Role of MTHFR C677T polymorphism in ischemic stroke

Inusha Panigrahi, Tathagata Chatterjee, Arijit Biswas, Madhuri Behari*, Prakash Ved Choudhry, Renu Saxena

Departments of Hematology and *Neurology, All India Institute of Medical Sciences, Ansari Nagar, Delhi - 110 029, India

Background: Homozygosity for *MTHFR C677T* polymorphism can lead to significantly high homocysteine levels and hyperhomocysteinemia is an important risk factor for thrombotic events. **Aims:** The aim was to determine role of *MTHFR C677T* polymorphism in North Indians with ischemic stroke. **Settings and Design:** In a prospective study, the subjects of stroke were recruited from the neurology clinic of the hospital. Controls were healthy individuals from the Hematology clinic without any history of stroke. **Materials and Methods:** Plasma homocysteine levels were measured by enzyme immuno assay method after 3 months of acute episode. Serum folate and Vitamin B12 levels were estimated by competitive inhibition radioassay. *MTHFR* polymorphism was detected by PCR-RFLP using *Hinf I* enzyme. **Statistical analysis:** The analysis of significance of results was done using SPSS software package. A pvalue < 0.05 was taken as significant. **Results:** Thirty-two acute ischemic stroke patients (aged 1-44 years) were studied. Fourteen (43.8%) had recurrent stroke. Nine (28%) had multiple infarcts. Four of 32 patients (12.5%) had high homocysteine levels. Three out of these 4 hyperhomocysteinemia patients were homozygous (TT) for *MTHFR* polymorphism (2 with recurrent stroke). Two of three homozygous cases with TT genotype had low serum folate. Five of 32 stroke cases (18.8%) were heterozygous (CT) genotype. **Conclusions:** Primary hyper-homocysteinemia appears to be an important risk factor for ischemic stroke in North Indians, most due to *MTHFR C677T* homozygosity. Folate levels may modify the presentation of the *MTHFR* TT genotype.

Key words: Asian-Indians, cerebrovascular disease, homocysteine, methylene tetrahydrofolate reductase.

dependent enzyme activity and reduced re-methylation of homocysteine to methionine. Homozygotes for this polymorphism have significantly higher homocysteine, an important predisposing factor for thrombosis. Present study was conducted to determine prevalence of hyper-homocysteinemia in North Indians with ischemic stroke and, to determine its relationship with *MTHFR* genotype, if any.

Materials and Methods

Subjects included consecutive cases of ischemic stroke attending the Neurology clinic of the hospital from June 2003 to May 2004, enrolled in the prospective study. Patients with ischemic stroke and no "traditional" risk factors were included. Inclusion criteria were, a) age < 45 years at presentation, and b) absence of history of smoking or significant systemic disease and intake of anti-epileptics, anti-cancer drugs or exogenous vitamins. Ischemic stroke was documented on MRI and/or CT. Recurrent stroke was defined as more than one episode of stroke at presentation, including the present episode at least 1 year apart. Sixty unrelated age and sex-matched healthy individuals from the Hematology clinic with no history of stroke, served as controls. Informed consent was taken from the patients or parents (in children < 10 years). The Institute ethical committee approved the study.

Plasma homocysteine (Hcy) levels were measured on samples obtained 3 months after acute episode, by modified EIA (enzyme immuno assay) (Diazyme laboratories, San Diego, CA). Normal level of homocysteine was taken as 5-12 $\mu\text{mol/l}$. Serum folate and Vitamin B12 levels were assayed by competitive inhibition radioassay (SimulTRAC, Becton Dickinson, Orangeberg, NY). The normal levels for folate and Vitamin B12 were taken as greater than 2.5 $\mu\text{g/ml}$ and 200ng/l respectively.

The *MTHFR C677T* polymorphism was studied by PCR-RFLP on DNA samples,^[1] using enzyme *Hinf I*. Statistical analysis was done by Fisher's exact test and Chi Square test by SPSS software and $P < 0.05$ was taken as significant.

Introduction

MTHFR C677T polymorphism results in reduced folate-

Renu Saxena

Professor, Department of Haematology, All India Institute of Medical Sciences, IRCH Building 1st Floor, Ansari Nagar, New Delhi - 110 029, India, E-mail: renusax@hotmail.com

Results

Of thirty-two patients with ischemic stroke (M: F= 22:10; age 1-42 years, median 12 years) were studied [Table 1]. Ten (31.3%) showed infarcts in MCA territory alone, and nine (28%) patients had multiple brain infarcts. Fourteen patients (43.8%) had recurrent stroke. High plasma homocysteine ($>12 \mu\text{mol/l}$) was seen in four of thirty-two (12.5%) [Table 2], not seen in controls. Occurrence of hyper-homocysteinemia in patients was significant ($P= 0.02$).

MTHFR homozygous (TT genotype) was seen in three patients but in none of controls ($P=0.073$). Youngest case was 25 years age and one had multiple brain infarcts. *MTHFR* CT (heterozygous) genotype was seen in six of 32 cases (18.8%) but

4/60 (6.6%) controls ($P= 0.16$). None of heterozygous individuals showed high homocysteine. However, no significant association was found between single vs. recurrent stroke in patients with hyperhomocysteinemia ($P=0.41$), *MTHFR* CT genotype ($P=0.98$) and *MTHFR* TT genotype ($P=0.77$). When single stroke, multiple stroke and multifocal infarct were considered with variables as high homocysteine, *MTHFR* TT genotype and CT genotype, the p value did not achieve significance. The number of cases in each group being less, the comparison between the groups did not show any significant difference.

Discussion

Studies in Caucasians have not shown correlation between

Table 1: Clinical characteristics of the ischemic stroke cases in present study

Age (yr)/Sex	Single/recurrent stroke	MRI/CT findings/carotid doppler
3/M	Single	MRI: MCA infarct
4½/F	Single	MRI: MCA infarct
25/M	Recurrent	MRI: multifocal infarct
7/M	Single	MRI: MCA infarct
7/F	Single	MRI: MCA and PCA infarct
13/M	Single	CT: infarcts fronto temporal parietal areas
5/M	Single	MRI: diffuse swelling of cerebellum with infarct (left) thalamus
22/F	Recurrent	MRI: MCA infarct
24/M	Recurrent PCA stroke	MRI: Infarct B/L cerebellar and Rt occipital.
11/M	Single	MRI: (L) MCA infarct including caudate lobe and (L) parietal lobe.
25/M	Recurrent 3 episodes	MRI: (R) MCA infarct
17/M	Single	CT: infarct (R) internal capsule to (R) temporal region.
22/M	Recurrent	CT: Rt Parietal infarct
12/M	Recurrent 2 episodes	MRI: B/L frontoparietal infarct and basal ganglion, (L) MCA territory
9/F	Recurrent	MRI: MCA and ACA infarct (L)
21/M	Recurrent	CT: infarct in internal capsule involving white matter
7/F	Recurrent	CT: (R) frontoparietal infarct with midline shift
21/M	Recurrent	MRI: infarct in internal capsule
2/M	Single	CT: (R) Basal ganglia and caudate lobe subacute infarct
15/M	Single	CT: Acute infarct (L) ACA territory
9/M	Single	CT: head: infarct (R) occipital and (L) frontal lobe
40/M	Recurrent	CT: Left temporal, pontine, midbrain infarct
9/M	Single	CT: infarct (R) putamen
21/M	Single	MRI: Infarct in internal capsule
2/M	Single	CT: infarct (L) MCA territory
42/F	Single	CT: infarct (R) MCA territory
40/F	Recurrent	MRI: Infarct in (L) internal capsule and basal ganglia, temporal and parietal lobe
22/M	Recurrent	CT: (R) basal ganglia and (L) pontine infarct
1/M	Single	CT: multiple infarcts B/L MCA and PCA territory
4/F	Recurrent 2 episodes	CT: multiple focal hypodense lesions in cerebral hemispheres
5/F	Single	CT: (R) temporo occipital infarct
2/M	Single	CT: (L) MCA territory infarct

Table 2: The laboratory characteristics* of the ischemic stroke cases (n=32)

Parameter (no. of cases)	Single stroke	Recurrent stroke	Multifocal infarct
*Hyperhomocysteinemia (4)	1	3	1
*Normal homocysteine (28)	17	11	6
*MTHFR CT genotype (6)	3	3	4
*MTHFR TT genotype (3)	1	2	1
*MTHFR CC genotype (23)	14	9	3
Low serum folate (5)	4	1	1
Low serum B12 (1)	1	0	0

Note*: Normal values for ser. homocysteine, ser. folate and ser. Vit B12 were $5-16 \mu\text{mol/l}$, $> 2.5 \text{ ug/ml}$ and $>200 \text{ pg/l}$ respectively. The *P* values were 0.41 (*vs.*), 0.098 (*vs.**), and 0.774 (*vs.**).

MTHFR C677T polymorphism^[2] and vascular disease. In present study, high incidence of heterozygosity (6.6%) was observed in controls. The ischemic stroke cases were young or middle aged patients, with median age of 12 years.

Serum homocysteine concentration was significantly higher in TT genotype compared to CC (normal) and CT (heterozygous) genotypes,^[3] in a Turkish study. The prevalence of homozygous gene mutation for *MTHFR* was high,^[4] in Turkish stroke patients. In present study, 4/32 (12.5%) of young stroke had hyperhomocysteinemia, of which 3 had TT genotype. A previous Indian study has reported CT genotype,^[5] in 54.5% patients with hyperhomocysteinemia and coronary artery disease (CAD). In study from Hyderabad,^[6] TT genotype was found in only 1.4% of hyperhomocysteinemia cases with low background prevalence for heterozygosity (2.08%). In a study in Tamilians,^[7] CT genotype (heterozygosity) was seen in 18.1% (13/72) and TT genotype in 1.38%. The *MTHFR* A1298C polymorphism is even more prevalent in Tamilians (47.2%). Some selection bias could have been due to analysis of a heterogenous population, comprising of 52 acute myocardial infarction cases and 20 normal individuals. In a study from Mumbai,^[8] heterozygote frequency was high in CAD (38%) though comparable to controls (31%) and significant association with disease was found in females. In another study from Mumbai in venous thrombembolism,^[9] CT and TT genotypes were present in 14.9% and 1.2%. Thus there is marked geographic variation in frequency of C677T polymorphism in Indians. Present study reflects high prevalence of *MTHFR* polymorphism in North Indians and a relatively greater contribution to hyperhomocysteinemia especially in young aged 20-40 years. All three homozygous TT genotype cases were in this age group, youngest being 25 years age.

Co-occurrence of homozygous methylenetetrahydrofolate reductase (*MTHFR*) TT and angiotensin-converting enzyme (ACE) D/D genotypes yielded a highly significant moderate risk of leukoaraiosis (ischemic white matter demyelination),^[10] in a Hungarian study.

In present study, no significant correlation was found between the studied factors (hyperhomocysteinemia, TT and CT genotypes) and single vs recurrent stroke. Larger studies may resolve this issue.

The effect of genetic polymorphism can be influenced by environmental factors. Synergistic effects between the ACE D/D

and *MTHFR* TT genotypes and drinking or smoking,^[11] has been found. *MTHFR* genotype could also be an important risk factor with low folic acid intake. In present study, 15.6% patients were folate deficient, including two of three homozygous cases. In previous study,^[5] 32% of CAD cases with hyperhomocysteinemia were folate deficient. It is thus suggested that in adult patients with homozygous *MTHFR* C677T polymorphism, higher doses of folic acid/B-complex could overcome defect due to TT genotype and may serve as a feasible option for stroke prevention.

Acknowledgements

We sincerely thank Mr. Suresh Kumar for technical support and residents in Neurology for help in conducting the relevant studies.

References

1. Frosst P, Blom HJ, Milos R, Goyette P, Sheppard CA, Matthews RG, et al. A candidate genetic risk factor for vascular disease: a common mutation in methylene tetrahydrofolate reductase. *Nat Genet* 1995;10:111-3.
2. Brattstrom L, Wileken DEL, Öhrvik J, Brudin L. Common methylene tetrahydrofolate reductase gene mutation leads to hyperhomocysteinemia but not to vascular disease. The result of a meta-analysis. *Circulation* 1998;98:2520-6.
3. Yilmaz H, Isbir S, Agaahan B, Ergen A, Farsak B, Isbir T. C677T mutation of methylene tetrahydrofolate reductase gene and serum homocysteine levels in Turkish patients with coronary artery disease. *Cell Biochem Funct* 2005; Jan 13; [Epub ahead of print].
4. Ucar F, Sommez M, Ovali E, Ozmenoglu M, Karti SS, Yilmaz M, et al. *MTHFR* C677T polymorphism and its relation to ischemic stroke in the Black Sea Turkish population. *Am J Hematol* 2004;76:40-3.
5. Nair KG, Nair SR, Ashavaid TF, Dalal JJ, Eghlim FF. Methylene tetrahydrofolate reductase gene mutation and hyperhomocysteinemia as risk factor for coronary artery disease in the Indian population. *J Assoc Phys India* 2002;50:9-15.
6. Alluri V, Mohan V, Komandur S, Chawda K, Chaudhuri JR, Hasan Q. *MTHFR* C 677T gene mutation as a risk factor for arterial stroke: a hospital based study. *Eur J Neurol* 2005;12:40-4.
7. Angeline T, Jeyaraj N, Granito S, Tsongalis GJ. Prevalence of *MTHFR* gene polymorphisms (C677T and A1298C) among Tamilians. *Exp Mol Pathol*. 2004;77:85-8.
8. Mukherjee M, Joshi S, Bagadi S, Dalvi M, Rao A, Shetty KR. A low prevalence of the C677T mutation in the methylenetetrahydrofolate reductase gene in Asian Indians. *Clin Genet* 2002;61:155-9.
9. Ghosh K, Shetty S, Madkaiker M, Pawar A, Nair S, Khare A, et al. Venous thromboembolism in young patients from western India: a study. *Clin Appl Thromb Hemost* 2001;7:158-65.
10. Szolnoki Z, Somogyvari F, Kondacs A, Szabo M, Fodor L, Bene J, et al. Specific APO E genotypes in combination with the ACE D/D or *MTHFR* 677TT mutation yield an independent genetic risk of leukoaraiosis. *Acta Neurol Scand* 2004;109:222-7.
11. Szolnoki Z, Somogyvari F, Kondacs A, Szabo M, Fodor L, Bene J, et al. Evaluation of the modifying effects of unfavourable genotypes on classical clinical risk factors for ischaemic stroke. *Neurol Neurosurg Psychiatr* 2003;74:1615-20.

Accepted on 11-01-2006

Invited Comments

Over the last decade, convincing evidence has been gathered on the relation between moderate elevation of plasma total homocysteine (tHcy) and ischemic stroke. If a causal association really exists, then the practical implication is that the intervention of increasing folate intake at a population level could lead to a decrease in stroke risk. However, causality has yet to be established. At least theoretically, the tHcy-stroke association might be a spurious epidemiological finding and elevated tHcy could be interpreted as an epiphenomenon secondary to the vascular disease itself.^[1]

Recently, evidence that tHcy may be a causal risk factor for ischemic stroke was provided by a large meta-analysis of literature, based on the principle of Mendelian randomization.^[2] However, most of the studies included in this meta-analysis were from Europe or North America and focused on subjects with mean age of 40 years or older, with relatively few data from studies conducted in other continents and from younger groups.

In this issue of *Neurology India*, NAME OF THE FIRST AUTHOR *et al*^[3] report on a hospital-based, case-control study including a group of 1- to 42-year-old patients (median age: 12 years) with ischemic stroke and no history of major "traditional" cardiovascular risk factors. Among the 32 patients who were recruited over one year, these investigators found a higher prevalence of hyperhomocysteinemia in comparison to the group of 60 controls, and a trend toward a significant association between the *TT677 MTHFR* genotype and disease. Such findings were independent on clinical phenotype (single infarct vs recurrent infarcts). Furthermore, given the inclusion criteria, the influence of a confounding effect on the reported association seems negligible.

Although the small sample size precludes extensive analysis, this study highlights the role of homocysteine as a potential risk factor for ischemic stroke at young age in this specific ethnic group.

Whether increased tHcy is as a causal risk factor for ischemic stroke remains to be proven. However, while awaiting for conclusive evidence from powered randomized trials of multi-vitamin supplementation,^[4] the implication of these findings may be that measuring tHcy levels and screening of *MTHFR* genotypes in stroke patients of this ethnic group is warranted, since the benefits of the empirical practice of treating selected high-risk patients (such as those included in NAME OF THE FIRST AUTHOR *et al* study) with folic acid-based multivitamin therapy, outweigh the potential risks.

Alessandro Pezzini

*Clinica Neurologica, Università degli Studi di Brescia,
P.le Spedali Civili, 1, 25125 - Brescia, Italy
E-mail: ale_pezzini@hotmail.com*

References

1. Ueland PM, Refsum H, Beresford SA, Vollset SE. The controversy over homocysteine and cardiovascular risk. *Am J Clin Nutr* 2000;72:324–32.
2. Casas PJ, Bautista LE, Smeeth L, Sharma P, Hingorani AD. Homocysteine and stroke: evidence on a causal link from Mendelian randomization. *Lancet* 2005;365:224–32.
3. Panigrahi I, Chatterjee T, Biswas A, Behari M, Choudhry PV, Saxena R. Role of *MTHFR C677T* polymorphism in ischemic stroke. *Neurol India* 2006;54:48–52.
4. The VITATOPS Trial Study Group. The VITATOPS (Vitamin To Prevent Stroke) trial: rational and design of an international, large, simple, randomised trial of homocysteine-lowering multivitamin therapy in patients with recent transient ischemic attack or stroke. *Cerebrovasc Dis* 2002;13:120–6.

Invited Comments

A strong association has been suggested between the folic acid level and the occurrence of a congenital neuronal tube defect.^[1] Various epidemiological studies have confirmed the presumption that periconceptional folic acid supplementation can reduce the frequency of such a defect.^[2] This has led to the introduction of an extensive program of prevention in the United States, in which cereal food productions are fortified with folic acid.

There are several other aspects of the daily folic acid intake in adults, which can influence the homocysteine level,^[3,4] a high homocysteine level being a strong predictor of death in patients with coronary artery disease.^[5] A paper published in this issue of the journal raises the importance of folic acid intake on the basis of genotype against ischaemic stroke too.^[6]

I approach the importance of the folic acid supply from the aspect of the disability caused by a cognitive deficiency. In addition to Alzheimer disease, the frequent causes of a cognitive deficiency can be ischaemic stroke and leukoaraiosis.

Leukoaraiosis is indicated by bilateral patchy or diffuse areas of hypointensity in CT scans, or hyperintensity in T2-weighted MRI scans. It involves only the white matter. One-quarter of subjects aged 65 years or over are affected by some degree of white matter changes. Leukoaraiosis can cause a mild cognitive impairment or severe subcortical dementia. Ischemic demyelination and small-vessel disease seem to be features of the underlying pathological process of this entity. Age and hypertension have been proved to be the main clinical risk factors. Diabetes mellitus

and cardiac diseases, which can be associated with cerebrovascular diseases, have not proved to involve direct significant risks of leukoaraiosis directly. Clinical observations have suggested that vasoregulatory disturbances and chronic cerebral hypoperfusion may be the pathological states which can lead to leukoaraiosis.

A number of genetic susceptibility factors for leukoaraiosis have been put forward. With the assumption of polygenic etiological factors, it has been demonstrated that the 5,10-methylenetetrahydrofolate reductase (MTHFR) 677C-T mutation and angiotensin-converting enzyme insertion/deletion (ACE I/D) polymorphism play important roles in leukoaraiosis. Both the ACE D and MTHFR 677T alleles are frequent in the normal population, but can be associated with an unfavorable vasoregulatory effect on the circulatory system. The former can lead to an elevated angiotensin II level in the serum and the tissues and the latter to an elevated serum homocysteine level. Leukoaraiosis has been stated to be positively correlated with the serum homocysteine level and inversely correlated with the serum folate level. It has been earlier reported that a clustering of the homozygous MTHFR 677TT and ACE D/D mutations in one person can mean a moderate (approximately 5-fold risk) but highly significant ($P < 0.0005$) risk of leukoaraiosis.^[7] Alone, however, neither of the mutations proved to be a risk factor for leukoaraiosis. It has also been suggested that low doses of ACE inhibitor drugs can reduce the risk of stroke or a cardiovascular event. The finding of the interaction of two unfavourable mutations that alone are insignificant might open up a new clinical strategy for the prevention or treatment of leukoaraiosis. Although further clinical studies are clearly needed to investigate the clinical and preventive effects of different doses of folic acid supplementation on leukoaraiosis associated with a cognitive disability, I suggest regular folic acid supplementation in the middle aged and elderly if they carry the ACE D/D and homozygous MTHFR 677TT mutations together. It has been demonstrated that this genotype constellation implies a moderate genetic risk for leukoaraiosis.^[7]

The further question arises of whether co-administration of an ACE inhibitor and folic acid could reduce occurrence of leukoaraiosis.

Ischaemic stroke is a very regularly occurring entity underpinned by several genetic factors and clinical risk factors. The MTHFR 677TT genotype and decreased serum folic acid level can be risk factors for ischaemic stroke. The folic acid supplementation can be a preventive measure against ischaemic stroke too.^[6] This question is also open and needs further studies. A regular folic acid intake seems to be important health issue in a wide range of nations in general. Its supplementation against general circulatory disorders raises hopes in a broad health issue. The new findings on the roles of folic acid and the genetic background might permit a selective prevention of leukoaraiosis and ischaemic stroke hence the cognitive decline they cause.

Zoltán Szolnoki

*Departments of Neurology and Neurophysiology, Pándy Kálmán County Hospital, H-5700 Gyula, Semmelweis 1, Hungary, H-5600 Békéscsaba, Pipacs köz 9, Hungary
E-mail: szolnoki99@hotmail.com*

References

1. Hibbard ED, Smithells RW. Folic acid metabolism and human embryopathy. *Lancet* 1965;1:1254.
2. Czeizel AE, Dudás I. Prevention of the first occurrence of neural-tube defects by periconceptional vitamin supplementation. *N Engl J Med* 1992;327:1832-5.
3. Frosst P, Blom HJ, Milos R, Goyette P, Sheppard CA, Matthew RG, et al. A candidate genetic risk factor for vascular disease: a common mutation in methylenetetrahydrofolate reductase. *Nat Gen* 1995;10:111-3.
4. Welch GN, Lasczko J. homocysteine and atherothrombosis. *N Engl J Med* 1998;338:1042-50.
5. Nygard O, Nordrehaug JE, Refsum H, Ueland PM, Furstad M, Vollset SE. Plasma homocysteine levels and mortality in patients with coronary artery disease. *N Engl J Med* 1997;337:230-6.
6. Panigrahi I, Chatterjee T, Biswas A, Behari M, Choudhry VP, Saxena R. Role of MTHFR C677T polymorphism in ischemic stroke. *Neurol India* 2006;54:48-52.
7. Szolnoki Z, Somogyvári F, Kondaes A, Szabó M, Fodor L. Evaluation of the roles of common genetic mutations in leukoaraiosis. *Acta Neurol Scand* 2001;104:281-7.