The patient group were consisted of 512 consecutive patients with ischemic stroke (310 cases were male and 202 cases were female, mean age years 60±10.2 years) who were recruited to the Hospital of Henan province from December 2004 to July 2005 and the control group were consisted of 500 healthy volunteers (274 cases were male and 226 cases were female, mean age 56±9.8 years). Diagnosis of ICVD was made by clinical manifestations and CT or MRI scans. Severe systemic diseases such as epilepsy, neoplastic, liver or renal disease were in the range of exclusion both patient and control group. All of them were unrelated, and were from the Chinese Han population.

#### 2.2 Genotyping of MS A2756G polymorphism

Blood samples were drawn from cases and controls in the fasting state and collected in EDTA tubes. Genomic DNA were extracted from peripheral-blood lymphocytes

by the standard phenol-chloroform method. The MS gene A2756G polymorphism was identified after amplification

and 5'-GAACTAGAAGACAGAAATTCTCTA-3') according to the previously described by Leclerc et al [4] and the conditions of the PCR reaction were thirty-five cycles (92°C for 60 seconds, 56°C for 60 seconds, and 72°C for 90 seconds) were used to amplify 189-bp products. PCR reaction mixture 25µl contains 100ng genoMic DNA, 10µmol of the primer, 1.5mol/L MgCL<sub>2</sub>, 50mol/L KCL, 10mol/L Tris-HCL(PH8.3), 0.2mol/L of each dNTP and 2.0U DNA polymerase. Following amplification, a restriction digestion was performed to detect the MS A2756G sequence polymorphism with the enzyme *Hae*III under the condition of 37°C during 16 hours. These products and PCR products were separated using electrophoresis through 2% agarose gel and staining with ethidium bromide and visualized under UV light.

### 2.3 Statistical analysis

The frequencies of the alleles and genotypes were counted and compared by the Chi-square test. Odds Ratios(OR) and their 95% confidence intervals (95%CI) were used to estimate the risk association to the genotype. All statistical procedures were performed with SPSS 11.0 software package. *P* values below 0.05 were considered statistically significant.

## 3 Results

### 3.1 Mutation Identification

The pattern of heterozygous MS A2756G mutation

with primers (5'-CATGGAAGAATATGAAGATATTAGAC-3'

(AG) showed three bands of 189, 159 and 30bp. The mutant homozygote (GG) showed two bands of 159 and 30 bp; the wild type (AA) showed only one band of 189 bp (fig1. the 23-bp band was out of gel).

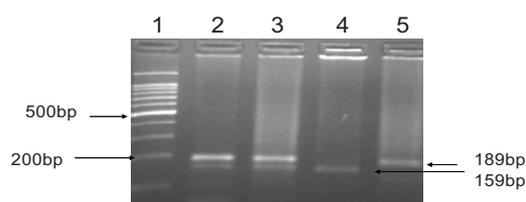


Fig.1 Genotyping of the MS A2756G gene

Lane 1:100bp DNA ladder

Lane 2,3:AG heterozygous

Lane 4:GG homozygous

Lane 5:AA homozygous

### 3.2 Genotype distributions and allele frequencies

The genotype distribution for both patients and controls was consistent with Hardy-Weinberg equilibrium by Chi-square test ( $P > 0.05$ ) (Tab1). The frequency of MS A2756G genotypes in study and control is shown in (Tab2). As can be seen, the frequency of genotypes and G alleles was no significantly difference in the study group compared with controls.

The data summarized in Table 3 demonstrate that the genotypes and allele distribution of MS A2756G in Henan Han population was consistent with the other areas in Chinese and differences with most foreigner of white races .

Table 1. The Hardy-Weinberg equilibrium of MS A2756G genotypes in patients and controls

genotypes	Patient group		Control group	
	object	expect	object	expect
AA	455	452	423	423.2
AG	55	57.8	75	73.6
GG	2	1.8	2	3.2
	$\chi^2=0.090$ P=0.75		$\chi^2=0.207$ P=0.825	

Table 2. Prevalence of the MS A2756G genotypes and allele frequency in patients and controls

groups	subjects(n)	Genotype frequency n (%)			allele frequency (%)	
		AA	AG	GG	A	G
Patient	512	455(88.9)	55(10.7)	2(0.39)	965(94.2)	59(5.8)
control	500	423(84.6)	75(15)	2(0.4)	921(92.1)	79(7.9)
		$\chi^2=4.101$ P > 0.05			$\chi^2=3.64$ P > 0.05	

Table 3. Methionine Synthase A2756G Genotypes and alleles in Case-Control and Reference Panels

Genotype frequencies	China (Henan)	China <sup>[5]</sup> (Guangzhou)	China <sup>[5]</sup> (Hunan)	American <sup>[8]</sup>	Finnish <sup>[4]</sup>	Austrilian <sup>[6]</sup>	Spanish <sup>[7]</sup>
AA	84.6	80.09	82	70.88	59	62.75	66.87
AG	15	18.02	17	25.82	38	33.22	30.12
GG	0.4	1.89	1	3.38	3	4.03	3.01
		$\chi^2=2.424$ P>0.05	$\chi^2=1.179$ P>0.05	$\chi^2=7.208$ P<0.05	$\chi^2=17.64$ P<0.001	$\chi^2=14.020$ P<0.001	$\chi^2=10.13$ P<0.001
Allele frequencies							
A(%)	92.1	89.10	90.50	83.79	78.39	79.36	81.93
G(%)	7.9	10.90	9.5	16.21	21.62	20.64	18.07
		$\chi^2=0.523$ P>0.05	$\chi^2=0.223$ P>0.05	$\chi^2=3.93$ P<0.05	$\chi^2=7.686$ P<0.01	$\chi^2=5.413$ P<0.05	$\chi^2=4.423$ P<0.05

### 3.3 Risk estimation

In subjects with the methionine synthase (AG+GG) genotype, the odds ratio (OR) and 95% confidence interval (CI) for patients with ischemic cerebrovascular diseases to controls were 0.682(0.47-0.98) and 0.93(0.13-6.63), respectively (P>0.05).

### 4. Discussion

Epidemiological evidence suggested that ischemic cerebrovascular disease (ICVD) shares many risk factors, such as age, hypertension, and smoking, et al. Recently, Cattaneo et al<sup>3</sup> reported that Hyperhomocysteinemia, which corresponds to the C677T mutation in the MTHFR gene, may be an inherited risk factor for coronary artery disease and ischemic cerebrovascular diseases. It is also possible that the features of the hemostatic system may influence the development of ICVD, as has been suggested for IHD. Genetic variations play a role in the ICVD.

Homocysteine is a sulfur amino acid generated as an intermediate product in methionine metabolism and

occurs at the intersection of two metabolic pathways, remethylation and transsulfuration. These pathways are known to be regulated by 3 key enzymes: cystathionine  $\beta$ -synthase, homocysteine methyltransferase (methionine synthase), and 5, 10-methylenetetrahydrofolate reductase (MTHFR), as well as by the cofactors folate, vitamin B6, and vitamin B12.

Methionine synthase is an key enzyme in molulation of plasma homocysteine by converting it into methionine. The mutation of Methionine synthase (MS A2756G ) leads to a reduction in enzyme activity and subsequent elevation of plasma homocysteine. Leclerc et al<sup>[4]</sup> Interestingly, they also showed that this mutation is common in the general population and inferred that it might lead to mild hyperhomocysteinemia with a consequent impact on vascular disease. Thus, analysis of the genetic polymorphism of methionine synthase might provide us with an explanation for elevated homocyst(e)ine levels in those cases that cannot be explained by other causes, such as MTHFR genotype. The most prevalent mutation of the MS gene is the

A2756G transition, which results in the substitution of aspartic acid by glycine [8]. Leclerc et al<sup>[4]</sup> found 3 mutations in Canadian patients with deficiencies in methionine synthase activity and among Western Caucasian populations, the prevalence of heterozygous and homozygous MS A2756G carriers has been reported to be around 32% and 4%, respectively. In our study, prevalence of the MS A2756G AG and GG genotypes in patients and controls is 57 and 79 relatively, while the allele frequency of G in patients is 59 and in controls is 79, which does not exhibit a significant difference between patients with ICVD patients and healthy controls. we found no evidence to suggest an association between this methionine synthase mutation and ischemic cerebrovascular diseases. The information indicates that the MS A2756G gene may not be an independent risk factor for ischemic cerebrovascular diseases in the Henan Han population. Differences in allelic frequencies had been reported in difference ethnic groups. In our study, the frequency of G allele in cases and controls was 5.8% and 7.9%, respectively. The G allele frequency of the MS A2756G polymorphism in Henan Han population was similar to that of Guangzhou and Hunan ( $P > 0.05$ )<sup>[8]</sup> significantly lower than that of the Americans ( $P < 0.05$ ). It was also lower than frequencies of the Spanish and Australian ( $P < 0.05$ )<sup>[4,6]</sup>, which suggested that the prevalence of gene mutation of MS A2756G varied with different ethnic group or geographic regions.

We didn't measure plasma levels of homocyst(e)ine and folate in the participants with ischemic cerebrovascular disease, which is thought to be another limitation of this study.

## 5 Conclusion

Our preliminary data indicates lack of significant association between MS A2756G polymorphism with ICVD; MS A2756G gene mutation may not be an independent risk factor for ischemic cerebrovascular disease. The polymorphisms of MS A2756G display an ethnic or geographic difference.

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