Hyperhomocysteinemia, C677T MTHFR Polymorphism and Ischemic Stroke In Tunisian Patients

Olfa Ben Salem-Berrabah, Nejiba Fekih-Mrissa, Mabrouka El Oudi*, Sami Layouni, Ines Louati**, Zied Aouni*, Chakib Mazigh*, Brahim N'siri, Abdelhamid Ben Hamida***, Salem Machghoul*, Ridha Mrissa** and Nasreddine Gritli.

Hematology Department. Laboratory of Molecular Biology. - *Biochemistry Department. - **Neurology Department.
Military Hospital. 1008 Mont-Fleury. Tunis, Tunisia.
***Epidemiology Department. University of Medicine.1007 La Rabta. Tunis, Tunisia.

O. Ben Salem-Berrabah, N. Fekih-Mrissa, M. El Oudi, S. Layouni, I. Louati, Z. Aouni, C. Mazigh, B. N'siri, A. Ben Hamida, S. Machghoul, R. Mrissa, N. Gritli.

Hyperhomocystéinémie, Polymorphisme C677T de la MTHFR et Accidents Vasculaires Cérébraux Ischémiques chez des Patients Tunisiens

LA TUNISIE MEDICALE - 2010 ; Vol 88 (n°09) : 655 - 659

O. Ben Salem-Berrabah, N. Fekih-Mrissa, M. El Oudi, S. Layouni, I. Louati, Z. Aouni, C. Mazigh, B. N'siri, A. Ben Hamida, S. Machghoul, R. Mrissa, N. Gritli.

Hyperhomocysteinemia, C677T MTHFR Polymorphism And Ischemic Stroke In Tunisian Patients

LA TUNISIE MEDICALE - 2010 ; Vol 88 (n°09) : 655 - 659

RÉSUMÉ

Prérequis : L'hyperhomocystéinémie est considérée comme un facteur de risque indépendant des accidents vasculaires cérébraux (AVC). La mutation C677T du gène de la 5,10–méthyltétrahydrofolate réductase (MTHFR) est associée à une augmentation de l'homocystéine plasmatique totale.

But: Rechercher si l'hyperhomocystéinémie et/ou la mutation C677T de la MTHFR sont associées aux accidents vasculaires cérébraux ischémiques (AVCI).

Méthodes: Nous avons mené une étude cas-témoins composée de 50 patients atteints d'ACVI et 97 témoins. Les mesures des concentrations plasmatiques de l'homocystéine (Hcy) et le génotypage de la mutation MTHFR C677T ont été effectuées. D'autres facteurs comme l'hypertension, l'obésité, la dyslipidémie, le diabète, les AVCI récidivantes, le tabac et l'alcool ont été analysés.

Résultats: Les concentrations moyennes de l'homocystéine plasmatique (Hcy) étaient significativement plus élevés chez les patients atteints d'AVCI que chez les témoins (15,83±10.60 μmol/L contre 13,78±6,29 μmol/L, p=0,04). Alors qu'aucune association de la mutation C677T de la MTHFR variante n'a été observée; ni avec la maladie ni avec l'Hcy. Le risque de développer un accident ischémique cérébral chez les sujets hyperhomocystéinémiques est 2,4 fois plus que chez les sujets ayant des taux normaux en Hcy (OR=2,4, IC 95%: de 1,13 à 5,06, p<0,05).

Conclusion: Ces données préliminaires montrent que l'Hcy a un effet sur les accidents vasculaires cérébraux ischémiques indépendamment du polymorphisme C677T en Tunisie.

Mots-clés

Hyperhomocystéinémie, polymorphisme C677T de la MTHFR, accidents vasculaires cérébraux ischémiques, facteur de risque, Tunisie.

SUMMARY

Background: Hyperhomocysteinaemia has been identified as a strong risk factor for ischemic stroke (IS). A point mutation in methylene tetrahydrofolate reductase (MTHFR C677T) has been associated with increased plasma homocysteine (Hcy) levels.

Aim: This preliminary study aimed to investigate whether hyperhomocysteinaemia and/or MTHFR C677T mutation are associated with ischemic stroke.

Methodes: A case-control study including 50 consecutive patients with confirmed IS and 97 controls was performed. Fasting plasma homocysteine levels, MTHFR C677T genotypes were assessed. Other factors such as hypertension, obesity, dyslipidemia, diabetes mellitus, recurrent stroke tobacco and alcohol were investigated.

Results: Mean plasma homocysteine levels were significantly higher in IS patients than in controls (15.83±10.60) μmol/L vs 13.78±6.29 μmol/L, p=0.04), while no association of MTHFR C677T variant was observed even with homocysteine. The risk to develop ischemic stroke in hyperhomocysteinemic subjects was 2.4 times more than in subjects with normal Hcy levels (OR= 2.4; 95% CI: 1.13-5.06; p<0.05).

Conclusion: Our findings suggest that high levels of homocysteine but not MTHFR C677T polymorphism represent risk factors for arterial ischemic stroke in Tunisian subjects.

Key-words

Homocysteine levels, MTHFR C677T polymorphism, risk factor, Ischemic Stroke, Tunisia.

Stroke is the leading cause of death among neurologic diseases [1] and the second leading cause of death among all medical conditions [2]. Several case control and prospective studies demonstrated that moderate elevation of plasma homocysteine (Hcy) is a risk factor cardiovascular disease and venous and arterial thrombosis including stroke [3-6]. However, its role in stroke is still unclear. Methylenetetrahydrofolate reductase (MTHFR) is an important enzyme in the metabolism of homocysteine [7]. A C677T mutation in this enzyme leads to a reduction in enzyme activity and an elevation of plasma Hcy [8]. Some studies reported that C677T mutation is associated to Ischemic Stroke (IS) but others did not [9-11]. In the present study, we intended to investigate the association between total plasma homocysteine, the C677T polymorphism of the MTHFR gene and ischemic stroke in Tunisian patients.

MATERIALS AND METHODS

Subjects

In the present case control study we recruited 50 IS patients who were diagnosed at the Department of Neurology of the Military Hospital of Tunis. The stroke was confirmed by clinical assessment, computed tomography (CT)/magnetic resonance imaging (MRI) scan, or a combination of both. Patients with intracerebral hemorrhage (ICH), or sub-arachnoid hemorrhage (SAH) that was documented either by CT-scan or MRI were not included in the present study. Clinical and anthropometric data were subscribed for all patients. Of the 50 patients, 28 were hypertensive, 24 had type 2 diabetes, 13 had dyslipidemia and 10 had recidival cerebrovascular disease. The patients' mean age was 57.62 years. Control group consisted of 97 healthy subjects without any complications or history of cerebral ischemia and they were aged from 30 to 70 years.

Homocysteine assay

Blood samples were withdrawn from subjects after an overnight fast. The plasma was separated within one hour of sampling by centrifugation (3500 cycles/min for 15 min). The blood for measuring total homocysteine (tHcy) was collected in tube containing EDTA and was kept on ice until centrifuged and the plasma was immediately stored at (-20°C). The concentrations of tHcy in plasma were evaluated based on a competitive immunoassay using an automatic analyzer (Immulite 1000 DPC, Los Angeles, USA). In our study, the normal range of plasma homocysteine was taken as 5-15 μmol/L.

Genotyping of MTHFR C677T

DNA was extracted from peripheral lymphocytes using (QIA amp DNA Blood Mini kit Qiagen) and the analysis of the C677T MTHFR mutation was performed by PCR using FV-PTH-MTHFR Strip A (Vienna Lab, Labordiagnostika GmbH, Austria) kit.

Statistical analysis

Results were statistically analyzed by SPSS 11.5 for Windows. Continuous variables are presented as (means ± SD), and qualitative variables as relative frequencies. We used t-test to compare quantitative variables and Chi-square test to compare qualitative variables. The odds ratio was used to assess the relative risk with a confidence interval at 95%. Correlation between variables was determined by Spearman test. The differences were significant if P value was less than 0.05.

RESULTS

The clinic characteristics of population study were shown in the table 1. We found higher prevalence of obesity (14% vs 11.34%) and smokers (36% vs 24.74%) in patient group. The demographic and clinical characteristics of the ischemic stroke

Table 1 : Baseline characteristics of patients and controls

	All			Men			Women		
	Case (n = 50)	Control (n = 97)	P	Case (n = 32)	Control (n = 51)	P	Case (n = 18)	Control (n = 46)	P
Hypertension, n (%)	28 (56.00)	0	< 0.001	13 (40.62)	0	< 0.001	15 (83.33)	0	< 0.001
Dyslipidemia, n (%)	13 (26.00)	0	< 0.001	5 (15.62)	0	0.007	8 (44.44)	0	< 0.001
Diabetes mellitus, n (%)	24 (48.00)	0	< 0.001	13 (40.62)	0	< 0.001	11 (61.11)	0	< 0.001
Obesity, n (%)	7 (14.00)	11 (11.34)	0.641	5 (15.62)	2 (3.92)	0.074	2 (11.11)	9 (19.56)	0.343
Smokers, n (%)	18 (36.00)	24 (24.74)	0.152	16 (50.00)	22 (43.13)	0.541	2 (11.11)	2 (4.34)	0.313
Alcohol consumers, n (%)	6 (12.00)	0	0.001	6 (18.75)	0	0.002	0	0	NA
Recurrent disease, n (%)	10 (20.00)	0	< 0.001	5 (15.62)	0	0.007	5 (27.77)	0	0.001

Smokers were divided into subjects who currently smoke and who never did.
Alcohol consumers were divided into subjects who currently drink and who never did.
Obesity and overweight estimated according to Body Mass Index (BMI) above 25 kg/m2.
NA: not applicable

patients and the control group are given in Table 1. There were significant differences for the frequencies of hypertension (p<0.001), dyslipidemia (p<0.001), diabetes mellitus (p<0.001), alcohol consumers (p=0.001) and recurrent disease (p<0.001) between patient and control groups. According to gender, the same results were found in men and in women.
Homocysteine levels were not statistically different between men and women in patient group, although men had elevated Hcy concentrations (16.73 ± 12.45 vs 14.03 ± 5.23μmol/L). Similar results were observed in the control group (14.70 ± 6.03 vs 12.78 ± 6.47μmol/L). Our findings showed that the risk to develop ischemic stroke in hyperhomocysteinemic subjects was 2.4 times more than in subjects with normal tHcy levels (OR= 2.4; 95% CI: 1.13-5.06; p<0.05) (Table 2).

Table 2 : Distribution of plasma homocysteine concentrations in population study.

	n	tHcy (μmol/l)	p
All			
Control	97	13.78 ± 6.29	
Patient	50	15.83 ± 10.60	0.04
Men			
Control	50	14.7 ± 6.03	
Patient	30	16.73 ± 12.45	0.33
Women			
Control	46	12.78 ± 6.47	
patient	15	14.03 ± 5.23	0.50

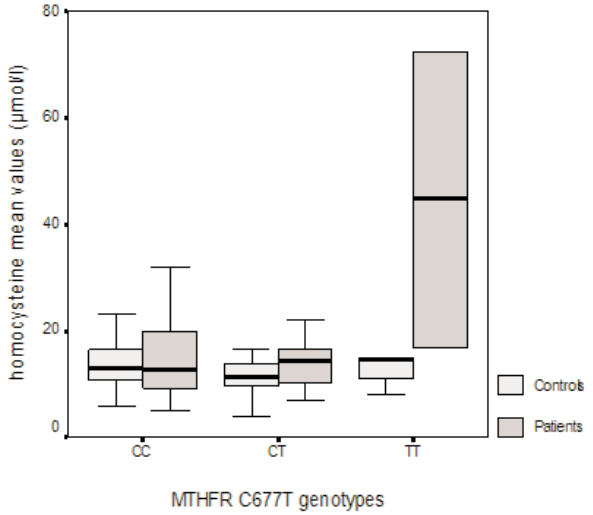
Odds ratio were calculated for the three genotypes of C677T MTHFR mutation and for T allele frequency in patients compared to controls. No significant results were found even with a separate analysis for sex (Table 3).

Table 3 : Genotype distribution and allele frequencies of the MTHFR C677T variant in ischemic stroke patients and controls.

	Case	Control	OR (95% CI) and p value
All	(n=50)	(n=97)	
CC (%)	33 (66)	57 (58.76)	1.00
CT (%)	15 (30)	35 (36.08)	0.74 (0.33-1.65) p=0.426
TT (%)	2 (4)	5 (5.15)	0.69 (0.09-4.37) p=0.505
T (%)	19 (19)	45 (23.2)	0.78 (0.41-1.47) p=0.409
C (%)	81(81)	149 (76.8)	
Men	(n=32)	(n=51)	
CC (%)	22 (68.75)	30 (58.82)	1.00
CT (%)	9 (28.12)	19 (37.25)	0.65 (0.22-1.87) p=0.373
TT (%)	1 (9.12)	2 (3.92)	0.68 (0.02-10.61) p=0.624
T (%)	11 (17.19)	23 (22.55)	0.71 (0.30-1.69) p=0.405
C (%)	53 (82.81)	79 (77.45)	
Women	(n=18)	(n=46)	
CC (%)	11 (61.11)	27 (58.69)	1.00
CT (%)	6 (33.33)	16 (34.78)	0.92 (0.24-3.42) p=0.889
TT (%)	1 (5.55)	3(5.52)	0.82(0.0-10.80) p=0.680
T (%)	8 (22.22)	22 (23.91)	0.91 (0.33-2.48) p=0.839
C (%)	28 (77.77)	70 (76.08)	

Further investigations of homocysteine distribution according to C677T MTHFR genotype was determined (Figure 1). Patient's carriers of TT and CT genotypes have the elevated Hcy levels when compared to controls (44.66 ± 39.11 μmol/L vs 16.44 ± 10.1 μmol/L and 14.10 ± 4.56 vs 11.94 ± 3.93 respectively for TT and CT genotypes).

Figure 1 : Distribution of homocysteine means values according to MTHFR C677T genotypes in population study.



DISCUSSION

The principal findings of the present study were first that plasma tHcy was elevated in patients compared to controls, with a prevalence of hyperhomocysteinemia of 40% in patients versus 24.7% in controls. Second, plasma tHcy was associated to the occurrence of the cerebrovascular disease.
Relation between Hcy and stroke conflicts greatly as shown in several epidemiologic data. Such studies confirmed that Hcy was an evident causal factor for stroke [3,12-13]. In other clinical studies, authors either did not found an association or found a moderate one [14-16]. These differences in results might be due to the ethnic groups and the conditions in which the studies were done. Although tHcy levels are higher in patients with homozygous and heterozygote MTHFR C677T mutation, these genotypes are not related to ischemic stroke in our study population. The small sample size in our study may limit the power of testing a non significant association between mild MTHFR C677T genotypes and ischemic stroke for the whole sample, the men and woman were 25%, 17% and 7%, respectively. We can conclude that Hcy may be a risk factor for ischemic stroke independently of MTHFR polymorphism.
Data 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 remain conflicting. The MTHFR 677C>T polymorphism, whether in the homozygous

or the heterozygous state, is not always associated with an increased homocysteine level. Meta-analyses have supported these findings. For example, Kelly et al. suggested that the MTHFR 677TT genotype might have a small influence in determining susceptibility to ischemic stroke [17]. In our study, we failed to find an association between the C677T MTHFR variant and ischemic stroke as several previous studies [18-22]. In Japanese population, the relevance of the MTHFR T/T mutation appears to be restricted to women [22]. However, controversial studies reported a significant or moderate association between MTHFR polymorphism and ischemic stroke [14,23,24]. All these controversial associations between the C677T polymorphism and stroke might be mainly due to ethnic group and small sample size.

In recent years, many epidemiological studies have given new insights into old and new lifestyle factors that influence the risk of cerebrovascular events. In our study, we investigated the relationship between stroke and different factors such as hypertension, dyslipidemia, diabetes mellitus, obesity, smokers, alcohol consumption and recurrent history of stroke. We found that tobacco and obesity was not associated to the onset of stroke ($P>0.05$). Recent data suggest that smoking, hypertension, dyslipidemia and diabetes mellitus are considered as major risk factors for stroke and might be considered as main targets for primary and secondary prevention of stroke [25]. Active and passive smokings are independent risk factors, and a smoking ban in public places has already reduced cardiovascular events in the short term [26]. In China, second hand smoke exposure in women is highly prevalent. In addition to being a risk factor for coronary heart disease, this should be considered an important risk factor for ischemic stroke and peripheral arterial disease in nonsmoking women [27]. Obesity is known to be associated with diverse disease outcomes; however, the effect of body weight on the occurrence of stroke remains controversial and has not been studied sufficiently. In our study, we did not found association between Body Mass Index (BMI > 25 Kg/m2) and the incidence of stroke. According to a recent study, this association was modified by age, with a weaker association only at higher ages [28]. Gállego et al reported that obesity contribute to the relatively high incidence of stroke [29]. The association of light to moderate alcohol consumption with risk of Ischemic Stroke remains uncertain. Light and moderate average alcohol use was generally not associated with an increased risk for ischemic stroke while intake of more than two drinks per day may be associated with a higher risk for ischemic stroke [30].

In conclusion, our data have been obtained on a limited number of patients so that they should be considered as preliminary; hence further study is needed in larger population to confirm our findings. Our data suggest that high levels of homocysteine but not MTHFR C677T polymorphism are risk factors for arterial ischemic stroke in Tunisian subjects. Among life style factors, hypertension, dyslipidemia, diabetes mellitus and alcohol consumption may have an effect on the occurrence of ischemic stroke. As a consequence, this arterial thrombotic disease can be substantially reduced by an active lifestyle and a healthy diet.

Acknowledgement

We thank Assistant Professor Iyadh Douagi (Department of Microbiology, Karolinska Institutet, Stockholm, Sweden) for his helpful comments on the preparation of the manuscript.

Références

1. Strong K, Mathers C, Bonita R. Preventing stroke: Saving lives around the world. Lancet Neurol 2007;6:182-7.
2. Murray CJL, Lopez AD. Mortality by cause for eight regions of the world: Global burden of disease study. Lancet 1997;349:1269-1276.
3. Wald DS, Law M, Morris JK. Homocysteine and cardiovascular disease: evidence on causality from a meta-analysis. BMJ 2002;325: 1202-6.
4. Lee R, Frenkel EP. Hyperhomocysteinemia and thrombosis. Hematol Oncol Clin North Am 2003;17:85-102.
5. Ray JG. Meta-analysis of hyperhomocysteinemia as a risk factor for venous thromboembolic disease. Arch Intern Med 1998 158:2101-6.
6. Moller J, Nielsen GM, Tvedegaard KC, Andersen NT, Jorgensen PE. A meta-analysis of cerebrovascular disease and hyperhomocysteinaemia. Scand J Clin Lab Invest 2000;60:491-9.
7. Matthews RG, Sheppard C, Goulding C. Methylenetetrahydrofolate reductase and methionine synthase: Biochemistry and molecular biology. Eur J Pediatr 1998;157 (Suppl 2):S54-9.
8. Engbersen AM, Franken DG, Boers GH, Stevens EM, Trijbels FJ, Blom HJ. Thermolabile 5,10-methylenetetrahydrofolate reductase as a cause of mild hyperhomocysteinemia. Am J Hum Genet 1995;56:142-50.
9. Soriente L, Coppola A, Madonna P, Cerbone AM, Di Minno G, Orefice G et al. Homozygous C677T mutation of the 5,10 methylenetetrahydrofolate reductase gene and hyperhomocysteinemia in Italian patients with a history of early-onset ischemic stroke. Stroke 1998;29:869-71.
10. Pezzini A, Del Zotto E, Archetti S, Negrini R, Bani P, Albertini A et al. Plasma homocysteine concentration, C677T MTHFR genotype, and 844ins68bp CBS genotype in young adults with spontaneous cervical artery dissection and atherothrombotic stroke. Stroke 2002;33:664-9.
11. Lalouschek W, Aull S, Serles W, Schnider P, Mannhalter C, Lang T et al. Genetic and nongenetic factors influencing plasma homocysteine levels in patients with ischemic cerebrovascular disease and in healthy control subjects. J Lab Clin Med 1999;133:575-82.
12. Tantirittisak T, Sura T, Moleerergpoom W, Hanchaipiboolkul S. Plasma homocysteine and ischemic stroke patients in Thailand. J Med Assoc Thai 2007;90:1183-7.
13. Biswas A, Ranjan R, Meena A, Akhter MS, Yadav BK, Munisamy M et al. Homocystine levels, polymorphisms and the risk of ischemic stroke in young Asian Indians. J Stroke Cerebrovasc Dis 2009;18:103-10.

14. Moe KT, Woon FP, De Silva DA, Wong P, Koh TH, Kingwell B et al. Association of acute ischemic stroke with the MTHFR C677T polymorphism but not with NOS3 gene polymorphisms in a Singapore population. Eur J Neurol 2008;15:1309-14.
15. Ntaios G, Savopoulos C, Hatzitolios A, Ekonomou I, Destanis E, Chryssogonidis I, et al. Homocysteine and carotid intima-media thickness in ischemic stroke patients are not correlated. Neuropsychiatr Dis Treat 2008;4:477-9.
16. Sabino A, Fernandes AP, Lima LM, Ribeiro DD, Sousa MO, de Castro Santos ME et al. Polymorphism in the methylenetetrahydrofolate reductase (C677T) gene and homocysteine levels: a comparison in Brazilian patients with coronary arterial disease, ischemic stroke and peripheral arterial obstructive disease. J Thromb Thrombolysis 2009;27:82-7.
17. Kelly PJ, Rosand J, Kistler JP, Shih VE, Silveira S, Plomaritoglou A et al. Homocysteine, MTHFR 677C-->T polymorphism, and risk of ischemic stroke: results of a meta-analysis. Neurology 2002;59: 529-36.
18. Uçar F, Sönmez 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.
19. McColl MD, Chalmers EA, Thomas A, Sproul A, Healey C, Rafferty I et al. Factor V Leiden, prothrombin 20210G-->A and the MTHFR C677T mutations in childhood stroke. Thromb Haemost 1999;81:690-4.
20. Lopaciuk S, Bykowska K, Kwiecinski H, Mickielewicz A, Czlonkowska A et al. Factor V Leiden, prothrombin gene G20210A variant, and methylenetetrahydrofolate reductase C677T genotype in young adults with ischemic stroke. Clin Appl Thromb Hemost 2001;7:346-50.
21. Marie SK, Shinjo SK, Oba-Shinjo SM, da Silva R, Barbosa KC, Yamamoto F et al. Methylenetetrahydrofolate reductase gene polymorphism is not related to the risk of ischemic cerebrovascular disease in a Brazilian population. Clinics (Sao Paulo) 2007;62:295-300.
22. Wu Y, Tomon M, Sumino K. Methylenetetrahydrofolate reductase gene polymorphism and ischemic stroke: sex difference in Japanese. Kobe J Med Sci 2001;47:255-62.
23. Harmon DL, Doyle RM, Meleady R, Doyle M, Shields DC, Barry R et al. Genetic analysis of the thermolabile variant of 5,10 methylenetetrahydrofolate reductase as a risk factor for ischemic stroke. Arterioscler Thromb Vasc Biol 1999;19:208-11.
24. Li CM, Zhang C, Lu XL, Feng HY, Su QX, Zeng Y et al. Relationship between methylenetrahydrofolate reductase gene and ischemic stroke. Zhongguo Wei Zhong Bing Ji Jiu Yi Xue 2006;18:264-7.
25. Khan NI, Naz L, Mushtaq S, Rukh L, Ali S, Hussain Z. Ischemic stroke: prevalence of modifiable risk factors in male and female patients in Pakistan. Pak J Pharm Sci 2009;22:62-7.
26. Galimanis A, Mono ML, Arnold M, Nedeltchev K, Mattle HP. Lifestyle and stroke risk: a review. Curr Opin Neurol 2009;22:60-8.
27. He Y, Lam TH, Jiang B, Wang J, Sai X, Fan L et al. Passive smoking and risk of peripheral arterial disease and ischemic stroke in Chinese women who never smoked. Circulation 2008; 118:1535-40.
28. Park JW, Lee SY, Kim SY, Choe H, Jee SH. BMI and stroke risk in Korean women. Obesity (Silver Spring) 2008;16:396-401.
29. Gállego J, Martínez Vila E, Muñoz R. Patients at high risk for ischemic stroke: identification and actions. Cerebrovasc Dis 2007;24 (Suppl 1):49-63.
30. Mukamal KJ, Ascherio A, Mittleman MA, Conigrave KM, Camargo CA, Kawachi I et al. Alcohol and risk for ischemic stroke in men: the role of drinking patterns and usual beverage. Ann Intern Med 2005;142:11-9.