CLINICAL SCIENCES

METHYLENETETRAHYDROFOLATE REDUCTASE GENE POLYMORPHISM IS NOT RELATED TO THE RISK OF ISCHEMIC CEREBROVASCULAR DISEASE IN A BRAZILIAN POPULATION

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PURPOSE: Data are conflicting concerning the risk for ischemic stroke associated with a common polymorphism in the gene encoding 5,10-methylenetetrahydrofolate reductase C677T, which predisposes carriers to hyperhomocysteinemia. A meta-analysis study suggested that the 5,10-methylenetetrahydrofolate reductase 677TT genotype might have a small influence in determining susceptibility to ischemic stroke.
METHODS: We analyzed the 5,10-methylenetetrahydrofolate reductase 677TT genotype polymorphism in Brazilian subjects with ischemic stroke, using a case-control design.
RESULTS: We compared 5,10-methylenetetrahydrofolate reductase genotypes in groups of subjects presenting ischemic stroke (n = 127) and normal control (n = 126) and found an odds ratio of 1.97 (95% CI, 0.84-4.64) in a multivariate analysis in which results were adjusted to baseline clinical characteristics of study participants.
CONCLUSION: We found that the homozygous 5,10-methylenetetrahydrofolate reductase C677T genotype was not a risk factor for ischemic stroke in these Brazilian subjects.

KEYWORDS: Genotype. Homocysteine. Ischemic stroke. Polymorphism. Risk factor.

INTRODUCTION

Ischemic stroke, which results from the abrupt interruption of focal cerebral blood flow[1,2] for more than 24 hours, plays an important role in death and disability worldwide. It is the third leading cause of death and the leading cause of serious long-term disability in the United States. Approximately 750,000 strokes occur annually, with a mortality rate exceeding 150,000[3–6] in a year.

Because stroke is a complex disease comprising a heterogeneous group of disorders with multiple risk factors, research into the genetics of stroke presents unique challenges. Among many genetic factors that have been studied in this context, the common polymorphism in the gene encoding 5,10-methylenetetrahydrofolate reductase (*MTHFR C677T*) may be of importance. The enzyme MTHFR catalyzes the reduction of 5,10-methylenetetrahydrofolate to 5-methyltetrahydrofolate, the predominant circulating form of folate and carbon donor for the remethylation of homocysteine to methionine and plays a crucial role in regulating the levels of homocyteine. This thermolabile variant form of MTHFR appears to facilitate the manifestation of hyperhomocysteinemia, which increases the risk of vascular disease and stroke[7–14] up to 2.5-fold, according to a meta-analysis study.[10]

However, other studies fail to demonstrate any association

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between ischemic stroke and the MTHFR 677TT genotype.[15,16] According to Markus et al,[16] the homozygous thermolabile genotype was not associated with ischemic cerebrovascular disease, despite the significant association of the genotype with elevated homocysteine in the patient group. Kelly et al[17], in another meta-analysis study demonstrated the association between mild-to-moderate hyperhomocysteinemia and ischemic stroke, and more recently this polymorphism has been suggested as a genetic risk factor for ischemic stroke.[18] In contrast, other authors have reported that the MTHFR 677TT genotype may have a small influence in determining susceptibility to ischemic stroke.[16]

In the present work, we investigated the prevalence of MTHFR genotypes in Brazilian patients with ischemic stroke, to assess likehood they represent a risk factor for this disease.

SUBJECTS AND METHODS

Study population

Stroke was defined as an episode of sudden-onset lateralized neurological deficit and was confirmed by objective evidence of ischemia on computed tomography brain scan. After approval by the Research Ethics Committee of Hospital das Clínicas, São Paulo University Medical School, informed consent was obtained from 127 patients with stroke in the period of 1999 to 2004. The following information regarding known risk factors for stroke was obtained from each patient (through patient/caregiver interview, patients' medical records, and laboratory tests and clinical exams): hypertension, current smoking, history of previous stroke, cardiovascular disorders (ischemic heart disease, atrial fibrillation, and congestive cardiac failure), diabetes mellitus, dyslipidemia, and chronic renal insufficiency. Control subjects (n = 126) were recruited from the same hospital. The exclusion criteria for control subjects were a past history of symptomatic stroke. Neuroimaging was not performed for control patients, so that it is possible that controls with silent lesions could have been included. Patients with cerebral hemorrhage, transient ischemic attack, or cerebral venous thrombosis were not included in the present work.

MTHFR C677T genotyping

DNA was extracted from peripheral venous blood collected with EDTA by a salting out method.[19] Polymerase chain reaction (PCR) amplification of the DNA samples was peformed following the method described by Frosst et al.[11] Restriction analysis with the enzyme H*in*fI (Boehringer Mannheim, Germany) was performed according to the manufacturer's recommendation. Digestion fragments were separated by 8% polyacrilamyde gel electrophoresis and visualized under ultraviolet light following ethidium bromide staining.

Statistical analysis

Differences among the genotype groups were examined with the use of Pearson's χ^2 test or the unpaired Student *t* test when appropriate. Significance was considered when $P < 0.05$. The 95% confidence intervals (95% CI) of the percentage were calculated by assuming a normal distribution. Goodness-of-fit of genotype distribution to Hardy-Weinberg equilibrium was examined. All calculations were performed using the computer program STATA version 7.0 (STATA Corp., College Station, TX, USA).

RESULTS

Demographic characteristics of the subjects participating in the study are shown in Table 1. Mean age was

Table 1 - Clinical and demographic characteristics of ischemic stroke patients and control subjects studied

	Case	Control	*p* value
Average age, year	64.0	63.5	1.000
Male (%)	53 (41.7)	72 (57.2)	0.028
Ethnic origin			
White (%)	82 (64.6)	85 (67.5)	
Black (%)	32 (25.2)	31 (24.6)	0.060
Yellow (%)	13 (10.2)	10 (7.9)	
Hypertension (%)	53 (41.7)	28 (22.2)	0.000
Current smoking (%)	22 (17.3)	9 (7.1)	0.069
Atrial fibrillation (%)	12 (9.5)	2 (1.6)	0.078
Ischemic heart disease (%)	9 (7.1)	2 (1.6)	0.830
Dislipidemic (%)	50 (39.4)	52 (41.3)	0.758
Congestive cardiac failure (%)	6 (4.7)	1 (0.8)	0.057
Diabetes mellitus (%)	18 (14.2)	16 (12.7)	0.731
Chronic renal insufficiency (%)	6 (4.7)	3 (2.4)	0.341
Total (%)	127 (100)	126 (100)	

63.8 years (range, 16-93 years). The case and control groups are similar, except for statistically significant predominance of men in the control group and hypertension in the case group, supporting the latter as a risk factor for stroke.

The distribution of MTHFR genotypes in the case and control groups is shown in Table 2. The frequencies of MTFHR genotypes were calculated, and the Hardy-Weinberg equilibrium test for *MTHFR 677TT* showed no significant discrepancy in the genotype distribution of the allele frequency (χ^2=0.15, p = 0.70). The allele distribution was 0.65 for control and 0.35 for case groups. The prevalence of the homozygous thermolabile MTHFR 677TT genotype was higher in the ischemic stroke group (observed in 20 cases - 15.7%) than in the age-matched controls (present in only 12 control subjects (9.5%). However, the distribution of these values showed Pearson's χ^2=2.5767 and p = 0.276, meaning that the observed trend is not significant for the number of subjects included in the study (OR, 1.97; 95%CI, 0.84-4.64 for *MTHFR 677TT* and OR, 0.84; 95%CI, 0.49-1.43 for *MTFHR C677T*, compared to *MTHFR 677CC* as the reference).

Table 2 - Distribution of 5,10-methylenetetrahydrofolate reductase genotypes in ischemic stroke cases and control group

MTHFR genotype	Control group	Ischemic stroke
TT (%)	12 (9.5)	20 (15.7)
CT (%)	60 (47.6)	52 (41.0)
CC (%)	54 (42.8)	55 (43.3)
Total (%)	126 (100)	127 (100)

Pearson χ^2=2.5767 p=0.276; MTHFR: 5,10-methylenetetrahydrofolate reductase

DISCUSSION

Reported data concerning risk for ischemic stroke associated with the common polymorphism *C677T* in the gene encoding MTHFR are thus far conflicting.

In the present case-control study, we investigated the relationships between *MTHFR C677T* polymorphism and other variables well known to be associated with an atherosclerotic risk in a Brazilian population.

We have shown that the homozygous MTHFR 677TT genotype is not a risk factor for ischemic stroke in these Brazilian subjects. In a meta-analysis study, Kelly et al[17] analyzed 19 published works (2788 stroke and 3962 nonstroke cases) on *MTHFR* polymorphism and concluded that the MTHFR 677TT genotype can be considered a mild-to-moderate risk factor for ischemic stroke. These authors found a small influence (pooled OR, 1.23; 95% CI, 0.96-1.58) of the MTHFR 677TT genotype on stroke risk, which showed a trend toward disease, but did not reach the threshold for statistical significance. In a subsequent meta-analysis study, Cronin et al[18] showed a gradual increase in ischemic stroke risk with increasing *MTHFR 677T* allele (OR, 1.48; 95% CI, 1.22-1.80), suggesting an influence of this polymorphism as a genetic stroke risk factor.

The association between the genotype and homocysteinemia is dependent, at least in part, on serum folate levels. Harmon et al[13] and Jacques et al[20] found that elevated homocysteine was only associated with the genotype in individuals with folate levels below the population median. Therefore, the activity of the thermolabile variant of this enzyme must be influenced by the availability of folate. Individuals who are homozygous for the thermolabile variant of MTHFR and who have low plasma folate levels may be particularly prone to moderate/intermediate homocysteinemia. According to Guttormsen et al,[21] plasma homocysteine levels in such individuals can be lowered by using folic acid. In the present study, the folate and homocysteine status of the population has not been determined.

Frequencies of the thermolabile MTFHR genotype vary dramatically depending on different ethnic background, from 0[25] to 16.7%[26]. For white populations in US and Australia, the frequency is about 11.5%[27]. In Brazil, this genotype frequency is 14.9% and 11.8% in white individuals with stroke and without stroke, respectively; in black individuals, these frequencies are lower, being 10.3% and 1.9% in patients with stroke and without stroke, respectively[28]. According to Carod-Artal et al,[29] the thermolabile MTFHR genotype occurred in 5.05% and 10% of elderly and young individuals with stroke, respectively, admitted to a rehabilitation hospital. On the other hand, it has been shown that the frequency of this genotype is higher in Italy,[30] but paradoxically, cardiovascular complications are estimated to be relatively low. Different dietary habits could explain this paradox, where the typical Mediterranean diet comprises foods with high content of folate, such as green vegetables and orange juice. Another hypothesis could be a possible protective role of the thermolabile MTHFR variant in the presence of other genetic disorders, which are particularly prevalent in littoral people of Mediterranean region.[31] In the present work, we found prevalence of the thermolabile MTHFR variant in 15.7% in the case group compared with 9.5% in the control group. Therefore, in general, the prevalence of the thermolabile MTHFR variant in these Brazilian subjects was much higher than that described in the literature.

In summary, we have shown that the homozygous MTHFR 677TT genotype does not appear to be a risk factor for ischemic stroke in Brazilians. Although the present result is not statistically powerful because of the small number of subjects included, it should be considered as a relevant preliminary data collection program, leading to further future studies, including dietary information, such as folate intake and serum measurements of homocysteine and folate levels, to better evaluate the possible risk factors involved in stroke. Further studies are necessary with larger case-control samples that are better matched for gender to confirm our results.

RESUMO

Nagahashi Marie SK, Shinjo SK, Oba-Shinjo SM, da Silva R, Barbosa KC, Yamamoto F, Scaff M. Polimorfismo no gene de metilenetetrahidrofolato redutase não está relacionado com o risco de doença cerebrovascular isquêmica em uma população brasileira. Clinics. 2007;62(3):295-300.

OBJETIVO. Os dados são conflitantes em relação a risco de acidente cerebrovascular associado a polimorfismo do gene 5,10-metilenetetrahidrofolato redutase C677T, o qual predispõe a hiperhomocisteinemia. Um estudo de meta-análise sugere que o genotipo 5,10-metilenetetrahidrofolato redutase 677TT poderia ter uma pequena influência em

determinar susceptibilidade a acidente cerebrovascular.
MÉTODOS: Analisamos este polimorfismo em indivíduos brasileiros com acidente cerebrovascular isquêmico, baseando-se em um estudo de caso-controle.
RESULTADOS: Comparamos os genótipos 5,10-metilenetetrahidrofolato redutase em grupos de indivíduos com acidente cerebrovascular isquêmico (n=127) e controle normal (n=126), e encontramos Odds Ratio de 1,97 (IC 95% 0,84 - 4,64) em uma análise multivariada, na qual os resultados foram ajustados a características clínicas basais dos indivíduos estudados.
DISCUSSÃO: Nossos estudos indicam que o genótipo 5,10-metilenetetrahidrofolato redutase C677T não é um fator de risco para acidente cerebrovascular isquêmico entre indivíduos brasileiros.

UNITERMOS: Genótipo, Homocisteína, Acidente cerebrovascular isquêmico, Polimorfismo, Fator de risco.

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