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***MTHFR* 677C>T and *ACE* D/I Polymorphisms and Migraine Attack Frequency in Women**

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Abstract

Data on the association of the *MTHFR* 677C>T and *ACE* D/I polymorphisms with migraine severity, measured by attack frequency, are scarce. We performed an association study among 24,961 women, participating in the Women's Health Study. Migraine, aura status, and attack frequency were self-reported. We used multinomial logistic regression to investigate the genotype-migraine association. Among the 3,186 migraineurs with complete genotype and attack frequency data, 1,270 reported migraine with aura (attack frequency: 76 $\geq$ weekly; 219 monthly; 123 every other month; 852 <6 times/year) and 1,916 migraine without aura (attack frequency: 85 $\geq$ weekly; 414 monthly; 208 every other month; 1,209 <6 times/year). The *MTHFR* 677TT genotype was associated with a reduced risk for migraine with aura, which only appeared for attacks <6 times/year (age-adjusted odds ratio=0.78; 95% confidence interval=0.61-0.99). We did not find a specific pattern of association of the *ACE* D/I polymorphism with attack frequency for migraine with or without aura.

Keywords

migraine; migraine severity; attack frequency; *MTHFR* 677C>T polymorphism; *ACE* D/I polymorphism

Introduction

Migraine is a common chronic neurological disorder affecting 10-20% of the population, predominantly women (1). Typically migraine presents with recurrent headache attacks and various combinations of gastrointestinal and autonomic nervous system symptoms (2,3). Up to one third of migraine patients experience an aura prior to or during the migraine headache characterized by neurological symptoms most often involving the visual field.

Migraine pathophysiology involves both multiple genetic and environmental factors (4). Current concepts view migraine as an inherited disorder of the brain, but vascular mechanisms are clearly implicated (5). Gene variants in the methylenetetrahydrofolate reductase gene (*MTHFR* 677C>T polymorphism, rs1801133) and in the angiotensin-converting enzyme gene (*ACE* D/I polymorphism, rs1799752) appear to play important roles in the vascular oxidative stress response (6,7). Oxidative stress may lead to endothelial dysfunction and explain – at least in part – pathological vascular reactivity among migraineurs (8) and the association between migraine and cardiovascular disease seen in epidemiological studies (9-11).

However, studies on the association between the *MTHFR* 677C>T (12-24) and the *ACE* D/I (24-32) polymorphism and migraine, including aura status, have yielded conflicting results. This may in part be due to the often heterogeneous clinical phenotypes among patients diagnosed with migraine. One attempt to create more homogeneous groups may be to categorize patients according to migraine severity. Migraine attack frequency, in addition to aura status, is considered a marker of migraine severity. Certain gene variants may reveal a differential association with migraine depending on attack frequency, as recently suggested (33).

We have recently reported a modestly reduced risk for migraine with aura among carriers of the *MTHFR* 677TT genotype (22) and a lack of association for the *ACE* D/I polymorphism (31) for both migraine with and without aura in a large cohort of Caucasian women participating in the Women's Health Study (WHS). This report presents additional analyses investigating whether our observed patterns of association differ according to migraine attack frequency.

Subjects and Methods

We have previously reported on the association between the *MTHFR* 677C>T (22) and *ACE* D/I (31) polymorphisms and migraine. For reasons of comprehensiveness we will report the important results again along with the new data. However, these may differ minimally from the original data since we have excluded additional 40 women with missing attack frequency data for the present analysis.

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 cardiovascular disease (CVD) and cancer. The design, methods, and results have been described in detail previously (34,35). 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 and with reported CVD or angina prior to receiving the baseline questionnaire (n=505), 27,840 women remained in the dataset. We further excluded women with missing information on the *MTHFR* 677C>T and *ACE* D/I polymorphisms (n=1,411), non-Caucasian women (n=1,428), and those with missing information on attack frequency among active migraineurs (n=40) leaving 24,961 Caucasian women for analyses.

Assessment of migraine

Participants were asked on the baseline questionnaire: “Have you ever had migraine headaches?” and “In the past year, have you had migraine headaches?” 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 (11), we have shown good agreement of our classification with the 1988 International Headache Society (IHS) criteria for migraine (36) and we have shown excellent agreement between self-reported migraine and ICHD-II based migraine classification in a WHS subcohort (37). Participants who reported active migraine, were asked 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. In addition, participants with active migraine were asked about the “approximate frequency of their migraines” (daily, weekly, monthly, every other month, less than 6 times per year). We will refer to active migraine with aura as migraine with aura and to active migraine without aura as migraine without aura in this report.

Genotype determination of the *MTHFR* 677C>T (rs1801133) and the *ACE* D/I (rs1799752) polymorphisms

Genotyping was performed in the context of a multi-marker assay (Roche Molecular Systems) using an immobilized probe approach, as previously described (38). 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.

Dietary assessment

At baseline, participants completed a 131-item semiquantitative food frequency questionnaire (39). 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 (40). 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.

Statistics

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

We used multinomial logistic regression models to evaluate the association between *MTHFR* 677C>T and *ACE* D/I genotypes and migraine. We calculated odds ratios (ORs) and

95% confidence intervals (CIs) for migraine with and without aura as well as for frequency categories ($\geq$ weekly, monthly, every other month, <6 times per year). We built additive, dominant, and recessive models. The additive model assumes that the risk for migraine among carriers of the heterozygous genotype (*MTHFR* 677CT; *ACE* DI) is half way between carriers of the homozygous wild-type (*MTHFR* 677CC; *ACE* DD) and the homozygous mutant genotype (*MTHFR* 677TT; *ACE* II). The dominant model assumes that carriers of the heterozygous (*MTHFR* 677CT; *ACE* DI) and homozygous mutant (*MTHFR* 677TT; *ACE* II) genotypes have the same risk for migraine compared with carriers of the homozygous wild-type genotype (*MTHFR* 677CC; *ACE* DD). The recessive model assumes that carrying the homozygous mutant genotype (*MTHFR* 677TT; *ACE* II) is necessary to alter the risk for migraine compared with carriers of the heterozygous (*MTHFR* 677CT; *ACE* DI) and homozygous wild-type (*MTHFR* 677CC; *ACE* DD) genotypes. We built age-adjusted and multivariable-adjusted models. In the multivariable-adjusted model investigating the *ACE* D/I polymorphism we included the following covariates: 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 myocardial infarction prior to age 60 (yes, no). In the multivariable-adjusted model investigating the *MTHFR* 677C>T polymorphism we additionally included 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. 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.

Results

Baseline characteristics of women according to *MTHFR* 677C>T and *ACE* D/I genotypes are summarized in Table 1. With regard to the *MTHFR* 677C>T polymorphism women carrying a T allele were more likely to have a history of hypertension and tended to be more physically active. Further, as expected, women carrying the TT genotype had significantly higher serum homocysteine levels compared with carriers of the CC and CT genotypes. There was no difference for any of the other baseline covariates. With regard to the *ACE* D/I polymorphism homozygotes had higher median folate intake than heterozygotes. There was no difference for any of the other baseline covariates.

At baseline, 4,537 (18.2%) women reported any history of migraine; 3,186 of those reported active migraine. Among the 1,270 women with migraine with aura (39.9% of active migraineurs) 76 reported an attack frequency $\geq$ weekly, 219 of monthly, 123 of every other month, and 852 of <6 times/year. Among the 1,916 women with migraine without aura the numbers were: 85 (attack frequency $\geq$ weekly), 414 (monthly), 208 (every other month), and 1,209 (<6 times/year).

The genotype distribution for *MTHFR* 677C>T was in Hardy-Weinberg equilibrium among women with and without any history of migraine (chi-square with 1 degree of freedom: $p = 0.38$ and $p = 0.06$, respectively). The genotype distribution for the *ACE* D/I polymorphism deviated from Hardy-Weinberg equilibrium for women with and without history of migraine (chi-square with 1 degree of freedom: both $p < 0.0001$).

There was no difference in the genotype and allele distribution for *MTHFR* 677C>T and *ACE* D/I between women with and without migraine (Table 2).

Results from the age-adjusted and multivariable-adjusted models were very similar for both genotypes, thus we only report results from the age-adjusted models.

Regarding the *MTHFR* 677C>T polymorphism, carriers of the TT genotype (recessive model) were less likely to have active migraine with aura (age-adjusted OR=0.79; 95% CI=0.65-0.96) (Table 3). When we further looked at migraine attack frequencies, this pattern occurred only among the largest group of women with attacks <6 times/year (age-adjusted OR=0.78; 95% CI=0.61-0.99), but not among women experiencing attacks more frequently. There was no association between *MTHFR* 677C>T and migraine without aura.

Regarding the *ACE* D/I polymorphism there was no overall association with migraine with or without aura (Table 4). When we further looked at migraine attack frequencies, the I allele (dominant model) appeared to be associated with migraine with aura experienced every other month (age-adjusted OR=1.81; 95% CI=1.15-2.84). Further, the I allele seemed to be associated with migraine without aura experienced $\geq$ weekly (age-adjusted OR=0.62; 95% CI=0.40-0.96), while the II genotype (recessive model) seemed to be associated with migraine without aura experienced monthly (age-adjusted OR=0.77; 95% CI=0.60-0.98).

Taken together our results indicate that the *MTHFR* 677TT genotype reduces the risk for active migraine with aura, which only appears in women with attacks <6 times/year. In contrast, with regard to the *ACE* D/I polymorphism, the results do not show a specific pattern of association with frequency categories for migraine with or without aura.

Discussion

The results of this large study of Caucasian women indicate that the reduced likelihood of having migraine with aura among carriers of the *MTHFR* 677TT genotype (22) only appears among those with infrequent attacks <6 times/year. In contrast none of the *ACE* D/I genotypes were associated with migraine attack frequency irrespective of migraine aura status. Further investigation of frequency categories also did not reveal a specific pattern of association.

The association between migraine, in particular migraine with aura, and cardiovascular disease (CVD) is consistent in several studies (9-11,41). Alterations in the oxidative vascular stress response are important aspects in the development of CVD, that may also explain in part pathological vascular reactivity seen in young migraineurs (8). The *MTHFR* 677C>T and the *ACE* D/I polymorphism have been implicated in the vascular oxidative stress response in CVD and, hence, may also be important in migraine (6,7). However, results on the association between both the *MTHFR* 677C>T (12-24) and the *ACE* D/I (24-32) polymorphism and migraine, including aura status, are conflicting. The heterogeneous phenotype of migraine is likely an important reason for this. Migraine severity may be a useful parameter to further subclassify migraine patients into more homogeneous categories and can for example be measured by attack frequency, in addition to aura status. This is supported by recent publications suggesting that migraine behaves different depending on attack frequency. First, the association between migraine and CVD varies by migraine frequency, specifically in women with migraine with aura (42). Second, a study investigating markers of endothelial dysfunction among migraineurs found that the *ACE* DD genotype is associated with a higher attack frequency (33), which agrees with results of an earlier study on migraine without aura (30).

Our analyses, however, do not suggest that any of the *ACE* D/I genotypes is associated with migraine with or without aura (31) or a higher attack frequency (present analysis). Reasons for our different results may include that our cohort was population-based with a lower migraine attack frequency compared to the clinic-based studies with participants reporting more frequent attacks (30,33).

We have previously shown that migraine with aura is associated with an about two-fold increased risk for CVD, including ischemic stroke and myocardial infarction (11). This association differs by migraine attack frequency (42). While a high-attack frequency ($\geq$ weekly) is only associated with ischemic stroke, a low-attack frequency ($<$ monthly) is associated with any vascular event, including ischemic stroke, myocardial infarction, etc. Certain gene variants further add to this complexity. For example, the *MTHFR* 677TT genotype is associated with a modestly reduced likelihood for having migraine with aura (22); our present analysis suggests that this appears only among women with infrequent attacks <6 times/ year. In contrast, co-occurrence of migraine with aura and *MTHFR* 677TT genotype appears to further increase the risk for ischemic stroke, but not other vascular ischemic events (22). This apparent contradiction may be explained in light of the complex migraine pathophysiology involving both neuronal and vascular dysfunctions (5). Migraine aura is likely determined by many gene variants of which *MTHFR* 677C>T is just one. We may speculate that while the TT genotype may reduce the risk for migraine aura, migraine attack frequency may further modify the interaction with other tissue specific gene variants and/or epigenetic effects. This may cause different effects in brain neurons and vasculature endothelial cells. This hypothesis also agrees with the concept of a differential pathophysiology between ischemic stroke and myocardial infarction, which may also be true among migraineurs.

Homocysteine is elevated in carriers of the *MTHFR* 677TT genotype, as also seen in our data. However, homocysteine is unlikely to be involved in the *MTHFR*-migraine with aura association, as serum levels have shown no association with migraine, migraine aura status, or attack frequency in our cohort (43). Hence, the biology underlying the *MTHFR*-migraine association remains to be unveiled.

Our study has several strengths, including the large number of participants and women with migraine. Further, we collected information on a large number of baseline characteristics including medical conditions and lifestyle habits and the homogenous nature of the cohort may reduce confounding. However, several limitations should be considered. First, migraine, migraine aura, and attack frequency were self-reported and migraine could not be classified according to strict IHS criteria. Thus, non-differential misclassification of migraine, migraine aura, and attack frequency is possible. However, our prevalence of migraine (18.2%) and migraine aura (39.9%) is similar to those seen in other large population-based studies in the U.S. (44) and the Netherlands (45), although the data acquisition method was different. Furthermore, we have shown good agreement of our classification (11) with the 1988 IHS criteria for migraine (36) and excellent agreement between self-reported migraine and ICHD-II based migraine classification in the WHS (37). Second, the genotype distribution for the *ACE* D/I polymorphism deviated from Hardy-Weinberg Equilibrium. This occurred for both women with migraine and women without migraine, thus this is an unlikely indication for genotype-based differential survival. In addition, genotyping error is unlikely given our stringent genotyping protocol. However, this stringency together with the fact that participants were all Caucasian female health professionals age ≥ 45 , not representing all Caucasian women, most likely accounts for the deviation from Hardy-Weinberg Equilibrium. Thus generalizability may be limited and this may be one reason for the differing results between our study and previous studies on the *MTHFR* 677C>T and *ACE* D/I polymorphism and migraine. Third, we only had coarse categorical data on migraine attack frequency and most women reported infrequent attacks. Thus, in contrast to clinic-based studies, which allow more accurate determination of the number of headache days per month, we were not able to investigate potential differences within the group of women reporting monthly and weekly attacks. Fourth, an interaction between the *ACE* D/I and *MTHFR* 677C>T polymorphisms with regard to migraine risk has been suggested previously (6,28). However, when we grouped women according to genotype status the numbers in some of the cells were too small to run meaningful analyses. Thus, we cannot exclude interactions between these polymorphisms or

with other gene variants—not in linkage disequilibrium with the variants tested—, as well as epigenetic effects. Finally, genetic association studies cannot indicate whether an association is causal or not. Thus, the *MTHFR* 677TT genotype may be in linkage disequilibrium with other causative genetic variants in nearby genes and may only be a marker for having less frequent attacks among migraineurs.

Future studies investigating the association between the *MTHFR* 677C>T and *ACE* D/I polymorphism and migraine severity are warranted. Detailed information on attack frequency, aura status, and aura frequency may be important in addition to ICHD-2 criteria for migraine. Further, investigation in non-Caucasian population may yield different results.

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Table 1

Baseline characteristics of participants in the Women's Health Study according to *MTHFR* 677C>T and *ACE* D/I genotypes (N=24,961) *

Characteristic	MTHFR 677C>T polymorphism				ACE D/I polymorphism			
	CC (n=11,228)	CT (n=10,956)	TT (n=2,777)	p-value [†]	DD (n=7,312)	DI (n=11,503)	II (n=6,146)	p-value [‡]
Age, mean (SD), y	54.7 (7.1)	54.7 (7.1)	54.7 (7.2)	0.96	54.7 (7.1)	54.6 (7.1)	54.8 (7.2)	0.14
Body mass index, mean (SD), kg/m ²	25.9 (4.9)	25.9 (5.0)	26.0 (5.1)	0.82	25.9 (5.0)	25.9 (4.9)	25.9 (4.9)	0.49
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	10.47 (8.77-12.94)	10.49 (8.72-12.86)	10.42 (8.59-12.88)	0.12
Folate intake, μg/day, median (IQR)	350 (276-543)	351 (277-552)	348 (276-523)	0.39	351 (278-539)	348 (274-543)	353 (278-557)	0.03
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)	0.64	2.00 (1.59-3.06)	1.99 (1.59-3.11)	2.02 (1.60-3.15)	0.53
Vitamin B ₆ intake, mg/day, median (IQR)	2.27 (1.86-3.63)	2.28 (1.86-3.63)	2.29 (1.87-3.47)	0.67	2.28 (1.88-3.58)	2.28 (1.85-3.61)	2.29 (1.86-3.68)	0.60
Vitamin B ₁₂ intake, mg/day, median (IQR)	6.41 (4.68-10.03)	6.42 (4.68-10.06)	6.33 (4.57-9.76)	0.23	6.39 (4.67-9.94)	6.39 (4.66-10.00)	6.46 (4.69-10.05)	0.68
History of diabetes	2.2	2.2	2.5	0.64	2.3	2.2	2.1	0.65
History of hypertension	23.7	25.2	25.9	0.01	25.1	24.3	24.8	0.44
Physical activity								
Never	37.5	37.2	35.7		36.8	37.6	36.9	
<1/week	19.9	19.2	20.4		19.4	20.0	19.4	
1-3/week	31.6	32.2	30.9		32.3	31.2	32.1	
≥4/wk	11.0	11.5	13.0	0.04	11.5	11.3	11.6	0.64
Postmenopausal hormone therapy								
Never	47.9	48.2	49.5		48.1	48.0	48.7	
Past	8.7	9.3	9.4		9.3	9.1	8.7	
Current	43.4	42.5	41.1	0.17	42.6	42.9	42.7	0.77
History of oral contraceptive use								
No	30.2	30.4	29.0		30.2	29.9	30.5	
Yes	69.5	69.2	70.4		69.5	69.7	69.1	
Not sure	0.3	0.4	0.6	0.25	0.4	0.4	0.5	0.63
Alcohol consumption								
Never	43.9	42.8	43.6		43.1	44.2	42.4	

Characteristic	MTHFR 677C>T polymorphism				ACE D/I polymorphism			
	CC (n=11,228)	CT (n=10,956)	TT (n=2,777)	p-value [‡]	DD (n=7,312)	DI (n=11,503)	II (n=6,146)	p-value [‡]
1-3 drinks/month	13.1	13.4	13.1		13.5	13.1	13.3	
1-6 drinks/week	32.4	33.0	33.2		32.9	32.3	33.5	
≥1 drink/day	10.6	10.7	10.1	0.73	10.5	10.5	10.8	0.40
Smoking status								
Never	51.3	51.3	50.2		50.4	51.4	51.6	
Past	37.2	37.4	38.5		38.0	37.2	37.1	
Current <15 cigarettes/day	4.3	4.1	3.6		4.5	4.0	4.0	
Current ≥15 cigarettes/day	7.2	7.2	7.7	0.52	7.1	7.4	7.3	0.44
Family history of MI prior to age 60 yrs								
No	78.4	78.2	78.9		78.7	78.5	77.8	
Yes	11.9	11.6	11.3		11.7	11.5	12.2	
Unknown	9.7	10.2	9.7	0.64	9.7	10.1	10.0	0.56

IQR: interquartile range.

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

* data are expressed as percentages unless otherwise stated.

[‡] p-values for chi-square test for categorical variables, and Kruskal-Wallis test for continuous variables.

Genotype and allele distribution for the *MTHFR* 677C>T and *ACE* D/I polymorphisms among women with and without migraine in the Women's Health Study (N=24,961)

Table 2

<i>MTHFR</i> 677C>T polymorphism						
Migraine status	Genotype frequencies			Allele frequencies		
	CC (11,228)	CT (10,956)	TT (2,777)	p-value*	C (33,412)	T (16,510)
No history of migraine	44.9 (9,173)	43.8 (8,939)	11.3 (2,312)		0.67 (27,285)	0.33 (13,563)
Any history of migraine	45.3 (2,055)	44.5 (2,017)	10.2 (465)	0.11	0.68 (6,127)	0.32 (2,947)
Migraine with aura	46.4 (589)	44.5 (565)	9.1 (116)	0.05	0.69 (1,743)	0.31 (797)
Migraine without aura	44.0 (842)	44.9 (861)	11.1 (213)	0.61	0.66 (2,545)	0.34 (1,287)
<i>ACE</i> D/I polymorphism						
Migraine status	Genotype frequencies			Allele frequencies		
	DD (7,312)	DI (11,503)	II (6,146)	p-value*	D (26,127)	I (23,795)
No history of migraine	29.4 (5,996)	45.9 (9,374)	24.7 (5,054)		0.52 (21,366)	0.48 (19,482)
Any history of migraine	29.0 (1,316)	46.9 (2,129)	24.1 (1,092)	0.43	0.52 (4,761)	0.48 (4,313)
Migraine with aura	28.8 (366)	47.7 (606)	23.5 (298)	0.41	0.53 (1,338)	0.47 (1,202)
Migraine without aura	29.9 (573)	47.1 (902)	23.0 (441)	0.24	0.53 (2,048)	0.47 (1,784)

Genotype frequencies are in %, allele frequencies in decimals. Numbers are in parentheses.

Frequencies for any history of migraine comprise those for migraine with and without aura as well as prior migraine.

* p-value from chi-square test (2df) comparing each of the migraine categories with women with no history of migraine.

† p-value from chi-square test (1df) comparing each of the migraine categories with women with no history of migraine.

Table 3

Age-adjusted odds ratios (OR) and 95% confidence intervals (95% CI) for migraine attack frequency according to *MTHFR* 677C>T polymorphism (n=24,961). Women with no history of migraine (n=20,424) serve as the reference group.

Attack frequency category	Migraine with aura			Migraine without aura		
	OR	95% CI	p-value	OR	95% CI	p-value
overall		(n=1,270)			(n=1,916)	
additive mode	0.92	0.85-1.00	0.06	1.02	0.95-1.09	0.65
dominant mode	0.94	0.84-1.06	0.31	1.04	0.95-1.14	0.42
recessive mode	0.79	0.65-0.96	0.02	0.98	0.84-1.14	0.77
≥weekly		(n=76)			(n=85)	
additive mode	1.17	0.84-1.63	0.35	0.96	0.70-1.33	0.81
dominant mode	1.40	0.88-2.23	0.16	1.01	0.66-1.55	0.97
recessive mode	0.92	0.44-1.92	0.82	0.81	0.39-1.69	0.58
monthly		(n=219)			(n=414)	
additive mode	0.82	0.67-1.01	0.06	1.01	0.87-1.16	0.95
dominant mode	0.82	0.63-1.07	0.15	1.06	0.87-1.29	0.57
recessive mode	0.66	0.40-1.08	0.10	0.88	0.64-1.22	0.45
every other month		(n=123)			(n=208)	
additive mode	0.97	0.74-1.27	0.82	1.18	0.97-1.44	0.10
dominant mode	0.94	0.66-1.35	0.75	1.18	0.89-1.56	0.25
recessive mode	1.01	0.58-1.76	0.99	1.37	0.93-2.01	0.11
<6 times/year		(n=852)			(n=1,209)	
additive mode	0.92	0.83-1.02	0.11	1.00	0.92-1.09	0.96
dominant mode	0.94	0.82-1.08	0.40	1.01	0.90-1.14	0.83
recessive mode	0.78	0.61-0.99	0.04	0.96	0.80-1.16	0.66

Table 4

Age-adjusted odds ratios (OR) and 95% confidence intervals (95% CI) for migraine and attack frequency according to ACE D/I polymorphism (n=24,961). Women with no history of migraine (n=20,424) serve as the reference group.

	Migraine with aura			Migraine without aura		
	OR	95% CI	p-value	OR	95% CI	p-value
overall		(n=1,270)			(n=1,916)	
additive mode	0.99	0.91-1.07	0.75	0.96	0.90-1.02	0.21
dominant mode	1.03	0.91-1.16	0.70	0.97	0.88-1.08	0.59
recessive mode	0.94	0.82-1.07	0.34	0.92	0.82-1.02	0.12
≥weekly		(n=76)			(n=85)	
additive mode	1.09	0.80-1.48	0.58	0.77	0.57-1.03	0.08
dominant mode	1.09	0.66-1.80	0.75	0.62	0.40-0.96	0.03
recessive mode	1.17	0.71-1.93	0.55	0.82	0.49-1.38	0.46
monthly		(n=219)			(n=414)	
additive mode	0.98	0.82-1.17	0.81	0.92	0.80-1.05	0.20
dominant mode	1.18	0.87-1.60	0.29	0.99	0.80-1.23	0.94
recessive mode	0.77	0.55-1.07	0.12	0.77	0.60-0.98	0.03
every other month		(n=123)			(n=208)	
additive mode	1.25	0.98-1.59	0.07	0.97	0.81-1.17	0.77
dominant mode	1.81	1.15-2.84	0.01	0.85	0.64-1.14	0.29
recessive mode	1.07	0.72-1.61	0.73	1.10	0.81-1.50	0.53
<every 6 months		(n=852)			(n=1,209)	
additive mode	0.95	0.86-1.04	0.27	0.99	0.91-1.07	0.78
dominant mode	0.92	0.79-1.07	0.27	1.02	0.90-1.16	0.73
recessive mode	0.94	0.80-1.11	0.48	0.94	0.82-1.08	0.41