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Interrelationships Between the *MTHFR* 677C>T Polymorphism, Migraine, and Cardiovascular Disease

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Abstract

Background—Interrelationships between the *MTHFR* 677C>T polymorphism (rs1801133), migraine, and cardiovascular disease (CVD) are plausible but remain controversial.

Methods—Association study among 25,001 white U.S. women, participating in the Women's Health Study, with information on *MTHFR* 677C>T polymorphism. Migraine and migraine aura status were self-reported. Incident CVD events were confirmed after medical record review. We used logistic regression to investigate the genotype-migraine association and proportional hazards models to evaluate the interrelationships of genotype and migraine on incident CVD.

Results—At baseline, 4,577 (18.3%) women reported history of migraine; 39.5% of the 3,226 women with active migraine indicated aura. During a mean of 11.9 years of follow-up, 625 CVD

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Supplemental data: Table E-1.

M. Schürks (Div. of Preventive Medicine) and R.Y. Zee (Div. of Preventive Medicine) conducted the statistical analysis.

Full Disclosures

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Dr. Schürks and Dr. Zee take full responsibility for the data, the analysis and interpretation, and the conduct of the research; they had full access to all of the data; and they have the right to publish any and all data, separate and apart from the attitudes of the sponsor.

events occurred. Our results suggest a modestly reduced risk for migraine with aura in carriers of the TT genotype. The multivariable-adjusted relative risk (RR) in the recessive model was 0.79 (95% CI=0.65–0.96; $p=0.02$). The TT genotype did not increase the risk for CVD. In contrast, migraine with aura doubled the risk for CVD (multivariable-adjusted RR=2.06; 95% CI=1.53–2.78; $p<0.0001$). Co-existence of migraine with aura and the TT genotype selectively raised this risk (RR=3.66; 95% CI=1.69–7.90; $p=0.001$). This pattern was driven by a 4-fold increased risk for ischemic stroke (multivariable-adjusted RR=4.19; 95% CI=1.38–12.74; $p=0.01$) and not apparent for myocardial infarction.

Conclusions—Data from this large cohort of women suggest a modest protective effect of the *MTHFR* 677TT genotype on migraine with aura. The increased risk for CVD among migraineurs with aura was magnified for TT genotype carriers, which was driven by a substantially increased risk of ischemic stroke.

Keywords

[101] migraine; *MTHFR* polymorphism; [2] cardiovascular disease; [54] cohort study

Migraine is a common debilitating headache disorder with a complex etiology, in which heredity plays an important role.^{1, 2} Pathophysiological concepts involve neuronal and vascular dysfunctions.³ Vascular dysfunctions are of particular interest since epidemiological studies have established an increased risk for ischemic vascular events among patients with migraine, in particular migraine with aura.^{4–6} It has been suggested that this association may be related to the 677C>T polymorphism in the methylenetetrahydrofolate reductase (*MTHFR*) gene (rs1801133).^{6, 7}

The TT genotype of this polymorphism impairs enzyme activity, thus elevating homocysteine levels.⁸ Some,^{9–14} but not all studies^{15–17} have shown that carriers of the TT genotype are more likely to have migraine, in particular migraine with aura. However, data of the Women's Health Study (WHS) indicate that migraine or migraine with aura is not associated with increased homocysteine levels.¹⁸

The relationship between homocysteine, *MTHFR* 677C>T polymorphism, and CVD is equally controversial and may involve differential associations with ischemic stroke and myocardial infarction (MI).¹⁹ Some data suggest, that *MTHFR* TT genotype and elevated homocysteine levels increase the risk for incident ischemic stroke²⁰ and MI.²¹ However, previous data from the WHS did not support such associations.²²

The WHS collected information allowing us to investigate (1) whether the *MTHFR* 677TT genotype is associated with migraine or migraine aura status; (2) whether the previously found lack of association between the *MTHFR* 677TT genotype and incident CVD changed with longer-term follow-up; and (3) whether the increased risk of CVD among migraineurs with aura is modified by *MTHFR* 677C>T genotype status.

Subjects and Methods

Study population

The WHS was a randomized trial designed to test the benefits and risks of low-dose aspirin and vitamin E in the primary prevention of CVD and cancer. The design, methods, and results have been described in detail previously.^{23, 24} Briefly, a total of 39,876 U.S. female health professionals aged ≥ 45 years at study entry (1992–1995) without a history of CVD, cancer, or other major illnesses were randomly assigned to active aspirin (100 mg on alternate days), active vitamin E (600 IU on alternate days), both active agents, or both placebos. All participants provided written informed consent and the Institutional Review Board of Brigham

and Women's Hospital approved the WHS. Baseline information was self-reported and collected by a mailed questionnaire that asked about many cardiovascular risk factors and lifestyle variables.

Blood samples were collected in tubes containing EDTA from 28,345 participating women prior to randomization. After excluding participants with missing information on migraine, *MTHFR* 677C>T polymorphism, and with reported CVD or angina prior to receiving the baseline questionnaire, we were left with 26,429 women. We further excluded non-Caucasian women (n=1,428), leaving 25,001 Caucasian women for analyses.

Assessment of migraine

Participants were asked on the baseline questionnaire: "Have you ever had a migraine headache?" and "In the past year, have you had a migraine headache?" From this information, we categorized women into "any history of migraine;" "active migraine," which includes women with self-reported migraine during the past year; and "prior migraine," which includes women who reported ever having had a migraine but none in the year prior to completing the questionnaire. In a previous study,⁵ we have shown good agreement with 1988 International Headache Society (IHS) criteria for migraine.²⁵ Participants who reported active migraine were further asked whether they had an "aura or any indication a migraine is coming." Responses were used to classify those women into active migraine with aura and active migraine without aura.

Ascertainment of CVD

During follow-up, participants self-reported cardiovascular events. Medical records were obtained for all events and reviewed by an Endpoints Committee of physicians. Nonfatal stroke was confirmed if the participant had a new focal-neurologic deficit of sudden or rapid onset that persisted for >24 hours. Major stroke subtype classification (ischemic, hemorrhagic, or unknown) was based on available clinical and diagnostic information with excellent interrater agreement.²⁶ The occurrence of MI was confirmed if symptoms met World Health Organization criteria and if the event was associated with abnormal levels of cardiac enzymes or abnormal electrocardiograms. Cardiovascular deaths were confirmed by review of autopsy reports, death certificates, medical records, or information obtained from next of kin or family members.

We evaluated major CVD, a combined endpoint defined as the first of any of these events: nonfatal ischemic stroke, nonfatal MI, or death from ischemic CVD. We also evaluated any first ischemic stroke and any first MI. There were too few CVD deaths to run meaningful analyses.

Dietary assessment

At baseline, participants completed a 131-item semiquantitative food frequency questionnaire.²⁷ Nutrient intake was calculated based on the content of the portion sizes multiplied by the frequency of consumption and was adjusted for total energy intake by the residual method.²⁸ In addition, participants were asked to report the type and amount of vitamin supplement use. Intake of folate and B-vitamins from supplements was calculated with a comprehensive multivitamin database.

Homocysteine measurements

EDTA blood samples were stored in vapor phase liquid nitrogen (−170 °C). The concentration of homocysteine was determined using an enzymatic assay on the Hitachi 917 analyzer (Roche Diagnostics) using reagents and calibrators from Catch Inc, Seattle, Wash.

Genotype determination of the *MTHFR* 677C>T polymorphism (rs1801133)

Genotyping was performed in the context of a multi-marker assay (Roche Molecular Systems) using an immobilized probe approach, as previously described.²⁹ In brief, each DNA sample was amplified by polymerase chain reaction (PCR) with biotinylated primers. Each PCR product pool was then hybridized to a panel of sequence-specific oligonucleotide probes immobilized in a linear array. The colorimetric detection method was based on the use of streptavidin-horseradish peroxidase conjugate with hydrogen peroxidase and 3,3',5,5'-tetramethylbenzidine as substrates. Linear array processing was facilitated by the use of the AutoRELI-Mark II (Dynal Biotech). Genotype assignment was performed using the proprietary Roche Molecular Systems StripScan image processing software. To confirm genotype assignment, scoring was carried out by two independent observers. Discordant results (<1% of all scoring) were resolved by a joint reading, and where necessary, a repeat genotyping.

Statistics

We compared baseline characteristics of participants with respect to their *MTHFR* 677C>T genotype status using the chi-square test for categorical variables and the Kruskal-Wallis test for continuous variables.

We used logistic regression models to evaluate the association between *MTHFR* 677C>T genotypes and migraine. We calculated odds ratios (ORs) and 95% confidence intervals (CIs) for any history of migraine, active migraine with aura, active migraine without aura, and prior migraine. We built two multivariable-adjusted models. In Model 1, we adjusted for age (continuous), body mass index (continuous), exercise (never, <1/week, 1–3/week, ≥4/week), postmenopausal hormone use (never, past, current), history of oral contraceptive use (yes, no, not sure), history of hypertension (yes, no), history of diabetes (yes, no), alcohol consumption (never, 1–3 drinks/month, 1–6 drinks/week, ≥1 drinks/day), smoking (never, past, current <15 cigarettes/day, current ≥15 cigarettes/day), and family history of MI prior to age 60 (yes, no). In Model 2, we additionally adjusted for serum homocysteine levels (continuous), folate intake (continuous), vitamin B2 intake (continuous), vitamin B6 intake (continuous), and vitamin B12 intake (continuous). We incorporated a missing value indicator if the number of women with missing information on covariates was ≥100 or imputed a value otherwise.

We used Cox proportional hazards models to evaluate the association of *MTHFR* 677C>T genotypes and migraine with incident cardiovascular events. We built additive, dominant, and recessive models and calculated multivariable-adjusted hazard ratios (HRs) and their 95% CIs. The additive model assumes that the risk for the outcome among carriers of the CT genotype is half way between carriers of the CC and TT genotypes. While the dominant model assumes that carriers of the CT and TT genotypes have the same risk of developing the outcome compared with carriers of the CC genotype, a recessive model assumes that carrying the TT genotype is necessary to alter the risk for the outcome compared with carriers of the CT and CC genotypes. We also evaluated whether the association between migraine and CVD is modified by genotype by using the likelihood ratio test.

Furthermore, we calculated the attributable rate for ischemic stroke comparing women with migraine with aura vs. women without migraine and women with migraine with aura and the TT genotype vs. women without migraine and without the TT genotype.

All analyses were performed using SAS version 9.1 (SAS Institute Inc, Cary, NC). All p-values were two-tailed and we considered $p < 0.05$ as significant. Since we evaluated biologically plausible associations between only one polymorphism, migraine, and CVD, we did not further adjust p-values.

Results

Baseline characteristics of women according to *MTHFR* 677C>T genotype are summarized in Table 1. Age, body mass index, and history of diabetes were equally distributed among genotypes. We also found no differences regarding postmenopausal hormone use, history of oral contraceptive use, alcohol consumption, smoking habits, family history of premature MI, and in median daily intake of folate and B-vitamins. Women carrying a T allele were, however, more likely to have a history of hypertension and tended to be more physically active. As expected, women carrying the TT genotype had significantly higher serum homocysteine levels compared with carriers of the CC and CT genotypes.

At baseline, 4,577 (18.3%) women reported any history of migraine. Of the 3,226 women who reported active migraine, 39.5% indicated migraine aura. The observed genotype distribution for *MTHFR* 677C>T was in Hardy-Weinberg equilibrium among women with no history of migraine (chi-square with 1 degree of freedom: $p=0.057$). There was no difference in the genotype and allele distribution for *MTHFR* 677C>T between women with and without migraine (Table E-1).

In the recessive model, we found that carriers of the *MTHFR* 677TT genotype were less likely to have any history of migraine (age-adjusted OR=0.89; 95% CI=0.80–0.99; $p=0.04$), a result only apparent for women with active migraine with aura (age-adjusted OR=0.79; 95% CI=0.65–0.96; $p=0.02$) (Table 2). Multivariable adjustment, including homocysteine levels and vitamin use, did not change these findings. When we further controlled for income and education as indicators of socioeconomic status, the results also did not change (data not shown).

During a mean of 11.9 years of follow-up (296,865 person-years), 625 major CVD events, 275 ischemic strokes, and 268 MIs were confirmed. The *MTHFR* 677TT genotype was not associated with increased risk of any ischemic vascular event (Table 3).

In Table 4, we summarize the association between migraine and incident ischemic cardiovascular events. Compared with women without migraine, women with any history of migraine had increased risk for major CVD (multivariable-adjusted HR 1.29; 95% CI 1.06–1.58; $p=0.01$). This elevated risk was only apparent in women with active migraine with aura (multivariable-adjusted HR=2.06; 95% CI=1.53–2.78; $p<0.0001$), but not in women with active migraine without aura. This increased risk was apparent for ischemic stroke and MI. The stratified analysis shows that carrying the TT genotype as opposed to the CT or CC genotype further increases the risk for major CVD (multivariable-adjusted HR=3.66; 95% CI=1.69–7.90; $p=0.001$). The association between migraine aura status and major CVD was significantly modified by the *MTHFR* 677C>T genotype status, assuming an age-adjusted recessive model (p for interaction=0.03). This pattern was driven by a substantially increased risk for ischemic stroke (multivariable-adjusted HR=4.19; 95% CI=1.38–12.74; $p=0.01$) that was not seen for MI (multivariable-adjusted HR=2.88; 95% CI=0.84–9.88; $p=0.09$).

In the Figure, we summarize the association between the *MTHFR* 677TT genotype, migraine with aura, and ischemic stroke based on findings from this study. The figure also shows underlying associations of commonly described links between environmental factors and other gene variants with *MTHFR* 677TT genotype, migraine with aura, and ischemic stroke. Compared with women without migraine, there were 3.8 additional ischemic stroke events attributable to migraine with aura per 10,000 women per year. When contrasting women with migraine with aura carrying the TT genotype to women without migraine and without the TT genotype, the attributable rate of ischemic stroke was 24.0 per 10,000 women per year.

Discussion

The results of this large study of Caucasian women indicate that carriers of the *MTHFR* 677TT genotype were less likely to have a migraine with aura. However, if women with migraine with aura carry the TT genotype, the risk of ischemic stroke was substantially increased, a pattern not observed for MI. In this analysis with extended follow-up, we further confirm previous reports, that the *MTHFR* 677TT genotype does not alter the risk for incident CVD²² and that migraine with aura increases the risk of CVD, including MI.⁵

Most studies have reported an increased risk for migraine with aura among carriers of the *MTHFR* 677TT genotype.^{9–13} However, a German study of 656 patients with migraine with aura and 625 controls,¹⁷ a Finnish study of 898 patients with migraine with aura and 900 controls,¹⁶ and a small Italian study in a pediatric migraine population¹⁵ did not confirm an association. A recent meta-analysis¹⁴ found an increased risk only for migraine with aura in the recessive mode using both the fixed (OR=1.32; 95% CI=1.10–1.59) and the random effects models (OR=1.63; 95% CI=1.10–2.43). Data from our study suggest that carriers of the TT genotype are approximately 20% less likely to have migraine with aura. The direction and magnitude of association was similar in the Finnish study (OR=0.83; 95% CI=0.55–1.24), which, however did not reach significance likely because of the smaller sample.¹⁶

The complex relationship between *MTHFR* 677C>T, migraine, and CVD has been the focus of recent studies. In these studies, migraine with aura has been shown to increase the risk of ischemic stroke and other ischemic events.^{4, 5, 30} Further, homocysteine and the *MTHFR* 677TT genotype have been associated with increased risk of ischemic stroke²⁰ and MI.²¹ Moreover, a recent case-control study from Italy showed an increased risk for migraine with aura among carriers of the 677TT genotype and, in a different study population, that this genotype independently influences migraine with aura as well as ischemic stroke.⁶ These findings may suggest that the association between the 677TT genotype or homocysteine and ischemic stroke is modified by migraine with aura. In addition, it has recently been proposed that the relationship between the *MTHFR* 677TT genotype, homocysteine, and cardiovascular events may be a limited to ischemic stroke.¹⁹

However, previous findings from the WHS do not support a link between the *MTHFR* 677TT genotype or homocysteine levels and CVD. First, neither homocysteine levels nor the 677TT genotype were associated with incident overall or incident specific CVD events,²² a result that did not change with extended follow-up as reported in the present study. Second, homocysteine levels were not associated with migraine or migraine aura status.¹⁸

The increased risk of CVD for patients with migraine with aura is further magnified for carriers of the 677TT genotype. This pattern of association, however, was entirely driven by a substantially increased risk for ischemic stroke and was not apparent for MI. This may suggest that differential pathophysiological mechanisms are involved in the migraine with aura-ischemic stroke and migraine with aura-MI association. Since CVD pathophysiology is complex, involving aspects of altered coagulation, increased blood lipid levels, oxidative stress, endothelial dysfunction etc.,¹⁹ differential processes may indeed account for ischemic stroke and MI. This may be supported by findings that biomarkers like c-reactive protein are more closely related with ischemic stroke than with coronary heart disease.³¹ In addition, interactions of the *MTHFR* 677TT genotype with other genetic variants like the insertion/deletion polymorphism in the gene coding for the angiotensin converting enzyme (*ACE* D/I polymorphism) need to be considered. While the significance of this polymorphism for ischemic stroke and other cardiovascular events is controversial, the combined occurrence of the *ACE* DD and *MTHFR* 677TT genotypes seem to magnify the risk for migraine, in particular

migraine with aura.³² Furthermore, the 5-HT_{1D} receptor gene (*HTR1D*), implicated in migraine pathophysiology, is located close to the *MTHFR* gene on chromosome 1p36.

Thus the *MTHFR* 677TT genotype may differentially modify the risk for ischemic stroke and MI among migraineurs with aura depending on the interactions with variants in any of these genes or on epigenetic effects on different biological pathways. However, the mechanism by which the TT genotype confers additional risk for ischemic stroke among migraineurs with aura remains to be established.

Our study has several strengths, including the prospective design as well as large number of participants and outcome events. Further, we collected information on a large number of potential CVD risk factors and the homogenous nature of the cohort may reduce confounding. However, several limitations should be considered. First, migraine and migraine aura status were self-reported and could not be classified according to strict IHS criteria. Thus, non-differential misclassification of migraine and migraine aura is possible. However, our prevalence of migraine (18.3%) and migraine aura (39.5%) is very similar to those seen in other large population-based studies in the U.S.³³ and the Netherlands.³⁴ The 1-year prevalence of migraine and migraine aura for women in the U.S. was 18.2% and 37.0%, respectively³³ and in the Netherlands 25% and 31%, respectively.³⁴ Moreover, this potential non-differential misclassification would likely have obscured a genotype-migraine association. Furthermore, our migraine ascertainment allowed us to classify migraine according to modified 1988 IHS criteria, which showed good agreement.⁵ Second, participants were white female health professionals age ≥ 45 , thus generalizability may be limited. For example, some studies suggest that young women with migraine with aura are at particular risk for ischemic stroke.³⁵ In addition, homocysteine levels differ between men and women, thus gender specific effects are possible. Finally, genetic association studies cannot exclude the possibility that examination of a different polymorphism—not in linkage disequilibrium with the variant tested—might lead to a different result. Thus, the *MTHFR* 677TT genotype may only be a marker for an increased risk of ischemic stroke among patients with migraine with aura.

Our results warrant replication in other large cohorts with information on migraine and aura status according to the IHS criteria. In particular, age- and gender-specific effects need to be considered. Given the limited knowledge about the biological pathways involved between migraine with aura and ischemic vascular events, a genetic testing of migraineurs with aura seems premature. However, practitioners should focus on reducing cardiovascular risk factors and particularly advise young women with migraine with aura not to smoke and to reevaluate oral contraceptives use.³⁶

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References

1. Ligthart L, Boomsma DI, Martin NG, Stubbe JH, Nyholt DR. Migraine with aura and migraine without aura are not distinct entities: further evidence from a large Dutch population study. *Twin Res Hum Genet* 2006;9:54–63. [PubMed: 16611468]
2. Mulder EJ, Van Baal C, Gaist D, et al. Genetic and environmental influences on migraine: a twin study across six countries. *Twin Res* 2003;6:422–431. [PubMed: 14624726]
3. Pietrobon D, Striessnig J. Neurobiology of migraine. *Nat Rev Neurosci* 2003;4:386–398. [PubMed: 12728266]
4. Kurth T, Gaziano JM, Cook NR, et al. Migraine and risk of cardiovascular disease in men. *Arch Intern Med* 2007;167:795–801. [PubMed: 17452542]
5. Kurth T, Gaziano JM, Cook NR, Logroscino G, Diener HC, Buring JE. Migraine and risk of cardiovascular disease in women. *JAMA* 2006;296:283–291. [PubMed: 16849661]
6. Pezzini A, Grassi M, Del Zotto E, et al. Migraine mediates the influence of C677T MTHFR genotypes on ischemic stroke risk with a stroke-subtype effect. *Stroke* 2007;38:3145–3151. [PubMed: 17962595]
7. Lipton RB, Bigal ME. Migraine and cardiovascular disease. *JAMA* 2006;296:332–333. [PubMed: 16849667]
8. Frosst P, Blom HJ, Milos R, et al. A candidate genetic risk factor for vascular disease: a common mutation in methylenetetrahydrofolate reductase. *Nat Genet* 1995;10:111–113. [PubMed: 7647779]
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. *Brain Res Mol Brain Res* 2003;111:84–90. [PubMed: 12654508]
10. 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. [PubMed: 11121176]
11. Lea RA, Ovcarić M, Sundholm J, MacMillan J, Griffiths LR. The methylenetetrahydrofolate reductase gene variant C677T influences susceptibility to migraine with aura. *BMC Med* 2004;2:3. [PubMed: 15053827]
12. Oterino A, Valle N, Bravo Y, et al. MTHFR T677 homozygosis influences the presence of aura in migraineurs. *Cephalalgia* 2004;24:491–494. [PubMed: 15154859]
13. Scher AI, Terwindt GM, Verschuren WM, et al. Migraine and MTHFR C677T genotype in a population-based sample. *Ann Neurol* 2006;59:372–375. [PubMed: 16365871]
14. Rubino E, Ferrero M, Rainero I, Binello E, Vaula G, Pinessi L. Association of the C677T polymorphism in the MTHFR gene with migraine: a meta-analysis. *Cephalalgia*. 2007;10.1111/j.1468-2982.2007.01400.x
15. Bottini F, Celle ME, Calevo MG, et al. Metabolic and genetic risk factors for migraine in children. *Cephalalgia* 2006;26:731–737. [PubMed: 16686913]
16. Kaunisto MA, Kallela M, Hamalainen E, et al. 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. [PubMed: 17116097]
17. Todt U, Freudenberg J, Goebel I, et al. MTHFR C677T polymorphism and migraine with aura. *Ann Neurol* 2006;60:621–622. [PubMed: 16800002]author reply 622–623
18. Kurth T, Ridker P, Buring J. Migraine and biomarkers of cardiovascular disease. *Cephalalgia* 2008;28:49–56. [PubMed: 17986270]
19. Spence J. Homocysteine-lowering therapy: a role in stroke prevention? *Lancet Neurol* 2007;6:830–838. [PubMed: 17706567]
20. Casas JP, Bautista LE, Smeeth L, Sharma P, Hingorani AD. Homocysteine and stroke: evidence on a causal link from mendelian randomisation. *Lancet* 2005;365:224–232. [PubMed: 15652605]
21. Yamada Y, Matsuo H, Segawa T, et al. Assessment of genetic risk for myocardial infarction. *Thromb Haemost* 2006;96:220–227. [PubMed: 16894468]
22. Zee RY, Mora S, Cheng S, et al. Homocysteine, 5,10-methylenetetrahydrofolate reductase 677C>T polymorphism, nutrient intake, and incident cardiovascular disease in 24,968 initially healthy women. *Clin Chem* 2007;53:845–851. [PubMed: 17332146]

23. Rexrode KM, Lee IM, Cook NR, Hennekens CH, Buring JE. Baseline characteristics of participants in the Women's Health Study. *J Womens Health Gend Based Med* 2000;9:19–27. [PubMed: 10718501]
24. Ridker PM, Cook NR, Lee IM, et al. A randomized trial of low-dose aspirin in the primary prevention of cardiovascular disease in women. *N Engl J Med* 2005;352:1293–1304. [PubMed: 15753114]
25. Headache Classification Committee of the International Headache Society. Classification and diagnostic criteria for headache disorders, cranial neuralgias and facial pain. *Cephalalgia* 1988;8 (Suppl 7):1–96.
26. Atiya M, Kurth T, Berger K, Buring JE, Kase CS. Interobserver agreement in the classification of stroke in the Women's Health Study. *Stroke* 2003;34:565–567. [PubMed: 12574576]
27. Zhang SM, Moore SC, Lin J, et al. Folate, vitamin B6, multivitamin supplements, and colorectal cancer risk in women. *Am J Epidemiol* 2006;163:108–115. [PubMed: 16339055]
28. Willett, W. Nutritional epidemiology. New York: Oxford University Press; 1998.
29. Cheng S, Grow MA, Pallaud C, et al. A multilocus genotyping assay for candidate markers of cardiovascular disease risk. *Genome Res* 1999;9:936–949. [PubMed: 10523522]
30. Etminan M, Takkouche B, Isorna FC, Samii A. Risk of ischaemic stroke in people with migraine: systematic review and meta-analysis of observational studies. *BMJ* 2005;330:63. [PubMed: 15596418]
31. Everett BM, Kurth T, Buring JE, Ridker PM. The relative strength of C-reactive protein and lipid levels as determinants of ischemic stroke compared with coronary heart disease in women. *J Am Coll Cardiol* 2006;48:2235–2242. [PubMed: 17161253]
32. Tietjen EG. Migraine and ischaemic heart disease and stroke: potential mechanisms and treatment implications. *Cephalalgia* 2007;27:981–987. [PubMed: 17661875]
33. Lipton RB, Stewart WF, Diamond S, Diamond ML, Reed M. Prevalence and burden of migraine in the United States: data from the American Migraine Study II. *Headache* 2001;41:646–657. [PubMed: 11554952]
34. Launer LJ, Terwindt GM, Ferrari MD. The prevalence and characteristics of migraine in a population-based cohort: the GEM study. *Neurology* 1999;53:537–542. [PubMed: 10449117]
35. Kurth T, Diener H. Current views of the risk of stroke for migraine with and migraine without aura. *Current Pain and Headache Reports* 2006;10:124–220.
36. Kurth T. Migraine with aura and ischemic stroke: which additional factors matter? *Stroke* 2007;38:2407–2408. [PubMed: 17690307]

Figure.

MTHFR TT, *MTHFR* 677TT genotype; MA, migraine with aura; +, increased risk; −, decreased risk; +/-, unknown or increased as well as decreased risk; //, does not alter risk. For simplification further arrows indicating that an interaction between various exposures (e.g. other gene variants plus environmental factors) may further increase the risk for ischemic stroke, have been omitted.

Table 1

Baseline characteristics of participants in the Women's Health Study according to *MTHFR* 677C>T genotype (N=25,001) *

Characteristic	CC (N=11,243)	CT (N=10,977)	TT (N=2,781)	p-value
Age, mean (SD), y	54.7 (7.1)	54.7 (7.1)	54.7 (7.2)	0.94
Body mass index, mean (SD), kg/m ²	25.9 (4.9)	25.9 (5.0)	26.0 (5.1)	0.84
Homocysteine, μmol/L, median (IQR)	10.31 (8.59–12.57)	10.51 (8.72–12.97)	11.08 (9.10–13.82)	<0.0001
Folate intake, μg/day, median (IQR)	350 (276–543)	351 (277–552)	348 (276–523)	0.33
Vitamin B ₂ intake, mg/day, median (IQR)	2.00 (1.59–3.10)	2.00 (1.60–3.12)	1.99 (1.59–3.05)	0.60
Vitamin B ₆ intake, mg/day, median (IQR)	2.27 (1.86–3.63)	2.29 (1.86–3.63)	2.29 (1.87–3.47)	0.64
Vitamin B ₁₂ intake, mg/day, median (IQR)	6.41 (4.68–10.03)	6.43 (4.68–10.07)	6.33 (4.56–9.75)	0.20
History of diabetes	2.2	2.2	2.5	0.64
History of hypertension	23.7	25.2	25.9	0.01
Physical activity				
Never	37.5	37.2	35.8	
<1/week	19.9	19.2	20.4	
1–3/week	31.6	32.2	30.9	
≥4/wk	11.0	11.5	12.9	0.04
Postmenopausal hormone therapy				
Never	47.9	48.2	49.4	
Past	8.7	9.3	9.4	
Current	43.4	42.5	41.1	0.17
History of oral contraceptive use				
No	30.1	30.4	29.0	
Yes	69.5	69.2	70.4	
Not sure	0.3	0.4	0.6	0.18
Alcohol consumption				
Never	43.9	42.9	43.6	
1–3 drinks/month	13.1	13.4	13.1	
1–6 drinks/week	32.4	33.0	33.2	
≥1 drink/day	10.6	10.7	10.1	0.72
Smoking status				
Never	51.3	51.3	50.2	
Past	37.2	37.4	38.4	
Current <15 cigarettes/day	4.3	4.1	3.6	
Current ≥15 cigarettes/day	7.2	7.2	7.7	0.55
Family history of MI prior to age 60 yrs				
No	78.4	78.2	78.9	
Yes	11.9	11.6	11.3	
Unknown	9.8	10.2	9.7	0.67

* data are expressed as percentages unless otherwise stated.

† p-values for chi-square test for categorical variables, and Kruskal-Wallis test for continuous variables; comparing no history of migraine vs. any history of migraine.

IQR: interquartile range.

Proportions may not add up to 100 due to rounding or missing values.

Table 2

Age- and multivariable adjusted odds ratios (OR) and 95% confidence intervals (95% CI) for migraine according to *MTHFR* 677C>T polymorphism

<i>MTHFR</i> 677C>T polymorphism	OR	Age-adjusted 95% CI	p-value	OR	Model 1* 95% CI	p-value	OR	Model 2† 95% CI	p-value
Any history of migraine (n=4,577)									
additive mode	0.97	0.92–1.02	0.20	0.97	0.92–1.02	0.21	0.97	0.92–1.02	0.20
dominant mode	0.99	0.93–1.05	0.70	0.99	0.93–1.06	0.73	0.99	0.93–1.05	0.70
recessive mode	0.89	0.80–0.99	0.04	0.89	0.80–0.99	0.03	0.89	0.80–0.99	0.03
Migraine with aura (n=1,275)									
additive mode	0.92	0.85–1.01	0.06	0.92	0.85–1.01	0.07	0.92	0.85–1.01	0.07
dominant mode	0.94	0.84–1.06	0.32	0.95	0.85–1.06	0.36	0.95	0.85–1.06	0.36
recessive mode	0.79	0.65–0.96	0.02	0.79	0.65–0.96	0.02	0.79	0.65–0.96	0.02
Migraine without aura (n=1,951)									
additive mode	1.02	0.95–1.09	0.64	1.02	0.95–1.09	0.59	1.02	0.95–1.10	0.56
dominant mode	1.04	0.95–1.15	0.37	1.05	0.95–1.15	0.33	1.05	0.95–1.15	0.33
recessive mode	0.97	0.84–1.13	0.70	0.97	0.84–1.13	0.72	0.98	0.84–1.13	0.75
Past migraine (n=1,351)									
additive mode	0.95	0.87–1.03	0.18	0.94	0.87–1.02	0.15	0.94	0.86–1.02	0.14
dominant mode	0.95	0.85–1.06	0.36	0.95	0.85–1.06	0.31	0.94	0.84–1.05	0.30
recessive mode	0.88	0.73–1.05	0.16	0.87	0.73–1.05	0.14	0.87	0.72–1.05	0.14

* controlling for: age, body mass index, diabetes, physical activity, hormone replacement therapy, oral contraceptive use, history of hypertension, alcohol consumption, smoking categories, and family history of myocardial infarction.

† controlling for: age, body mass index, diabetes, physical activity, hormone replacement therapy, oral contraceptive use, history of hypertension, alcohol consumption, smoking categories, family history of myocardial infarction, serum homocysteine levels, folate intake, vitamin B2 intake, vitamin B6 intake, and vitamin B12 intake.

Table 3

Multivariable-adjusted* hazard ratios (HR) and 95% confidence intervals (95% CI) for ischemic vascular events according to *MTHFR* 677C>T polymorphism (n=25,001)

<i>MTHFR</i> 677C>T polymorphism	HR	95% CI	p-value
Major Cardiovascular Events (n=625)			
additive mode	0.99	0.88–1.12	0.90
dominant mode	1.01	0.86–1.18	0.90
recessive mode	0.94	0.73–1.21	0.65
Ischemic Stroke (n=275)			
additive mode	1.04	0.87–1.24	0.66
dominant mode	1.08	0.85–1.37	0.54
recessive mode	0.99	0.69–1.44	0.97
Myocardial Infarction (n=268)			
additive mode	0.94	0.79–1.13	0.51
dominant mode	0.89	0.70–1.13	0.35
recessive mode	1.01	0.70–1.47	0.94

* controlling for: age, body mass index, diabetes, physical activity, hormone replacement therapy, oral contraceptive use, history of hypertension, alcohol consumption, smoking categories, family history of myocardial infarction, serum homocysteine levels, folate intake, vitamin B₂ intake, vitamin B₆ intake, and vitamin B₁₂ intake.

Table 4

Multivariable-adjusted Hazard Ratios for Cardiovascular Events according to Migraine Status Stratified by *MTHFR* 677C>T genotype in the Women's Health Study (N=25,001)

Ischemic vascular events	No migraine history	Any history of migraine (N=4,577)	P value	Active migraine with aura (N=1,275)	P value	Active migraine without aura (N=1,951)	P value	Prior migraine (N=1,351)	P value
Major CVD*	N=504	N=121		N=48		N=32		N=41	
Multivariable-adjusted [†]									
Overall	1.00	1.29 (1.06–1.58)	0.01	2.06 (1.53–2.78)	<0.0001	0.97 (0.68–1.39)	0.86	1.10 (0.80–1.51)	0.57
CC genotype	1.00	1.29 (0.95–1.75)	0.10	2.39 (1.56–3.68)	<0.0001	0.77 (0.42–1.41)	0.39	1.08 (0.66–1.76)	0.77
CT genotype	1.00	1.17 (0.86–1.60)	0.31	1.48 (0.89–2.47)	0.14	0.95 (0.55–1.64)	0.86	1.17 (0.74–1.83)	0.50
TT genotype	1.00	1.78 (1.02–3.09)	0.04	3.66 (1.69–7.90)	0.001	1.65 (0.72–3.81)	0.24	0.80 (0.25–2.59)	0.71
Ischemic Stroke	N=228	N=47		N=20		N=14		N=13	
Multivariable-adjusted [†]									
Overall	1.00	1.11 (0.81–1.53)	0.52	1.88 (1.18–2.99)	0.007	0.96 (0.56–1.66)	0.88	0.77 (0.44–1.34)	0.35
CC genotype	1.00	1.12 (0.69–1.82)	0.65	1.49 (0.68–3.26)	0.32	1.13 (0.52–2.47)	0.75	0.87 (0.38–1.98)	0.73
CT genotype	1.00	0.88 (0.53–1.47)	0.63	1.85 (0.93–3.68)	0.08	0.59 (0.22–1.62)	0.31	0.58 (0.23–1.42)	0.23
TT genotype	1.00	2.00 (0.89–4.49)	0.09	4.19 (1.38–12.74)	0.01	1.38 (0.36–5.29)	0.64	1.35 (0.32–5.80)	0.68
Myocardial Infarction	N=217	N=51		N=22		N=13		N=16	
Multivariable-adjusted [†]									
Overall	1.00	1.23 (0.90–1.67)	0.20	2.12 (1.36–3.31)	0.0009	0.87 (0.49–1.53)	0.62	0.99 (0.59–1.65)	0.97
CC genotype	1.00	1.21 (0.76–1.91)	0.43	2.98 (1.68–5.29)	0.0002	0.44 (0.14–1.39)	0.16	0.81 (0.35–1.84)	0.61
CT genotype	1.00	1.21 (0.75–1.96)	0.44	1.10 (0.44–2.74)	0.83	1.03 (0.44–2.38)	0.95	1.43 (0.74–2.77)	0.29
TT genotype	1.00	1.37 (0.58–3.24)	0.48	2.88 (0.84–9.88)	0.09	1.98 (0.66–5.98)	0.22		

* defined as the first of any of these events: nonfatal ischemic stroke, nonfatal myocardial infarction, or death due to ischemic cardiovascular cause.

[†] controlling for: age, body mass index, diabetes, physical activity, hormone replacement therapy, oral contraceptive use, history of hypertension, alcohol consumption, smoking categories, family history of myocardial infarction, serum homocysteine levels, folate intake, vitamin B2 intake, vitamin B6 intake, and vitamin B12 intake.