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Polymorphisms in the Renin-Angiotensin System and Migraine in Women

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Abstract

Background—Recent findings suggest an association between the renin-angiotensin system and migraine. However, genetic studies are scarce and controversial.

Objective—To investigate the association between the *AGTR1* 1166A>C and *AGT* Met235Thr polymorphisms with migraine and migraine aura status.

Methods—We performed an association study among 25,000 Caucasian U.S. women, participating in the Women's Health Study, with information on the *AGTR1* 1166A>C and *AGT* Met235Thr polymorphisms. Migraine and migraine aura status were self-reported. We distinguished between any history of migraine, active migraine with aura, active migraine without aura, and prior migraine (history of migraine, but not in the year prior to baseline). We used logistic regression to investigate the genotype-migraine association.

Results—At baseline, 4,577 (18.3%) women reported any history of migraine; 39.5% of the 3,226 women with active migraine indicated aura. The polymorphisms were not associated with migraine

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Full Disclosures

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or migraine specific subgroups. We also did not find a significant interaction between the polymorphisms.

Conclusions—Data from this large cohort of Caucasian women do not suggest an association of polymorphisms in the renin-angiotensin system with migraine or aura status. Future studies should focus on haplotype analyses and additional gene-gene as well as gene-environment interactions.

Keywords

migraine; renin-angiotensin system; polymorphism; women

Introduction

Migraine is a very common debilitating disorder characterized by recurrent headache attacks, usually of unilateral location, pulsating quality, moderate to severe intensity, and aggravated by routine physical activity.¹ In addition, migraine attacks are typically associated with nausea and/or vomiting as well as photo- and phonophobia.¹ Some patients experience transient neurologic symptoms prior to or during the attacks that are known as migraine aura. Heredity plays an important role in migraine.^{2, 3} However, migraine etiology is complex, involving both multiple genetic and environmental factors.³ Current pathophysiological concepts are based on the 'neurovascular hypothesis'.⁴

The ubiquitous renin-angiotensin system (RAS) is involved in complex physiological processes affecting both cardiovascular⁵ and neuronal functions.⁶ In the RAS, renin converts angiotensinogen to angiotensin I, which is then turned into angiotensin II by the angiotensin-converting enzyme (ACE). Angiotensin II mediates most physiological functions of the RAS via the type I angiotensin receptors (AT1 receptors).⁷

Recent findings suggest that the RAS plays an important role in migraine pathophysiology.^{8, 9} First, there is some evidence suggesting that ACE inhibitors^{10, 11} and AT1 receptor blockers^{12, 13} may be effective in migraine prophylaxis. Second, some studies have associated the *ACED/I* polymorphism with overall migraine,¹⁴⁻¹⁶ migraine with^{15, 17} and without aura.¹⁸ However, we could not confirm these findings (unpublished data). Third, the Met235Thr polymorphism in the angiotensinogen gene (*AGT*; rs699) controls angiotensinogen levels,¹⁹ while the 1166A>C polymorphism in the gene coding for the AT1 receptor (*AGTR1*; rs5186) appears to control AT1 receptor levels.²⁰ Thus both gene variants modulate the RAS. Although variants in the genes coding for angiotensinogen and AT1 receptors, including the Met235Thr polymorphism and the 1166A>C polymorphism, have also been shown to impact various pathophysiological processes,²¹ none of these variants has been investigated with regard to migraine pathophysiology. Thus, the relevance of the RAS and its functional genetic variants in migraine pathophysiology remains to be established.

We sought to investigate the association of the 1166A>C polymorphism in the gene coding for the AT1 receptor (*AGTR1*; rs5186) and the Met235Thr polymorphism in the angiotensinogen gene (*AGT*; rs699) with migraine and migraine aura status in a large cohort of 25,000 Caucasian women participating in the Women's Health Study (WHS).

Subjects and Methods

Study population

The WHS was a randomized, placebo-controlled 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 among apparently healthy women. The design, methods, and results have been described in detail previously.^{22, 23} 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, *AGTR1* 1166A>C and *AGT* Met235Thr polymorphisms, and with reported CVD or angina prior to receiving the baseline questionnaire, a total of 26,428 women remained in the data set. We further excluded non-Caucasian women ($n=1,428$) to avoid race-specific genetic interaction, leaving 25,000 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 baseline questionnaire. To reduce the possibility of recall bias, only participants who reported active migraine were asked details about their migraine attacks, including attack duration of 4 to 72 hours; unilateral location of pain; pulsating quality; inhibition of daily activities; aggravation by routine physical activity; nausea or vomiting; sensitivity to light; and sensitivity to sound. In previous studies of the WHS²⁴, 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 women who reported active migraine into “active migraine with aura” and “active migraine without aura”. Distinguishing between prior migraine and active migraine in the analysis allowed us to answer two additional questions: First, whether there is a differential impact of the investigated polymorphisms on women whose migraine has stopped and women with ongoing migraine. Second, we only had information about migraine aura status for women with migraine in the past year (active migraine). Thus, we could only investigate a possible differential impact of the aura status on the gene-migraine association among women with active migraine.

Genotype determination of the *AGTR1* 1166A>C (rs5186) and *AGT* Met235Thr (rs699) polymorphisms

Genotyping was performed in the context of a multi-marker assay using an immobilized probe approach, as previously described (Roche Molecular Systems).²⁶ 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 *AGTR1* 1166A>C and *AGT* Met235Thr genotypes using the chi-square test for categorical variables and the Kruskal-Wallis test for continuous variables. Genotype and allele frequencies were compared according to migraine and migraine aura status.

We used logistic regression models to evaluate the association between *AGTR1* 1166A>C and *AGT* Met235Thr polymorphisms and migraine. We calculated odds ratios (OR) and 95% confidence intervals (CI) for 1) any history of migraine, 2) active migraine with aura, 3) active migraine without aura, and 4) prior migraine. We built age-adjusted and multivariable-adjusted models. In the multivariable-adjusted model, we considered 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), and family history of myocardial infarction (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.

We built additive models to investigate the polymorphism-migraine association. This model assumes that the risk for carriers of the heterozygous genotype for developing the outcome is half way between carriers of the homozygous genotypes. The advantage of such a model is that the strength of genotype-phenotype association is expressed in a single parameter (beta estimate) and statistical tests for detecting a relationship have only one degree of freedom.²⁷ We also checked for deviation from additivity by adding a ‘dominance’ variable to the model (extended model). We compared the overall fit of the additive and the extended model by comparing the $-2 \log L$ of both models.

We also explored interactions between the polymorphisms by including variables for both polymorphisms and adding an interaction term to the model.

All analyses were performed using SAS version 9.1 (SAS Institute Inc, Cary, NC). All p-values were two-tailed and we considered $p < 0.01$ as significant.

Results

The baseline characteristics of women according to *AGTR1* 1166A>C and *AGT* Met235Thr genotype are summarized in Table 1. Age, body mass index, and history of diabetes were equally distributed among genotypes. Women also did not differ regarding physical activity, history of oral contraceptive use, alcohol consumption, smoking habits, and family history of myocardial infarction. With regard to the *AGTR1* 1166A>C polymorphism women harboring the AA and CC genotypes were more likely to have never used postmenopausal hormone therapy compared to carriers of the AC genotype. Women with the TT genotype of the *AGT* Met235Thr polymorphism less frequently had a history of hypertension compared to the TC/CC genotypes.

At baseline 4,577 (18.3%) women reported any history of migraine. Of the 3,226 women who reported active migraine, 1,275 (39.5%) indicated migraine aura. The observed genotype distribution for the *AGTR1* 1166A>C and the *AGT* Met235Thr polymorphisms were in Hardy-Weinberg equilibrium among women with no history of migraine (chi-square with 1 degree of freedom: $p = 0.76$ and $p = 0.39$). There was no difference in the genotype and allele distribution

for both *AGTR1* 1166A>C and *AGT* Met235Thr between women with and without migraine (Table 2).

Results from the logistic regression analysis showed no association between the *AGTR1* 1166A>C or the *AGT* Met235Thr polymorphism and any history of migraine (Table 3). The age-adjusted odds ratio in the additive mode was 0.99 (95% CI=0.94–1.04; $p=0.68$) for *AGTR1* 1166A>C and 1.00 (95% CI=0.95–1.05; $p=0.92$) for *AGT* Met235Thr. Controlling for multiple covariates and investigating migraine specific subgroups did not change the results. There was no indication for deviation from the additivity assumption. In addition, our results do not suggest significant interaction between the two polymorphisms (all p for interaction >0.1).

Discussion

In this large study of Caucasian women we did not find an association of the *AGTR1* 1166A>C and the *AGT* Met235Thr polymorphisms with migraine or migraine aura status.

Several findings have suggested an association of the RAS in migraine pathophysiology. Such a link should be interpreted in the context of the association between migraine and vascular disorders.²⁸ For example, population based studies have reported an association between migraine, in particular migraine with aura, and CVD²⁴ and the RAS plays an important role in the pathophysiology of CVD.²¹ In addition, clinical trials have suggested that ACE inhibitors^{10, 11} and AT1 receptor blockers^{12, 13} may be effective in migraine prophylaxis. However, like with other migraine preventives only about 50% of patients benefit. This may indicate genetic heterogeneity of migraine, but also suggests a differential medication effect according to variants in genes coding for proteins in the RAS. In this regard, we have recently shown that effect modification by genotype status can be of importance in the association between migraine and certain outcomes.²⁹ Furthermore, the *ACE* D/I polymorphism has been associated with overall migraine,^{14–16} migraine with^{15, 17} and without aura.¹⁸ Limitations of these studies include that they were performed in clinic-based or clinic recruited populations, thus limiting generalizability. We could not confirm an association of the *ACE* D/I polymorphism with migraine in a large cohort of women participating in the WHS (unpublished data). However, other functionally relevant polymorphisms in the RAS have not been investigated so far. Among these are the *AGT* Met235Thr polymorphism controlling angiotensinogen levels,¹⁹ and the *AGTR1* 1166A>C polymorphism controlling AT1 receptor levels.²⁰ These gene variants have been shown to impact various pathophysiological processes.²¹ In the present study we did not find evidence for an association of these two genetic variants in the RAS with migraine and migraine aura.

Our study has several strengths, including the large number of participants with and without migraine and the homogenous nature of the cohort, which 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. However, the prevalence of migraine (18.3%) and the prevalence of migraine aura (39.5%) is very similar to those seen in other large population-based studies in the U.S.³⁰ and the Netherlands.³¹ Furthermore, our migraine ascertainment allowed us to classify migraine according to IHS criteria, which showed good agreement.²⁴ Second, participants were all white female health professionals age ≥ 45 , thus generalizability to other ethnicities and men may be limited. However, given the genetic heterogeneity in migraine, investigating well-defined populations free of admixture will facilitate detecting population-specific patterns of association. Third, we only investigated single nucleotide polymorphisms (SNPs) in two genes, accounting for only a part of the genetic variability in these genes. However, we only had information on the SNPs presented here. In addition, these two SNPs have been shown to be

important markers of various pathophysiological processes.²¹ 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.

In order to characterize the association between the RAS and migraine in more detail, future studies should focus on the following. First, the genetic variability of RAS genes needs to be captured more comprehensively. Methods involved are haplotype analyses using tag-SNPs and investigation of copy number variations of genes, which account for a large amount of genetic variability and have been associated with various disorders.³² This may also be important in migraine. Second, gene-gene and gene-environment interactions need to be considered, which may determine in part differences in incidence, prevalence, clinical features and medication response to ACE inhibitors and AT1 receptor blockers among different populations or individual migraine patients.

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Abbreviations

RAS, renin-angiotensin system; AT1, angiotensin 1; AGTR1, gene coding for the AT1 receptor; AGT, gene coding for angiotensinogen; WHS, Women's Health Study; IHS, International Headache Society.

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Table 1
Baseline characteristics of participants in the Women's Health Study according to *AGTR1* 166C>C and *AGT* Met235Thr genotypes (N=25,000)*

Characteristic	<i>AGTR1</i> 166C>C genotype				<i>AGT</i> Met235Thr genotype			
	AA (n=12,126)	AC (n=10,580)	CC (n=2,294)	P value	TT (n=8,534)	TC (n=12,122)	CC (n=4,344)	P value
Age, mean (SD), y	54.7 (7.1)	54.8 (7.2)	54.6 (7.0)	0.97	54.8 (7.2)	54.7 (7.1)	54.7 (7.1)	0.68
Body mass index, mean (SD), kg/m ²	26.0 (5.0)	25.8 (4.9)	25.9 (5.0)	0.21	25.9 (5.0)	25.9 (5.0)	25.8 (4.9)	0.80
History of diabetes	2.1	2.3	2.2	0.47	1.9	2.3	2.5	0.09
History of hypertension	24.9	24.5	24.0	0.65	23.5	25.1	25.4	0.01
Physical activity								
Never	36.6	37.6	38.2		37.7	37.0	36.6	
<1/week	19.7	19.6	19.2		19.4	19.5	20.3	
1–3/week	32.1	31.6	30.7		31.8	31.5	32.5	
≥4/wk	11.5	11.2	12.0	0.51	11.1	11.9	10.6	0.21
Postmenopausal hormone therapy								
Never	48.7	47.5	48.5		48.0	48.2	48.6	
Past	8.4	9.7	9.2		8.8	9.1	9.4	
Current	42.9	42.7	42.3	0.01	43.2	42.7	42.0	0.70
History of oral contraceptive use								
No	30.1	30.3	29.5		30.5	30.2	29.1	
Yes	69.5	69.4	69.9		69.2	69.4	70.5	
Not sure	0.4	0.3	0.6	0.54	0.3	0.4	0.5	0.40
Alcohol consumption								
Never	43.2	43.4	44.9		43.8	43.0	44.0	
1–3 drinks/month	13.2	13.2	13.5		13.1	13.3	13.3	
1–6 drinks/week	33.3	32.5	31.3		32.2	33.0	33.1	
≥1 drink/day	10.4	10.9	10.3	0.42	11.0	10.7	9.7	0.29
Smoking status								
Never	50.9	51.5	51.2		51.3	51.4	50.4	
Past	37.9	36.9	37.0		36.7	37.7	37.9	
Current <15 cigarettes/day	4.1	4.1	4.2		4.2	4.1	4.3	
Current ≥15 cigarettes/day	7.1	7.5	7.6	0.73	7.9	6.9	7.4	0.15

Characteristic	AGT <i>T</i> 166C>C genotype			AGT <i>T</i> Met235Thr genotype		
	AA (n=12,126)	AC (n=10,580)	CC (n=2,294)	TT (n=8,534)	TC (n=12,122)	CC (n=4,344)
Family history of MI prior to age 60 yrs						
No	78.3	78.5	77.5	78.5	78.3	78.3
Yes	11.6	11.7	12.2	11.9	11.6	11.6
Unknown	10.0	9.8	10.3	9.6	10.1	10.1
						0.75

† 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.
Proportions may not add up to 100 due to rounding or missing values.
* data are expressed as percentages unless otherwise stated.

Table 2
Genotype and allele distribution of the *AGTR1* 1166A>C and the *AGT* Met235Thr polymorphisms among women with and without migraine in the Women's Health Study (N=25,000)

<i>AGTR1</i> 1166A>C polymorphism						
Migraine status	Genotype frequencies		p-value*	Allele frequencies		p-value†
	AA (12,126)	AC (10,580)		A (34,832)	C (15,168)	
No history of migraine	48.4 (9,891)	42.4 (8,657)	9.2 (1,875)	0.70 (28,439)	0.30 (12,407)	
Any history of migraine	48.8 (2,235)	42.0 (1,923)	0.88	0.70 (6,393)	0.30 (2,761)	0.69
Active migraine with aura	48.5 (618)	42.6 (543)	0.96	0.70 (1,779)	0.30 (771)	0.88
Active migraine without aura	48.7 (951)	42.4 (827)	0.89	0.70 (2,729)	0.30 (1,173)	0.68
Prior migraine	49.3 (666)	40.9 (553)	0.52	0.70 (1,885)	0.30 (817)	0.88
<i>AGT</i> Met235Thr polymorphism						
Migraine status	Genotype frequencies		p-value*	Allele frequencies		p-value†
	TT (8,534)	TC (12,122)		T (29,190)	C (20,810)	
No history of migraine	34.2 (6,990)	48.3 (9,865)	17.5 (3,568)	0.58 (23,845)	0.42 (17,001)	
Any history of migraine	33.7 (1,544)	49.3 (2,257)	0.44	0.58 (5,345)	0.42 (3,809)	0.98
Active migraine with aura	33.6 (428)	49.3 (628)	0.80	0.58 (1,484)	0.42 (1,066)	0.86
Active migraine without aura	32.3 (630)	50.7 (990)	0.11	0.58 (2,250)	0.42 (1,652)	0.39
Prior migraine	36.0 (486)	47.3 (639)	0.41	0.60 (1,611)	0.40 (1,091)	0.20

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

* 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- and multivariable adjusted odds ratios (ORs) and 95% confidence intervals (95% CIs) for migraine according to *AGTR1* 1166A>C and *AGT* Met235Thr polymorphisms assuming an additive mode (n=25,000)

Polymorphism	Age-adjusted			Multivariable-adjusted*		
	OR	95% CI	p-value	OR	95% CI	p-value
Any history of migraine (n=4,577)						
<i>AGTR1</i> 1166A>C	0.99	0.94–1.04	0.68	0.99	0.94–1.04	0.60
<i>AGT</i> Met235Thr	1.00	0.95–1.05	0.92	0.99	0.95–1.04	0.81
Migraine with aura (n=1,275)						
<i>AGTR1</i> 1166A>C	0.99	0.91–1.08	0.86	0.99	0.90–1.08	0.77
<i>AGT</i> Met235Thr	1.01	0.93–1.09	0.90	1.00	0.92–1.09	0.97
Migraine without aura (n=1,951)						
<i>AGTR1</i> 1166A>C	0.99	0.92–1.06	0.67	0.98	0.91–1.06	0.63
<i>AGT</i> Met235Thr	1.03	0.96–1.10	0.43	1.03	0.96–1.10	0.48
Past migraine (n=1,351)						
<i>AGTR1</i> 1166A>C	0.99	0.91–1.08	0.88	0.99	0.91–1.08	0.79
<i>AGT</i> Met235Thr	0.95	0.88–1.03	0.22	0.95	0.87–1.03	0.17

* 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.