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A Candidate Gene Association Study of 77 Polymorphisms in Migraine

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Abstract

Population-based studies have established an association between migraine and cardiovascular disease (CVD). We sought to investigate whether genetic variants implicated in CVD are associated with migraine. We performed an association study among 25,713 women, participating in the Women's Health Study, with information on 77 previously characterized polymorphisms. Migraine and migraine aura status were self-reported. We used logistic regression to investigate the genotype-migraine association. At baseline, 4,705 (18.3%) women reported history of migraine; 39.6% of the 3,306 women with active migraine indicated aura. Regarding any history of migraine, the multivariable-adjusted odds ratios (95% confidence intervals) for *TNF rs673* were 0.52 (0.30-0.89), for *TGFB1 rs1800469* 0.93 (0.89-0.98), and for *CCR2 rs1799864* 1.12 (1.03-1.21). Among active migraine with aura the odds ratios (95% confidence intervals) were 1.35 (1.0-1.81) for *TNF rs1800750*, 1.13 (1.02-1.26) for *TNF rs1800629*, and 1.22 (1.07-1.40) for *CCR2 rs1799864*; among active migraine without aura 0.9 (0.84-0.97) for *TGFB1 rs1800469*, 1.13 (1.01-1.27) for *NOS3 rs3918226*, and 1.12 (1.02-1.24) for *IL9 rs2069885*. After correction for multiple testing using the

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false discovery rate, none of the results remained significant. Our data suggest an association of polymorphisms implicated in inflammatory pathways and migraine in women. *TNF, CCR2, TGFB1, NOS3, and IL9* warrant further investigation.

Perspective—This article presents results from an association study of 77 polymorphisms, implicated in CVD, and migraine. Variants in *TNF, CCR2, TGFB1, NOS3, and IL9* were found to be associated with migraine, but did not remain significant after adjustment for multiple testing. Variations in these genes warrant further investigation.

Keywords

epidemiology; genetics of migraine; polymorphisms; cardiovascular disease

Introduction

Migraine is a common debilitating disorder characterized by recurrent headache attacks associated with autonomic symptoms. Some patients also experience transient neurologic symptoms known as migraine aura. Heredity plays an important role in migraine.[22] However, migraine etiology is complex, involving both multiple genetic and environmental factors.[22] Current pathophysiological concepts are based on the 'neurovascular hypothesis'.[21] Vascular dysfunctions are of particular interest since population-based studies have established an increased risk for cardiovascular events among patients with migraine, in particular migraine with aura.[13] In addition, pathophysiological mechanisms of atherosclerosis and cardiovascular disease (CVD), including hypercoagulability and endothelial dysfunction, have also been implicated in migraine and may explain the increased risk for CVD among migraineurs.[30]

Available association studies have investigated single or few genetic variants from physiological pathways involved in CVD. Among these variants are polymorphisms involved in endothelial dysfunction like the *MTHFR* 677C>T[26, 27, 31] and the *ACE* D/I[10, 15] polymorphisms, hypercoagulability,[9] vasoreactivity,[3, 33] inflammation,[18, 23, 24, 32] and lipid[7, 20] and glucose metabolism.[19] However, the results have been disappointing, because they have either not been replicated,[3, 19, 20, 32, 33] were negative[9, 24] or provided contradicting results.[7, 10, 15, 18, 20, 23, 26, 27, 31, 32] Possible explanations include a limited sample size in most of these studies and investigations being performed in clinic populations of different ethnic backgrounds.

The Women's Health Study (WHS) provides a unique opportunity to investigate the genetics of migraine. First, it consists of a clearly defined population of predominantly Caucasian women. Second, genetic information is available for over 27,000 participants. Third, more than 4,700 women with genetic information reported migraine. Although at the time of WHS initiation, no validated diagnostic migraine questionnaire was available for self-administration, our migraine diagnosis showed good agreement with the 1988 criteria of the International Headache Society (IHS)[13] and we have shown excellent agreement between self-reported migraine and ICHD-II based migraine classification in the WHS (Schürks 2008). We thus sought to investigate the association of 77 polymorphisms from 52 genes implicated in biological pathways of CVD, including inflammation, cell adhesion, coagulation, and platelet function with migraine in this large cohort of women.

Materials 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 among apparently healthy women.

The design, methods, and results have been described in detail previously.[25] Briefly, a total of 39,876 U.S. female health professionals aged ≥45 years at baseline in 1993 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, complete missing genotype information, and with reported CVD or angina prior to receiving the baseline questionnaire, a total of 27,203 women remained in the data set. We further excluded non-Caucasian women (n=1,490), leaving 25,713 Caucasian women for analyses.

Selection of candidate genes and polymorphisms

We investigated the association between 77 previously known polymorphisms in 52 candidate genes and migraine. The findings for one of these polymorphisms has been published previously.[27] The candidate genes were selected for previous studies[4, 34] from biochemical pathways that have been implicated in the development and progression of CVD and the panel of polymorphisms were part of a validated Roche proprietary linear array assay.[4, 34] In addition, in these previous studies, the polymorphisms were chosen based on prior evidence of potential functionality, validated allele frequency and heterozygosity, and sequence-proven allelic variation. They focused broadly on the atherosclerotic pathway, including genes involved in inflammation, cell adhesion, coagulation, and platelet function.

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 order to reduce potential recall-bias, only participants who reported active migraine were asked further details about their migraine attacks. In a previous study,[13] we have shown good agreement of our classification with the 1988 International Headache Society (IHS) criteria for migraine[8] and we have shown excellent agreement between self-reported migraine and ICHD-II based migraine classification in the WHS (Schürks 2008). 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 women who reported active migraine into "active migraine with aura" and "active migraine without aura".

Genotype determination

Genotyping was performed in the context of a multi-marker assay using an immobilized probe approach, as previously described (Roche Molecular Systems).[4] 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. The average genotype completion rate per polymorphism was ≥95%.

Statistics

We compared baseline characteristics of participants with respect to their migraine status using the chi-square test for categorical variables and the Wilcoxon test for continuous variables.

We calculated allele frequencies and performed a Hardy–Weinberg equilibrium test using the Fisher exact test statistics.

We used logistic regression models to evaluate the association between polymorphisms and migraine. We calculated odds ratios (ORs) and 95% confidence intervals (CIs). We built additive models only, which assume that the risk for carriers of the heterozygous genotype for developing the outcome is half way between carriers of the homozygous genotypes. The advantage is that the strength of genotype-phenotype association is expressed in a single parameter (beta estimate) and statistical tests have only one degree of freedom.[5] For each polymorphism the most frequent genotype, as determined from the genotype distribution among women without migraine, was used as the reference.

We built age-adjusted and multivariable-adjusted models. Given the similar results we only present multivariable-adjusted models. The multivariable-adjusted models included the following covariates: age (continuous), body mass index (continuous), exercise (never, less than once/week, 1-3 times/week, 4 or more times/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), family history of premature myocardial infarction (yes, no), and randomized aspirin assignment (yes, no). We incorporated a missing value indicator if the number of women with missing information on covariates was ≥100 or imputed a value otherwise.

Our main outcome variable was “any history of migraine.” We also investigated “active migraine with aura” and “active migraine without aura” separately.

In further exploratory analyses we investigated the interaction between polymorphisms that were significantly associated with our main outcome variable “any history of migraine” and the covariates mentioned above by including an interaction term into the model. Only for this analysis did we categorize age (<55 years, ≥55 years) and body mass index (≤ 25 kg/m^2, 25-<30 kg/m^2, 30-<35 kg/m^2, ≥35 kg/m^2).

All analyses were performed using SAS version 9.1 (SAS Institute Inc, Cary, NC). To adjust for multiple hypothesis testing, we applied the false discovery rate (FDR).[2] We considered an FDR threshold of 0.20 as previously suggested.[29]

Results

At baseline 4,705 (18.3%) women reported any history of migraine. Of these, 3,306 (70.3%) reported active migraine and 1,399 prior migraine. Among active migraineurs 1,309 (39.6%) had migraine with aura and 1,997 (60.4%) migraine without aura. No history of migraine was reported by 21,008 women.

The baseline characteristics of women according to migraine status are summarized in Table 1. Women with any history of migraine were younger, less likely to have a history of diabetes, less physically active, less likely to consume alcohol, and less likely to smoke compared to

women without migraine. In contrast women with migraine had a slightly higher body mass index. Further, they more frequently had a history of hypertension, used postmenopausal hormone replacement therapy, had a history of oral contraceptive use, and had a family history of premature myocardial infarction.

Table 2 summarizes the minor allele frequencies and p-values from the Fisher's exact test for the Hardy Weinberg-Equilibrium for each of the 77 investigated polymorphisms. Six of the polymorphisms were not in Hardy Weinberg-Equilibrium based on a Bonferroni corrected p-value threshold of 0.00065 (*ACE rs1799752, FGB rs1800790, TNF rs673, MS4A2 rs569108, CCR5 rs333, CSF2 rs25882*, all $p<0.0001$).

Three of the 77 polymorphisms were significantly associated with our primary outcome any history of migraine at the p=0.05 level (Table 3). The OR (95% CI; p-value) was 0.52 (0.30-0.89; p=0.017) for *TNF rs673*, 0.93 (0.89-0.98; p=0.009) for *TGFB1 rs1800469*, and 1.12 (1.03-1.21; p=0.007) for *CCR2 rs1799864*. After correction for multiple testing the FDR values were 0.43 (*TNF rs673*), 0.35 (*TGFB1 rs1800469*), and 0.35 (*CCR2 rs1799864*). This pattern remained unchanged for active migraine with and without aura. When we only looked at active migraine with and without aura three polymorphisms were associated with each subgroup (Table 3). For active migraine with aura the odds ratios (95% confidence intervals; p-value) were 1.35 (1.0-1.81; p=0.049) for *TNF rs1800750*, 1.13 (1.02-1.26; p=0.018) for *TNF rs1800629*, and 1.22 (1.07-1.40; p=0.004) for *CCR2 rs1799864*. The FDR values were 0.87 (*TNF rs1800750*), 0.69 (*TNF rs1800629*), and 0.31 (*CCR2 rs1799864*). For active migraine without aura the OR (95% CI; p-value) for *TGFB1 rs1800469* was 0.9 (0.84-0.97; p=0.0076), for *NOS3 rs3918226* 1.13 (1.01-1.27; p=0.04), and for *IL9 rs2069885* 1.12 (1.02-1.24; p=0.02). (Table 3). The FDR value was 0.58 (*TGFB1 rs1800469*), 0.85 (*NOS3 rs3918226*), and 0.81 (*IL9 rs2069885*).

In summary, *TNF rs673* was suggestive of a reduced risk for any history of migraine, but was not associated with any of the subgroups. Two polymorphisms (*TNF rs1800750, TNF rs1800629*) were associated with an increased risk for active migraine with aura, and two polymorphisms (*NOS3 rs3918226, IL9 rs2069885*) with an increased risk for active migraine without aura. In addition, two polymorphisms (*TGFB1 rs1800469, CCR2 rs1799864*) were associated with any history of migraine and one of the subgroups. *TGFB1 rs1800469* appeared to reduce the risk for any history of migraine, a pattern that also occurred for active migraine without aura. In contrast, *CCR2 rs1799864* appeared to raise the risk for any history of migraine, a pattern that was slightly more pronounced for active migraine with aura. However, none of the polymorphisms remained significantly associated when applying the FDR value.

None of the interaction terms between polymorphisms associated with the main outcome any history of migraine (*TNF rs673, TGFB1 rs1800469, CCR2 rs1799864*) and any of the covariates investigated was statistically significant.

Discussion

Data from this large cohort of Caucasian women suggest an association between three (*TNF rs673, TGFB1 rs1800469, CCR2 rs1799864*) of the 77 polymorphisms and our main outcome any history of migraine. Furthermore, three of the polymorphisms were associated with active migraine with aura (*TNF rs1800750, TNF rs1800629, CCR2 rs1799864*) and active migraine without aura (*TGFB1 rs1800469, NOS3 rs3918226, IL9 rs2069885*). However, after correction for multiple testing none of the associations remained significant.

Our results suggest that genetic variants in inflammatory pathways (*TNF, CCR2, TGFB1, IL9*) and vasoreactivity (*NOS3*) may be associated with migraine. Based on the present pathophysiological model of migraine these findings are plausible.

Tumor necrosis factor (TNF) is a proinflammatory cytokine secreted predominantly by monocytes/macrophages with effects on lipid metabolism, coagulation, insulin resistance, and endothelial function. The A allele of the *TNF rs1800629* is associated with increased TNF-α levels and elevated TNF-α levels have been reported in 20 patients affected by migraine without aura when compared with 17 patients with chronic tension type headache.[6] Our results suggest that *TNF rs673* reduces the risk for the main outcome "any history of migraine," while *TNF rs1800750, TNF rs1800629* appeared to increase the risk for active migraine with aura. These results should be considered with caution, since *rs1800750* and *rs673* had allele frequencies lower than 5%, which may result in spurious associations. Previous studies on the association between the TNF −308G>A polymorphism (*rs1800629*) and migraine provided contradicting results. One study did not find an association,[32] a second reported an increased risk for carriers of the G allele,[23] and a third showed an increased risk among carriers of the A allele.[18] This may be due to the small sample size in the first study[32] and the different ethnic populations in the other two.[18, 23]

Chemokine receptor 2 (CCR2) is the receptor for the monocyte chemoattractant protein-1, which is produced by endothelial cells, smooth muscle cells, and macrophages in response to various mediators, including TNF-α, and is involved in inflammatory processes and chronic pain.[1] Transforming growth factor beta (TGFB) controls proliferation, differentiation, and inflammatory processes in many cell types. The -509C>T polymorphism (*rs1800469*) of the TGFB1 gene results in increased plasma levels of TGF-beta-1.[28] In addition, pathways involving TGFB1 signalling are crucial in regulating inflammation in the central nervous system.[17] In our cohort *CCR2 rs1799864* appeared to increase the risk for migraine, a pattern more pronounced for active migraine with aura, while the reduced risk of *TGFB1 rs1800469* for migraine was more pronounced for active migraine without aura.

Interleukin-9 (IL9) is a cytokine that serves as a regulator of lymphoid and myeloid systems and nitric oxide synthase (NOS) is an endothelial enzyme synthesizing NO. *IL9 rs2069885* and *NOS3 rs3918226* were suggestive of a reduced risk for active migraine without aura. In contrast to a previous report[3] one NOS3 polymorphism (rs1799983) was not associated with migraine. We are not aware of prior studies investigating the polymorphisms *CCR2 rs1799864, TGFB1 rs1800469, IL9 rs2069885, and NOS3 rs3918226* with regard to migraine.

Association studies investigating polymorphisms involved in endothelial dysfunction like *MTHFR* 677C>T[26, 27, 31] and *ACE* D/I[10, 15] have been conflicting. Among the reasons may be limited sample sizes, predominantly clinic populations, and different ethnic backgrounds. Both polymorphisms were not associated with migraine or aura status in our study. We have recently reported a protective association between the *MTHFR* TT genotype and migraine in the WHS,[27] however, this apparent discrepancy is due to the different genetic models employed in the former and in the present study. Studies investigating polymorphisms in pathways implicated in coagulation[9] and inflammation[24] were mostly negative. In accordance with these studies we did not find an association of polymorphisms in the genes coding for Factor II (rs1799963), Factor V (rs6025), Factor VII promoter (rs5742910), and Interleukin 6 (rs1800795) with migraine. Other studies focusing on vasoreactivity,[33] lipid[7, 20] or glucose metabolism[19] were not replicated[19, 33] or contradictory.[7, 20] These polymorphisms were not represented in the panel available for our study.

Our study has several strengths, including the large number of participants, detailed information on many potential CVD risk factors, and the vast number of polymorphisms implicated in CVD on our panel. In addition, the homogenous nature of the cohort, consisting only of white Caucasian women, may reduce confounding. However, several limitations should be considered. First, migraine and aura status were self-reported and were not classified according to strict IHS criteria. Thus, non-differential misclassification is possible, which may

in part explain some of our null findings. However, the prevalence of migraine (18.3%) and of migraine aura (39.5%) is very similar to those seen in other large population-based studies in the U.S.[16] and the Netherlands,[14] although the age distribution was somewhat different in these studies. The 1-year prevalence of migraine for women was 18.2% in the U.S. and 25% in the Netherlands, while migraine aura was reported by 37% in the U.S.[16] and 31% in the Netherlands.[14] Furthermore, our migraine classification showed good agreement with the 1988 IHS criteria[13] and there also is excellent agreement between self-reported migraine and ICHD-II based migraine classification in the WHS (Schürks 2008). Second, the genotype distribution for six of the investigated polymorphisms deviated from Hardy-Weinberg Equilibrium after Bonferroni correction. Genotyping error is unlikely given our stringent genotyping protocol. However, this stringent protocol, the large sample size, and the fact that participants do not represent all white women, most likely accounts for the deviation from Hardy-Weinberg Equilibrium. Third, since we only looked at white female health professionals age ≥45, generalizability may be limited. While there is no reason to believe, that migraine pathophysiology is different between women and men, the phenotypic expression among migraineurs may differ by gender and age. For example, this is suggested by data showing that the deep white matter lesion load is only increased among women, but not men,[11] and that the migraine-ischemic stroke association is greater among younger women than older women, which may depend on a changing pattern in cardiovascular risk profile.[12] Fourth, we only considered an additive model of transmission. This model would also have sufficient power to capture dominant modes of transmission given our sample size. While recessive modes of transmission may be missed, we consider this a minor disadvantage because (i) recessive modes of transmission are rare, (ii) mostly relevant for monogenic disorders, and (iii) because of potential loss of power due to very low minor allele frequencies. Finally, we cannot exclude that examination of different polymorphisms–not in linkage disequilibrium with the variants tested–might lead to a different result.

None of the polymorphisms we identified remained significant after correction for multiple testing using FDR. Thus, we did not identify a clear association of genetic variants from pathways implicated in CVD with migraine. However, based on some relatively low p-values, an association is possible, thus the *TNF, CCR2, TGFRB1, IL9, and NOS3* genes warrant further investigation. Further, the odds ratios are of small effect size. This is in line with the notion that migraine is a complex and heterogeneous disorder. Moreover, the genes and polymorphisms investigated here only represent a fraction of all genes and variants that are relevant for CVD. In addition, many other pathways for example involving serotonin and dopamine are important in migraine pathophysiology and deserve further investigation.

Our results may suggest the following for future studies: First, biological pathways implicated in migraine need to be more comprehensively addressed by methods capturing genetic variants more densely, ideally using a whole genome scan. Second, large well-defined cohorts are needed with standardized information on migraine and aura status, gender, ethnicity, risk factors, and other medical conditions. Finally, gene-gene and gene-environment interactions need to be explored.

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Table 1

Baseline characteristics of participants in the Women's Health Study according to migraine status (N=25,713)*

Characteristic	No migraine (N=21,008)	Any migraine (N=4,705)	p-value
Age, mean (SD), y	55.0 (7.2)	53.7 (6.5)	<0.0001
Body mass index, mean (SD), kg/m^2	25.9 (4.9)	26.1 (5.1)	0.02
History of diabetes	2.3	1.8	0.04
History of hypertension	24.4	25.7	0.06
Physical activity			
Never	37.0	38.2	
<1/week	19.2	21.5	
1-3/week	32.2	30.0	
≥4/wk	11.7	10.3	<0.0001
Postmenopausal hormone therapy			
Never	49.1	44.6	
Past	9.0	9.3	
Current	41.9	46.2	<0.0001
History of oral contraceptive use			
No	31.2	25.0	
Yes	68.4	74.4	
Not sure	0.4	0.6	<0.0001
Alcohol consumption			
Rarely/never	42.7	46.5	
1-3 drinks/month	13.0	14.3	
1-6 drinks/week	33.1	30.9	
≥1 drink/day	11.2	8.4	<0.0001
Smoking status			
Never	50.7	53.4	
Past	37.8	35.8	
Current <15 cigarettes/day	4.2	3.9	
Current ≥15 cigarettes/day	7.4	7.0	0.01
Family history of MI prior to age 60 yrs			
No	78.6	77.4	
Yes	11.5	12.8	
Unknown	10.0	9.8	0.04

*data are expressed as percentages unless otherwise stated.

†p-values for chi-square test for categorical variables, and Wilcoxon test for continuous variables.

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

Table 2

Minor allele frequencies and Hardy Weinberg-Equilibrium p-values for the genetic polymorphisms among women without migraine in the Women's Health Study (N=21,008)

Gene	*Symbol*	Polymorphism	Chromosome	MAF	HWE*
Adducin 1 (alpha)	*ADD1*	*rs4961*	4p16.3	0.194	0.473
Angiotensin II receptor type 1	*AGTR1*	*rs5186*	3q21-25	0.304	0.7485
Angiotensinogen	*AGT*	*rs699*	1q42-43	0.416	0.411
Angiotensin-converting enzyme 1	*ACE*	*rs1799752*	17q23	0.477	<0.0001
Beta-2 adrenergic receptor	*ADRB2*	*rs1042713*	5q31-32	0.369	0.51
		rs1042714		0.435	0.1425
		rs1800888		0.014	0.0205
Beta-3 guanine nucleotide-binding protein	*GNB3*	*rs5443*	12p13	0.306	0.479
CD14 molecule	CD14	*rs2569190*	5q31.1	0.475	0.275
Chemokine receptor 2	*CCR2*	*rs1799864*	3p21	0.086	0.289
Chemokine receptor 3	*CCR3*	*rs5742906*	3p21.3	0.003	1
Chemokine receptor 5	*CCR5*	*rs333*	3p21	0.104	<0.0001
		rs1799987		0.446	0.6105
Chemokine ligand 11	*CCL11*	*rs3744508*	7q21.1-21.2	0.176	0.2275
		rs4795895		0.189	0.8865
Chemokine ligand 12	*CXCL12*	*rs1801157*	10q11.1	0.197	0.559
Coagulation factor II	*F2*	*rs1799963*	11p11-q12	0.014	0.015
Coagulation factor V	*F5*	*rs6025*	1q23	0.027	0.5075
Coagulation factor VII	*F7*	*rs5742910*	13q34	0.119	0.9705
		rs6046		0.109	0.917
Colony stimulating factor 2	*CSF2*	*rs25882*	5q31.1	0.199	<0.0001
Complement component 3	*C3*	*rs2230199*	19p13.3-p13.2	0.209	0.0045
Complement component 5	*C5*	*rs17611*	9q33-q34	0.438	0.1615
Cystathionine-beta-synthase	*CBS*	*rs12329790*	21q22.3	0.002	1
Cytotoxic T-lymphocyte-associated protein 4	*CTLA4*	*rs5742909*	2q33	0.094	0.9625
		rs231775		0.381	0.882
Fibrinogen beta	*FGB*	*rs1800790*	4q28	0.213	<0.0001
Group-specific component (vitamin D binding protein)	*GC*	*rs7041*	4q12-q13	0.437	0.0705
		rs4588		0.284	0.1325

Gene	*Symbol*	Polymorphism	Chromosome	MAF	HWE*
Integrin, alpha 2	*ITGA2*	*rs1062535*	5q23-31	0.396	0.7
Integrin, beta3	*ITGB3*	*rs5918*	17q21.32	0.153	0.184
Intercellular adhesion molecule 1	*ICAM1*	*rs5491*	19p13.3-13.2	0.003	1
		rs1799969		0.116	0.918
Interleukin 1 alpha	*IL1A*	*rs1800587*	2q14	0.299	0.1815
Interleukin 1 beta	*IL1B*	*rs16944*	2q14	0.330	0.8035
		rs1143634		0.232	0.8025
Interleukin 4	*IL4*	*rs2243250*	5q31.1	0.151	0.4905
Interleukin 4 receptor	*IL4R*	*rs1805010*	16p11.2-12.1	0.450	0.9665
		rs1805015		0.164	0.485
		rs1801275		0.207	0.6415
Interleukin 5 receptor, alpha	*IL5RA*	*rs2290608*	3p26-24	0.256	0.6535
Interleukin 6	*IL6*	*rs1800796*	7p21	0.053	0.155
		rs1800795		0.422	0.358
Interleukin 9	*IL9*	*rs2069885*	5q31.1	0.133	0.4655
Interleukin 10	*IL10*	*rs1800872*	1q31-32	0.230	0.454
Interleukin 13	*IL13*	*rs1295686*	5q31	0.203	0.6385
Leukotriene C4 synthase	*LTC4S*	*rs730012*	5q35	0.285	0.87
Lymphotoxin alpha	*LTA*	*rs1041981*	6p21.3	0.343	0.157
		rs909253		0.337	0.5555
Matrix metallopeptidase 3	*MMP3*	*rs3025058*	11q22.3	0.489	0.008
Membrane-spanning 4-domains, subfamily A, member 2	*MS4A2*	*rs569108*	11q13	0.026	<0.0001
5,10-methylenetetrahydrofolate reductase	*MTHFR*	*rs1801133*	1p36.3	0.332	0.0465
Natriuretic peptide precursor A	*NPPA*	*rs5063*	1p36.21	0.050	0.2285
		rs5065		0.147	0.7865
Nitric oxide synthase 2A	*NOS2A*	*rs1137933*	17q11.2-q12	0.224	0.4825
Nitric oxide synthase 3	*NOS3*	*rs1800779*	7q36	0.374	0.098
		rs3918226		0.081	0.33
		rs1799983		0.327	0.651
Secretoglobin, family 1A, member 1	*SCGB1A1*	*rs3741240*	11q12.3-13.1	0.351	0.278
Selectin E	*SELE*	*rs5361*	1q22-25	0.105	0.4835
		rs5355		0.041	0.9235

Gene	*Symbol*	Polymorphism	Chromosome	MAF	HWE*
Selectin P	*SELP*	*rs6131*	1q22-25	0.187	0.1585
		rs6133		0.117	0.399
Serpin peptidase inhibitor, clade E, member 1	*SERPINE1*	*rs1799768*	7q21.3-22	0.457	0.28
		rs7242		0.437	1
Sodium channel, nonvoltage-gated 1 alpha	*SCNN1A*	*rs5742912*	12p13	0.023	0.8805
		rs2228576		0.331	0.5065
Transcription factor 7	*TCF7*	*rs5742913*	5q31.1	0.105	0.388
		rs244656		0.136	0.36
Transforming growth factor, beta 1	*TGFB1*	*rs1800469*	19q13.1	0.304	0.5275
Tumor necrosis factor	*TNF*	*rs1800750*	6p21.3	0.014	0.001
		rs1800629		0.173	0.556
		rs673		0.003	<0.0001
		rs361525		0.051	0.005
Vascular cell adhesion molecule 1	*VCAM1*	*rs1041163*	1p32-31	0.160	0.9415
Vitamin D (1,25-dihydroxyvitamin D3) receptor	*VDR*	*rs2228570*	12q13.11	0.388	0.284
		rs1544410		0.406	0.604

MAF: minor allele frequencies in decimals.

HWE: p-value from Fisher's Exact test for Hardy Weinberg-Equilibrium

Table 3

Multivariable-adjusted odds ratios for migraine according to polymorphisms with p-values <0.05 in the Women' Health Study (n=25,713) assuming an additive model

Polymorphism	Any history of migraine N=4,705				Active migraine with aura N=1,309				Active migraine without aura N=1,997			
	OR	**95% CI**	***p*-value**	**FDR**	**OR**	**95% CI**	***p*-value**	**FDR**	**OR**	**95% CI**	***p*-value**	**FDR**
NOS3 rs3918226	1.02	0.94-1.11	0.58	0.94	1.00	0.86-1.16	0.98	1.0	**1.13**	**1.01-1.27**	**0.04**	0.85
IL9 rs2069885	1.01	0.94-1.08	0.88	0.94	0.95	0.84-1.08	0.46	1.0	**1.12**	**1.02-1.24**	**0.02**	0.81
TNF rs1800750	1.13	0.94-1.36	0.19	0.94	**1.35**	**1.00-1.81**	**0.049**	0.87	0.920	0.69-1.23	0.57	0.92
TNF rs1800629	1.02	0.96-1.09	0.46	0.94	**1.13**	**1.02-1.26**	**0.018**	0.69	0.967	0.89-1.06	0.46	0.92
TNF rs673	**0.52**	**0.30-0.89**	**0.017**	0.43	0.396	0.13-1.23	0.11	1.0	0.588	0.28-1.24	0.16	0.92
TGFB1 rs1800469	**0.93**	**0.89-0.98**	**0.009**	0.35	0.954	0.87-1.04	0.31	1.0	**0.90**	**0.84-0.97**	**0.0076**	0.58
CCR2 rs1799864	**1.12**	**1.03-1.21**	**0.007**	0.35	**1.22**	**1.07-1.40**	**0.004**	0.31	1.117	0.99-1.25	0.06	0.85