Migraine Mediates the Influence of C677T MTHFR Genotypes on Ischemic Stroke Risk With a Stroke-Subtype Effect

Alessandro Pezzini, Mario Grassi, Elisabetta Del Zotto, Alessia Giossi, Roberto Monastero, Giorgio Dalla Volta, Silvana Archetti, Paola Zavarise, Cecilia Camarda, Roberto Gasparotti, Mauro Magoni, Rosolino Camarda and Alessandro Padovani

Stroke 2007;38;3145-3151; originally published online Oct 25, 2007;
DOI: 10.1161/STROKEAHA.107.491506
Stroke is published by the American Heart Association. 7272 Greenville Avenue, Dallas, TX 72514
 Print ISSN: 0039-2499. Online ISSN: 1524-4628

Migraine Mediates the Influence of *C677T MTHFR* Genotypes on Ischemic Stroke Risk With a Stroke-Subtype Effect

Alessandro Pezzini, MD; Mario Grassi, PhD; Elisabetta Del Zotto, MD; Alessia Giossi, MD; Roberto Monastero, MD, PhD; Giorgio Dalla Volta, MD; Silvana Archetti, MD; Paola Zavarise, MD; Cecilia Camarda, MD; Roberto Gasparotti, MD; Mauro Magoni, MD; Rosolino Camarda, MD; Alessandro Padovani, MD, PhD

Background and Purpose—The objective was to investigate the role of *C677T MTHFR* polymorphism in migraine pathogenesis and in the migraine–ischemic stroke pathway.

Methods—A first genotype–migraine association study was conducted on 100 patients with migraine with aura (MA), 106 with migraine without aura (MO), and 105 subjects without migraine, which provided evidence in favor of association of the *TT677 MTHFR* genotype with increased risk of MA compared with both control subjects (OR, 2.48; 95% CI, 1.11 to 5.58) and patients with MO (OR, 2.21; 95% CI, 1.01 to 4.82). Based on these findings, mediational models of the genotype–migraine–stroke pathway were fitted on a group of 106 patients with spontaneous cervical artery dissection, 227 young patients whose ischemic stroke was unrelated to a spontaneous cervical artery dissection (noncervical artery dissection), and 187 control subjects, and a genotype–migraine partial mediation model was selected.

Results—Both migraine and the *TT* genotype were more strongly associated to the subgroup of patients with spontaneous cervical artery dissection (OR, 4.06; 95% CI, 1.63 to 10.02 for MA; OR, 5.45; 95% CI, 3.03 to 9.79 for MO; OR, 2.87; 95% CI, 1.45 to 5.68 for *TT* genotype) than to the subgroup of patients with noncervical artery dissection ischemic stroke (OR, 2.22; 95% CI, 1.00 to 4.96 for MA; OR, 1.81; 95% CI, 1.02 to 3.22 for *TT* genotype) as compared with controls.

Conclusions—Migraine may act as mediator in the methylenetetrahydrofolate reductase–ischemic stroke pathway with a more prominent effect in the subgroup of patients with spontaneous artery dissection. **(*Stroke*. 2007;38:3145-3151.)**

Key Words: genetics ■ migraine ■ risk factors ■ stroke in young adults

Despite lively debate in the past as to whether a migraine–stroke relation really exists, there is currently mounting epidemiological evidence demonstrating that migraine increases the risk of cerebral ischemia,[1] which appears to be higher among the young but may persist in the elderly,[2,3] and is also associated with subclinical brain white matter abnormalities.[4,5] Migraine is known to run in families, suggesting a condition that is at least partly regulated by genetics. It occurs disproportionately more frequently in association with other inherited disorders. Several large-scale epidemiologic studies have confirmed that genetic factors play an important role in migraine.[6,7] More recently, the results of some genetic-association studies have reinforced this assumption and suggested the involvement of specific candidate genes. Among them, the gene for methylenetetrahydrofolate reductase (*MTHFR*) is currently the focus of much investigation. In particular, the common single nucleotide polymorphism in which cytosine (*C*) is replaced by thymidine (*T*) at base position 677, resulting in a thermolabile variant of the enzyme with approximately half-normal activity, has been reported to increase the risk of migraine with aura (MA) in some case-control and cohort studies.[8–12] Because of these findings, despite some conflicting results,[13,14] the *C677T MTHFR* polymorphism seems to be a promising candidate to migraine susceptibility. The polymorphism is also one of the most extensively investigated candidates in cerebral ischemia. In the largest meta-analysis to date of studies examining the association with ischemic stroke, Casas et al[15] found an increased risk among individuals homozygous for the T allele, although the size of the effect seems to be modest

Received April 17, 2007; final revision received May 17, 2007; accepted May 30, 2007.

From Dipartimento di Scienze Mediche e Chirurgiche (A.P., M.M.), Stroke Unit, Neurologia Vascolare, Spedali Civili di Brescia, Brescia, Italia; Dipartimento di Scienze Sanitarie Applicate (M.G.), Sezione di Statistica Medica e Epidemiologia, Università di Pavia, Pavia, Italia; Dipartimento di Scienze Mediche e Chirurgiche (E.D.Z., A.G., A.P.), Clinica Neurologica, Università degli Studi di Brescia, Brescia, Italia; Dipartimento di Neuroscienze Cliniche (R.M., C.C., R.C.), Divisione di Neurologia e Riabilitazione Neurologica, Università degli Studi di Palermo, Palermo, Italia; Unità Operativa di Neurologia (G.D.V., P.Z.), Centro Cefalee, Istituto Clinico Città di Brescia, Brescia, Italia; III Laboratorio di Analisi (S.A.), Biotecnologie, Università degli Studi di Brescia, Brescia, Italia; Dipartimento di Diagnostica per Immagini (R.G.), Neuroradiologia, Università degli Studi di Brescia, Brescia, Italia.

Correspondence to Alessandro Pezzini, Stroke Unit, Neurologia Vascolare, Spedali Civili di Brescia, P. le Spedali Civili, 1, 25100 Brescia, Italia. E-mail ale_pezzini@hotmail.com

Stroke **is available at http://stroke.ahajournals.org** **DOI: 10.1161/STROKEAHA.107.491506**

compared with those of classic cardiovascular risk factors. More recently, sparse reports indicated that the polymorphism might be more strongly associated to spontaneous cervical artery dissection (sCAD),[16,17] a vascular disorder for which a specific relation with migraine has been suggested,[18,19] than to other pathogenic stroke subtypes, thus leading to the hypothesis of a differential influence on the risk of cerebral ischemia.

Taken together, these findings prompt to speculate on the existence of a relationship between this genetic variant and migraine on stroke occurrence. Because both the *C677T MTHFR* polymorphism and migraine are closely related to ischemic stroke risk, and because the *C677T MTHFR* polymorphism could influence migraine susceptibility, we hypothesized that the 2 factors might work together in the pathogenic process leading to cerebral ischemia, and might confer a higher disease risk.

We therefore performed 2 independent analyses in which we genotyped the *C677T MTHFR* polymorphism in patients with migraine and patients with ischemic stroke. First, to investigate associations with migraine subtypes, we studied a series of patients with MA, patients with migraine without aura (MO), and control subjects. Second, to determine whether genotype–migraine association may have an influence on the risk of stroke as a whole and on individual stroke subtypes, we studied a series of well-phenotyped ischemic stroke and control subjects.

Subjects and Methods

The study was approved by the local Ethics Committee. Informed consent was provided by all study participants.

Study Population

Genotype–Migraine Association Study

To test the hypothesis of an association between the *C677T MTHFR* genotypes and migraine, we considered a group of unrelated migraine patients and control subjects, recruited from July 2005 to September 2006. Migraine cases were selected from those consecutively referred for outpatient examination of headache at the Headache Center of the Istituto Clinico Città di Brescia, Brescia, and the 2 subtypes (MA and MO) frequency matched. Control subjects, taken from the hospital staff, were included if they had no personal history of headache, matched migraine cases by sex and age (in 3-year bands), and were from the same geographical area and ethnic background.

Genotype–Migraine Mediation Study

The hypothesis of a mediational effect of the *C677T MTHFR* genotypes via migraine on the risk of ischemic stroke was explored in a case-control analysis including a group of unrelated patients consecutively admitted to the Department of Neurology of the University Hospital of Brescia between January 1999 and September 2006 and a group of control subjects. Because of the higher prevalence of migraine as well as of the *677TT MTHFR* genotype in the subgroup of patients with cervical artery dissection as compared with other stroke subtypes observed in previous studies,[16–19] the following 2 pathogenic subgroups of patients were selected: patients with sCAD and patients with first-ever acute ischemic stroke occurring before the age of 45, not related to a cervical artery dissection (non-CAD). Dissections were classified as spontaneous when occurring spontaneously or in association with a minor trauma, and dichotomized in "single-vessel dissection" and "multiple-vessel dissection," according to the number of involved cervical arteries.

Patients with non-CAD ischemic stroke were those in whom a diagnosis of CAD was excluded. A detailed description of the standard diagnostic workup, as well as stroke subtype classification, data collection, and risk factor definition have been presented previously.[16]

Control Subjects

Subjects from the staff members of our hospital with no known history of vascular disease aged younger than 45 years were invited to participate in the study as controls. This group of control subjects was different from the one selected for the genotype–migraine association study. Both cases and controls were from the same geographic area and ethnic background.

Assessment of Migraine History

Personal history of headache was assessed in all subjects by 4 investigators (E.D.Z., G.D.V., R.M., C.C.), during a face-to-face interview, based on a semi-structured questionnaire.[19] In stroke cases, migraine was diagnosed retrospectively. History of migraine before stroke occurrence was considered for the present analysis. The diagnosis of MO and MA was made according to the diagnostic criteria of the International Headache Society.[20]

Genetic Analysis

C677T MTHFR genotypes were determined according to the method of Frosst et al,[21] using polymerase chain reaction amplification and restriction digestion with Hinf I to distinguish mutant from wild-type alleles.

Statistical Analysis

To test the hypothesis of association between *C677T MTHFR* genotypes and migraine subtypes, a bivariate (cross-tabulation) analysis of the Genotype–Migraine Association Study data set was performed. Because a potential *TT677 MTHFR*-migraine relation was observed in this part of the study (that is, the 2 variables were statistically associated), we applied a mediation modeling strategy to the Genotype–Migraine Mediation Study data set to investigate the hypothesis of a mediational effect of these 2 factors on the risk of ischemic stroke. Actually, according to Baron and Kenny,[22] a mediator (the environment; here migraine, E) is an intermediate variable that occurs in a causal pathway from an independent variable (the genotype, G) to a dependent variable (the disease, D). It determines variation in the dependent variable, and it is caused to vary by the independent variable. Logically, G must temporally precede E, so that E mediates G.

Two mathematical equations were fitted: a "disease equation," in which both G and E have a direct effect on D, and an "environment equation," in which G has a direct effect on E. Fixing specific equation parameters equal to 0, 4 genotype–migraine mediation models were obtained:[23] (1) genotype–migraine partial mediation; (2) genotype–migraine complete mediation; (3) genotype as proxy of migraine and stroke; and (4) genotype–migraine overlapping (independent) effects (Figure 1).

The modeling strategy assumed disease status (sCAD, non-CAD, and controls) as outcome variable (D), migraine status (MO, MA, and no migraine) as environment variable (E), and codominant (A=*CC*, B=*CT*, C=*TT*), additive (0=*CC*; 1=*CT*; 2=*TT*), or recessive (0=*CC* or *CT*; 1=*TT*) *C677T MTHFR* coding as genotype variable (G).

Accordingly, "disease equation" and "environment equation" were categorical (multinomial) logistic regression models. Assessment of mediation models without and with adjustment for covariates (age, sex, smoking habit, hypertension, and hypercholesterolemia) was performed. Diabetes mellitus was not entered in the final analysis as covariate, because of the low frequency of this condition in the present series. The regression models were fitted using maximum likelihood estimates with robust standard errors computed by "sandwich" estimator.[24] To compare these competing models, the Akaike Information Criterion (Akaike Information Criterion=−2×model log-likelihood+2×number of model parameters), and the Bayesian

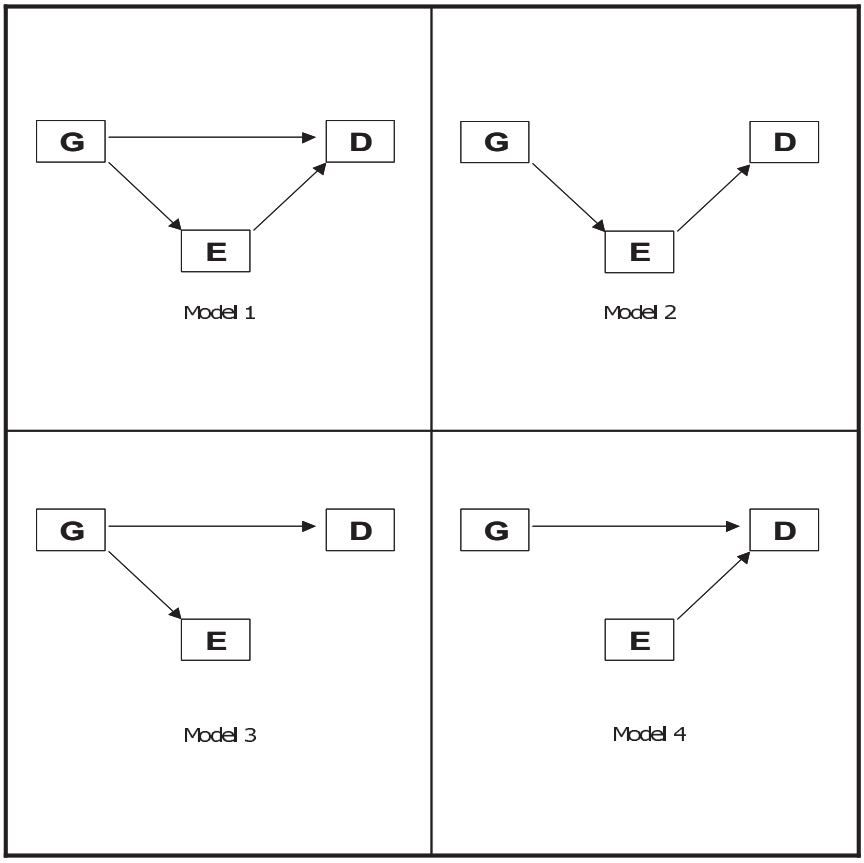

Figure 1. Genotype–migraine mediation models. G=genotype (*C677T MTHFR*); E=environment (migraine); D=disease (stroke). (1) Genotype–migraine partial mediation; (2) genotype–migraine complete mediation; (3) genotype as proxy of migraine and stroke; (4) genotype–migraine independent effects.

Information Criterion (Bayesian Information Criterion=−2×model log-likelihood+log(*n*)×number of model parameters) were computed. The selected model was the one minimizing either Akaike Information Criterion or Bayesian Information Criterion.[25] The regression parameter estimates were re-expressed as ORs, and 95% CIs using robust standard errors. Direct, indirect, and total ORs effects of genotype on disease risk were computed from maximum likelihood estimates, as proposed by Huang et al.[26] Probability values of the test z (parameter estimate/standard error) were performed to test the null effect (OR=1) across groups. The significance level was set at $P<0.05$ (2-sided). Multivariate modeling were performed by Mplus 4.1 software (www.statmodel.com), whereas bivariate statistics (χ^2 testing for Hardy-Weinberg), equilibrium, heterogeneity, and trend with probability values (computed by Monte Carlo approximation method) were performed by Stata 9 software (www.stata.com).

Results

Genotype–Migraine Association Study

The study group consisted of 102 patients with MA (mean age, 34.7±10.2 years; 68% women), 106 with MO (mean age, 33.6±8.6 years; 72.6% women), and 105 subjects without migraine (mean age, 34.4±7.4 years; 63.8% women). Because DNA could not be amplified in 2 patients with MA, data from 100 individuals were entered into the final analysis. No migraine patient had personal history of stroke.

Genotype frequencies did not differed significantly from those predicted by the Hardy-Weinberg equilibrium (χ^2 [df]=0.963; $P=0.326$)[1] within all the subgroups. There were no significant differences in the *TT677 MTHFR* genotype distribution between patients with MO and control subjects (OR, 1.13; 95% CI, 0.48 to 2.66). In contrast, a significant difference was observed between patients with MA and control subjects (OR, 2.48; 95% CI, 1.11 to 5.58), as well as between patients with MA and patients with MO (OR, 2.21; 95% CI, 1.01 to 4.82), in both cases because of an overrepresentation of the *TT* genotype in the subgroup of patients with MA. The frequency of the *T* allele was also significantly higher in the group of patients with MA than in the other 2 groups (OR, 1.46; 95% CI, 0.98 to 2.18 as compared with patients with MO; OR, 1.50; 95% CI, 1.01 to 2.24 as compared with control subjects; Table 1).

Genotype–Migraine Mediation Study

The study population consisted of 107 patients with sCAD (confirmed by MRI/MRA [n=46], digital subtraction angiography [n=17], or both [n=44]), 81 (75.7%) with ischemic stroke, 232 patients with non-CAD ischemic stroke, and 187 control subjects. Because DNA could not be amplified in 6 patients, the group of cases was composed of 106 patients with sCAD (16 with multiple-vessel dissection) and 227 patients with non-CAD ischemic stroke. Demographic characteristics and prevalence of selected risk factors are presented in Table 2. Patients with non-CAD ischemic stroke more often had hypertension and hypercholesterolemia, and were more often smokers compared with control subjects. Patients with sCAD more often had hypertension than control subjects. A history of migraine was diagnosed more frequently in the subgroup of patients with sCAD (n=57, 53.7%) than in the subgroup of patients with non-CAD ischemic stroke (n=55, 24.2%) and the group of control subjects (n=34,

Table 1. Frequency Distribution, Bivariate ORs (95% CI) of the *C677T MTHFR* Genotypes, and *C677T MTHFR* Alleles According to Migraine Subtypes in the Genotype–Migraine Association Study

	MO (n=106)	MA (n=100)	No Migraine (n=105)	MO vs No Migraine		MA vs No Migraine		MA vs MO	
				OR	95% CI	OR	95% CI	OR	95% CI
C677T MTHFR Genotypes									
CC	42 (39.6)	33 (33.0)	41 (39.0)	1		1		1	
CT	49 (46.2)	41 (41.0)	51 (48.6)	0.94	0.52–1.68	1.00	0.54–1.85	1.06	0.57–1.97
TT	15 (14.2)	26 (26.0)	13 (12.4)	1.13	0.48–2.66	2.48	1.11–5.58	2.21	1.01–4.82
C677T MTHFR Alleles									
C	133 (62.7)	107 (53.5)	133 (63.3)	1		1		1	
T	79 (37.3)	93 (46.5)	77 (36.7)	1.03	0.69–1.53	1.50	1.01–2.24	1.46	0.98–2.18

18.2%). Allele frequencies were in Hardy-Weinberg equilibrium (χ^2 [df]=1.242; P=0.286)[1] within the study subgroups. Both the *TT677 MTHFR* genotype and the *T* allele were more represented in the subgroup of patients with sCAD (n=32, 30.2%; n=109, 51.4%) than in the other 2 subgroups (n=49, 21.6% and n=210, 46.3% in patients with non-CAD ischemic stroke; n=27, 14.5%, and n=141, 37.7% in the group of control subjects).

Akaike Information Criterion index signed the "*C677T MTHFR* and migraine partial mediation model" (model 1, Figure 1) for codominant and additive ("counting alleles") genotype coding, whereas Bayesian Information Criterion index signed "*C677T MTHFR* and migraine independent effects model" (model 4, Figure 1) for all genotype coding. Because model 1 had the highest log-likelihood in all coding systems, and in the genotype–migraine association study we signed a statistically significant association between genotype and migraine, this model was selected as the best in predicting the effect of *C677T MTHFR* genotype and migraine on stroke risk (data not shown).

Direct effects estimates, re-expressed as ORs, of the "environmental equation" and "disease equation" of the selected model considering codominant genotype coding are displayed in Table 3. The *677TT MTHFR* genotype was significantly more represented in the subgroup of patients with MA as compared with subjects without migraine (OR, 2.28; 95% CI, 1.00 to 5.21), and as compared with patients with MO (OR, 2.53; 95% CI, 1.06 to 6.02), whereas the comparison between patients with MO and subjects without migraine gave no significant differences (Table 3, top). Patients with MA had >4-fold risk for development of sCAD (OR, 4.06; 95% CI, 1.63 to 10.02), and >2-fold risk for

Table 2. Demographics and Cardiovascular Risk Factors Profiles in Patients with sCAD, Patients With Non-CAD Ischemic Stroke, and Control Subjects

Characteristic	sCAD (n=106) mean±SD	N -CAD Ischemic Stroke (n=227) mean±SD	Control Subjects (n=187) mean±SD	*P* (MC)
Age, y	42.9±10.2	34.9±7.8	36.4±7.5	<0.001
	n (%)	n (%)	n (%)	
Male	52 (49.0)	122 (53.4)	99 (52.9)	0.751
Current smokers	34 (32.0)	109 (48.0)	47 (25.1)	<0.001
Hypertension	32 (30.2)	40 (17.6)	13 (6.9)	<0.001
Diabetes mellitus	4 (3.8)	8 (3.5)	7 (3.7)	0.995
Hypercholesterolemia	29 (27.4)	65 (28.6)	36 (19.2)	0.060
Personal history of migraine				<0.001
No migraine	49 (46.2)	169 (76.4)	153 (81.8)	
MO	44 (41.5)	31 (13.7)	25 (13.4)	
MA	13 (12.3)	24 (10.6)	9 (4.8)	
C677T MTHFR genotype frequency				0.011
CC	29 (27.3)	66 (29.1)	73 (39.0)	
CT	45 (42.5)	112 (49.3)	87 (46.5)	
TT	32 (30.2)	49 (21.6)	27 (14.5)	
C677T MTHFR allele frequency				0.003
C	103 (48.6)	244 (53.7)	233 (62.3)	
T	109 (51.4)	210 (46.3)	141 (37.7)	

P values (MC) are *P* values of ANOVA F test or χ^2 tests (2-tailed) for the 2×3 tables (df=2) and 3×3 tables (df=4) computed by Monte Carlo approximation method.

Table 3. Maximum Likelihood Estimates of Direct Effects Re-expressed as ORs (95% CI) in the Environmental Equation (Top) and in the Disease Equation (Bottom) of the Selected *C677T MTHFR* and Migraine Partial Mediation Model Using Codominant Genotype Coding

	MA vs No Migraine		MO vs No Migraine		MA vs MO	
	OR	95% CI	OR	95% CI	OR	95% CI
C677T MTHFR genotypes						
CC	1		1		1	
CT	1.14	0.54–2.42	0.78	0.47–1.29	1.42	0.62–3.23
TT	2.28	1.00–5.21	1.25	0.68–2.28	2.53	1.06–6.02
	sCAD vs Non-CAD		sCAD vs Controls		Non-CAD vs Controls	
	OR	95% CI	OR	95% CI	OR	95% CI
Personal history of migraine						
No migraine	1		1		1	
MO	5.01	2.86–8.77	5.45	3.03–9.79	1.19	0.62–1.91
MA	1.83	0.87–3.85	4.06	1.63–10.2	2.22	1.00–4.96
C677T MTHFR genotypes						
CC	1		1		1	
CT	1.03	0.87–3.85	1.36	0.76–2.44	1.32	0.86–2.03
TT	1.59	0.84–2.99	2.87	1.45–5.68	1.81	1.02–3.22

ORs of *C677T MTHFR* genotypes are adjusted for migraine status. ORs of migraine status are adjusted for *C677T MTHFR* genotypes.
Because history of migraine was unknown in 3 patients from the subgroup with non-CAD ischemic stroke, estimated values were obtained from 224 subjects.

development of non-CAD ischemic stroke (OR, 2.22; 95% CI, 1.00 to 4.96) compared with control subjects. A history of MO conferred a 5-fold risk of sCAD (OR, 5.01; 95% CI, 2.86 to 8.77) as compared with non-CAD ischemic stroke, and a 5.5-fold risk (OR, 5.45; 95% CI, 3.03 to 9.79) as compared with control subjects (Table 3, middle). The *TT677 MTHFR* genotype was associated with ≈3-fold risk of sCAD (OR, 2.87; 95% CI, 1.45 to 5.68) and ≈2-fold risk of non-CAD ischemic stroke (OR, 1.81; 95% CI, 1.02 to 3.22) in comparison with control subjects. No significant difference in genotype distribution was observed between the subgroup of patients with sCAD and the subgroup of patients with non-CAD ischemic stroke (Table 3, bottom).

These ORs were essentially unchanged when adjusted for potential confounders including age, sex, hypertension, smoking, and hypercholesterolemia (data not shown). Summing the direct effect of *C677MTHFR* gene with the indirect effect via migraine, the estimated total effect of the *TT*-genotype was 3.38 on the risk of sCAD, and 1.93 on the risk of non-CAD ischemic stroke, as compared with control subjects. The estimated increase in percentage of log–OR attributable to mediation effect were 16% and 6%, respectively.

The evidence of a role of migraine in mediating the effect of the *TT677 MTHFR* genotype on the risk of sCAD prompted to speculate a dose-dependent influence of these 2 variables on clinical phenotype. Actually, the prevalence of those with migraines carrying the *TT677 MTHFR* genotype turned out to be higher among patients with multiple-vessel dissection (3/16; 18.8%) than among those with single-vessel dissection (12/90; 13.3%) and control subjects (5/187; 2.7%), and the log–odds trend was statistically significant (χ^2 [df] for log–odds trend=11.2; P= 0.0008; Figure 2).

Discussion

Migraine and ischemic stroke are considered the end phenotype of polygenic disorders reflecting the influence of several genetic loci modulating different pathophysiological processes. Thus, it is reasonable to hypothesize that certain genes might have an effect on both diseases and influence their relation. At what levels in the migraine–stroke pathway these genetic influences might be operating to increase the propensity to cerebral ischemia and whether such effects might vary according to different stroke subtypes are important and still poorly investigated aspects of stroke pathogenesis. The results of our study put emphasis on the possibility to identify some of these genetic susceptibility factors.

First, the present analysis demonstrates that subjects with migraine, particularly MA, have an increased prevalence of

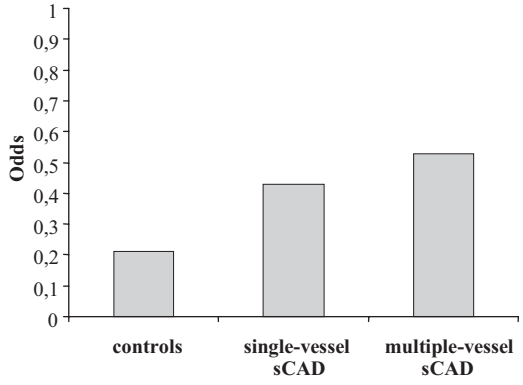

Figure 2. Odds=Pr (*TT* genotype and any migraine)/Pr (otherwise) for the subgroup of patients with single-vessel sCAD, the subgroup of patients with multiple-vessel sCAD, and the group of control subjects.

the homozygous *TT* genotype and of the *T* allele of the *C677T MTHFR* polymorphism. These findings confirm previous evidence in favor of a direct contributory role of *MTHFR* as a migraine gene.[10–14]

Second, a further confirmatory finding was that personal history of migraine is associated with increased risk of cerebral ischemia and the risk is strikingly different according to aura status.[1]

Third, although the existence of a direct genotype–stroke relation is likely, our results suggest that *MTHFR* contributes to increase the risk of ischemic stroke at least in part through its effect on migraine. In this regard, migraine should be considered an intermediate factor in the complex pathway from *MTHFR* to ischemic stroke, which mediates the effects of genotype, and may explain how the *C677T MTHFR* polymorphism works to influence ischemic stroke. If we look at this relation another way, the *C677T MTHFR* polymorphism may be one of the hitherto unknown factors linking migraine to cerebral ischemia.

Forth, an additional observation of the present study is the finding of a stroke subtype-dependency for the triangular *MTHFR*–migraine–stroke relation. In particular, stronger risk effects of genotype and migraine were found in the subgroup of patients with sCAD as compared with other pathogenic subtypes. The finding that the 2 factors are more represented in the subgroup of patients with multiple-vessel sCAD than in the subgroup of those with single-vessel sCAD, although statistically unstable because of the low frequencies in each category, indirectly reinforces such an observation. All these data confirm previous observations on the putative role of both migraine and *C677T MTHFR* polymorphism in the pathogenesis of sCAD,[16–19] but also extends such findings, pointing toward a mediational genotype–environmental effect between the 2 conditions on disease occurrence.

The biological links by which migraine may act as an intermediate factor in the relation between the *C677T MTHFR* polymorphism and ischemic stroke are likely to be complex and currently speculative. The gene is a plausible candidate in the migraine–stroke relation. Many studies using animal models and human subjects have demonstrated that it is actively involved in the maintaining of the normal homeostatic properties of vascular endothelium, which include endothelium-dependent regulation of vascular tone, hemostasis, and inflammation, a process relevant to both cortical spreading depression, the presumed mechanism behind migraine aura, and cerebral ischemia.[27,28] Furthermore, homocysteine-related dysfunction of the vascular endothelium has been demonstrated to activate trigeminal fibers, leading to an inflammatory reaction in the meninges and a dilation of the large cerebral vessels. This mechanism is thought to participate in the head pain associated with migraine.[29,30]

Our study has several strengths, including the relatively large number of subjects in each subgroup, the use of a standardized questionnaire and diagnostic workup, and the homogeneous nature of the study group, which may reduce confounding. Nevertheless, several limitations should be pointed out. Because of the retrospective migraine ascertainment in our stroke patients, a recall bias cannot be theoretically excluded. However, because cases were unaware of the hypothesis undergoing study, there is no reason why they should have reported migraine symptoms more completely than controls. An interviewer bias is also unlikely, because of the use of a structured questionnaire for the diagnosis of migraine. Furthermore, even taking into account such potential biases, we have no reason to believe that they have any influence on genotype distribution in each specific subgroup.

An additional limitation is that we had no information regarding the duration of migraine or frequency of migraine attacks in our series. Any consideration on differential effects according to migraine activity is, therefore, speculative based on our data. No detailed information is also available on the use of migraine-specific drugs. Because of the known vasoconstrictive effect of these molecules (particularly, ergotamines),[31] they might be theoretically associated with increased risk of cerebral ischemic events. The safety profiles of these compounds as well as their use by both subjects with MO and subjects with MA, however, make this an unlikely explanation of our findings. Finally, although we adjusted for major potential confounders, residual confounding is possible given the observational design of the study.

In conclusion, our findings reinforce the assumption that specific hereditary factors contribute to migraine–stroke susceptibility, with the relative contribution of these genetic determinants different for MA as compared with MO. In particular, they indicate that the *C677T MTHFR* polymorphism is associated with MA, that its predisposing influence on the risk of ischemic stroke is in part mediated by migraine, and that the magnitude of this effect is greater for arterial dissection than for other stroke mechanisms. The study indirectly supports the theory that genetic loci related to homeostatic properties of vascular endothelium are promising candidate sites in the complex migraine–stroke pathway.

Acknowledgments

The authors are grateful to a number of doctors who assisted in the ascertainment and recruitment of patients and control subjects: Dr Biagio Troianiello, Unità Operativa di Neurologia, Istituto Clinico S. Anna, Brescia; Dr Simona Griffini, Unità Operativa di Neurologia, Istituto Clinico Città di Brescia, Brescia; Dr Eugenio Magni, Unità Operativa di Neurologia, Casa di Cura Poliambulanza, Brescia; Dr Raffaella Spezi, Dipartimento di Scienze Mediche e Chirurgiche, Neurologia Vascolare, Stroke Unit, Spedali Civili, Brescia. The authors also acknowledge the technical assistance of Michela Cossandi, III Laboratorio di Analisi, Biotecnologie, Spedali Civili, Brescia, in performing laboratory assays. The authors express their gratitude to all the individuals who participated in the study.

Disclosures

None.

References

1. Etminan M, Takkouche B, Isorna FC, Samii A. Risk of ischemic stroke in people with migraine: systematic review and meta-analysis of observational studies. *BMJ*. 2005;330:63–66.
2. Kurth T, Slomke MA, Kase CS, Cook NR, Lee IM, Gaziano JM, Diener HC, Buring JE. Migraine, headache, and the risk of stroke in women. A prospective study. *Neurology*. 2005;64:1020–1026.
3. Stang PE, Carson AP, Rose KM, Mo J, Ephross SA, Shahar E, Szklo M. Headache, cerebrovascular symptoms, and stroke. The Atherosclerosis Risk in Communities Study. *Neurology*. 2005;64:1573–1577.

4. Schwartz RH, Kern RZ. Migraine is associated with magnetic resonance imaging white matter abnormalities. A meta-analysis. *Arch Neurol*. 2004; 61:1366–1368.
5. Porter A, Gladstone JP, Dodick DW. Migraine and white matter hyperintensities. *Curr Pain Headache Rep*. 2005;9:289–293.
6. Ferrari MD. Heritability of migraine. Genetic findings. *Neurology*. 2003; 60(Suppl 2):S15–S20.
7. Russell MB, Olesen J. Increased familial risk and evidence of genetic factor in migraine [see comment]. *BMJ* 1995;311:541–544; Comment in *BMJ*. 1995;311:1227.
8. Kowa H, Yasui K, Takeshima T, Urakami K, Sakai F, Nakashima K. The homozygous C677T mutation in the methylenetetrahydrofolate reductase gene is a genetic risk factor for migraine. *Am J Med Genet*. 2000;96: 762–764.
9. Kara I, Sazci A, Ergul E, Kaya G, Kilic G. Association of the C677T and A1298C polymorphisms in the 5,10 methylenetetrahydrofolate reductase gene in patients with migraine risk. *Mol Brain Res*. 2003;111:84–90.
10. Lea RA, Ovcaric M, Sundholm J, MacMillan J, Griffiths LR. The methylenetetrahydrofolate reductase gene variant C677T influences susceptibility to migraine with aura. *BMC Med*. 2004;2:3.
11. Oterino A, Valle N, Bravo Y, Munoz P, Sáncez-Velasco P, Ruiz-Alegia C, Castello J, Leyva-Cobián F, Vadillo A, Pascual J. MTHFR T677 homozygosis influences the presence of aura in migraineurs. *Cephalalgia*. 2004;24:491–494.
12. Scher AI, Terwindt GM, Verschuren WMM, Kruit MC, Blom HJ, Kowa H, Frants RR, van den Maagdenberg AMJM, Van Buchem M, Ferrari MD, Launer LJ. Migraine and MTHFR C677T genotype in a population-based sample. *Ann Neurol*. 2006;59:372–375.
13. Todt U, Freudenberg J, Goebel I, Netzer C, Heinze A, Heinze-Kuhn K, Gobel H, Kubisch C. MTHFR C677T polymorphism and migraine with aura. *Ann Neurol*. 2006;60:621–622.
14. Kaunisto M, Kallela M, Hamalainen E, Kilpikari R, Havanka H, Harno H, Nissila M, Sako E, Ilmavirta M, Liukkonen J, Teirmaa H, Tornwall O, Jussila M, Terwilliger J, Farkkila M, Kaprio J, Palotie A, Wessman M. Testing of variants of the MTHFR and ESR1 genes in 1798 Finnish individuals fails to confirm the association with migraine with aura. *Cephalalgia*. 2006;26:1462–1472.
15. Casas PJ, Bautista LE, Smeeth L, Sharma P, Hingorani AD. Homocysteine and stroke: evidence on a causal link from mendelian randomization. *Lancet*. 2005;365:224–232.
16. Pezzini A, Del Zotto E, Archetti S, Negrini R, Bani P, Alberini A, Grassi M, Assanelli D, Gasparotti R, Vignolo LA, Magoni M, Padovani A. Plasma homocysteine concentration, C677T MTHFR genotype and 844ins68bp CBS genotype in young adults with spontaneous cervical artery dissection and atherothrombotic stroke. *Stroke*. 2002;33:664–669.
17. Kloss M, Wiest T, Hyrenbach S, Werner I, Arnold M-L, Lichy C, Grond-Ginsbach C. MTHFR 677TT genotype increases the risk for cervical artery dissections. *J Neurol Neurosurg Psychiatry*. 2006;77: 951–952.
18. Tzourio C, Benslamia L, Guillon B, Aidi S, Bertrand M, Berthet K, Bousser MG. Migraine and the risk of cervical artery dissection: a case-control study. *Neurology*. 2002;59:435–437.
19. Pezzini A, Granella F, Grassi M, Bertolino C, Del Zotto E, Immovilli P, Bazzoli E, Padovani A, Zanferrari C. History of migraine and risk of spontaneous cervical artery dissection. *Cephalalgia*. 2005;25(8): 575–580.
20. Headache Classification Subcommittee of the International Headache Society. The international classification of headache disorders. *Cephalalgia* 2004;24(Suppl 1):24–36.
21. Frosst P, Blom HJ, Milos R, Goyette P, Sheppard CA, Matthews RG, Boers GJH, den Heijer M, Kluijtmans LAJ, van den Heuvel LP, Rozen R. A candidate genetic risk factor for vascular disease: a common mutation in methylenetetrahydrofolate reductase. *Nature Gen*. 1995;10:111–113.
22. Baron RM, Kenny DA. The moderator-mediator variable distinction in social psychological research: conceptual, strategic, and statistical considerations. *J Per Soc Psychol*. 1986;51:1173–1182.
23. Kraemer HC, Stice E, Kazdin A, Offord D, Kupfer D. How do risk factors work together? Mediators, moderators, and independent, overlapping, and proxy risk factors. *Am J Psychiatry*. 2001;158:848–856.
24. Skrondal A, Rabe-Hesketh S. *Generalized latent variable modeling. Multilevel, longitudinal, and structural equation models*. Boca Raton, FL: Chapman and Hall/CRC; 2004;8.3.2.
25. Burnham KP, Anderson DR. *Model Selection and Inference. A Practical Information Theoretic Approach,* 2nd ed. New York: Springer-Verlag; 2002.
26. Huang B, Sivaganesan S, Succop P, Goodman E. Statistical assessment of mediational effects for logistic mediational models. *Statist Med*. 2004; 23:2713–2728.
27. Lentz SR. Homocysteine and cardiovascular physiology. In: L, Jackobsen DW, eds. *Homocysteine in health and disease.* Cambridge, UK: Cambridge University Press; 2001.
28. Faraci FM, Lentz SR. Hyperhomocysteinemia, oxidative stress, and cerebral vascular dysfunction. *Stroke*. 2004;35:345–347.
29. Parsons AA, Strijbos PJ. The neuronal versus vascular hypothesis of migraine and cortical spreading depression. *Curr Opin Pharmacol*. 2003; 3:73–77.
30. Storer RJ, Goadsby PJ. Microiontophoretic application of serotonin (5HT)1B/1D agonists inhibits trigeminal cell firing in the cat. *Brain*. 1997;120:2171–2177.
31. Tietjen GE. The risk of stroke in patients with migraine and implications for migraine management. *CNS Drugs*. 2005;19:683–692.