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ANGIOTENSIN RECEPTOR GENE POLYMORPHISMS AND TWO-YEAR CHANGE IN HYPERINTENSE LESION VOLUME IN MEN

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Abstract

This longitudinal study examined the relationship between 2-year change in white matter hyperintense lesion (WML) volume and polymorphisms in genes coding for the angiotensin-II type 1 and type 2 receptors, *AGTR1* A1166C and *AGTR2* C3123A. 137 depressed and 94 nondepressed subjects age 60 years or older were enrolled. Standard clinical evaluations were performed on all subjects and blood samples obtained for genotyping. 1.5T MRI was obtained at baseline and approximately two years later. These scans were processed using a semi-automated segmentation process which allowed for the calculation of WML volume at each time point. Statistical models tested for the relationship between change in WML volume and genotype, while also controlling for age, sex, diagnostic strata, baseline WML volume, and comorbid cerebrovascular risk factors. In men, *AGTR1* 1166A allele homozygotes exhibited significantly less change in WML volume than 1166C carriers. We also found that men reporting hypertension with the *AGTR2* 3123C allele exhibit less change in WML volume than hypertensive men with the 3123A allele, or men without hypertension. There were no significant relationships between these polymorphisms and change in WML volume in women. No significant gene-gene or gene-depression interactions were observed. Our results parallel previously observed gender differences of the relationship between other renin-angiotensin system polymorphisms and hypertension. Further work is needed to determine if these observed relationships are secondary to

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polymorphisms affecting response to antihypertensive medication, and if antihypertensive medications can slow WML progression and lower the risk of morbidity associated with WMLs.

Keywords

MRI; Major Depressive Disorder; Volumetric Study; Cerebrovascular Disease; Renin-Angiotensin System; Genetic Polymorphisms

INTRODUCTION

Cerebral hyperintensities are regions of increased signal intensity observed on T2-weighted magnetic resonance imaging (MRI) which are associated with ischemia and advanced age 1 but also with cognitive impairment,1 motor disability,2 and depression.3 They are often described as subcortical ischemic disease and share risk factors with cerebrovascular disease.1,4 Little is known about the genes associated with complex multifactorial ischemic disease of the brain. There are likely many alleles across multiple pathways, each with small effect size, contributing ischemia risk.5

Genes in the renin-angiotensin system (RAS) are such candidates. The RAS regulates blood pressure and fluid homeostasis primarily through angiotensin II's (AII's) effect on two major receptor types: AT_1 and AT_2. AII acts peripherally and centrally, where it exhibits pressor effects and regulates secretion of antidiuretic hormone. Additionally, stimulation of AT_2 receptors may potentiate the expression of methyl methanesulfonate sensitive 2 (MMS2), a neuroprotective factor that may be protective against cerebral ischemia.6

Polymorphisms of the genes coding for these receptors may modulate AII's effect. The C allele variant of the A1166C SNP of the *AGTR1* gene is associated with hypertension,7 cardiac disease,7 and ischemic stroke.7, 8 The X chromosome-linked *AGTR2* gene is less well studied and its location raises the possibility of sex differences in gene effects. The A allele variant of the *AGTR2* C3123A polymorphism is associated with cardiac disease 9 and response of blood pressure to salt intake in men.10

We examined the relationship between these polymorphisms and change in white matter hyperintensity lesion (WML) volume over a two-year period in a cohort of depressed and nondepressed older subjects. We hypothesized that the *AGTR1* 1166C allele and the *AGTR2* 3123A allele would be associated with greater change in WML volume. As exploratory aims, we tested for gene-gene and gene-demographic interactions (including gene-diagnosis and gene-sex interactions) affecting WML volume change.

METHODS

Sample

Subjects were age 60 years or older and participants in the NIMH-sponsored Conte Center for the Neuroscience of Depression at Duke University Medical Center. Depressed subjects met DSM-IV criteria for Major Depressive Disorder at enrollment based on the NIMH Diagnostic Interview Schedule (DIS) 11 and by clinical interview. Exclusion criteria

included (1) another major psychiatric illness; (2) history of substance abuse or dependence; (3) primary neurologic illness, including dementia; and (4) MRI contraindications. Subjects were recruited primarily through clinical referrals, but also through limited advertising and self-referral.

Community-dwelling comparison subjects were recruited from Duke's Aging Center Subject Registry. Eligible comparison subjects had a non-focal neurological examination, no self-report of neurologic or psychiatric illness, no evidence of a psychiatric diagnosis based on the DIS, and no contraindication to MRI. The study was approved by the Duke University Medical Center Institutional Review Board and all subjects provided written informed consent.

We have previously published studies examining longitudinal change in hyperintense lesion volume in this sample.12–14 The current study was restricted to those subjects with both longitudinal neuroimaging data and genetic polymorphism data, and further restricted to Caucasian subjects as others have reported racial differences in *AGTR1* A1166C allele frequency.7 341 subjects had genetic data. Of those, 290 were Caucasian and only 231 had longitudinal MRI data. When compared with Caucasian subjects who remained in the study, subjects without follow-up MRI data were significantly older, less educated, with lower MMSE scores and higher WML volumes (data not shown). Depressed subjects were more likely to not have follow-up MRI data but there were no differences based on sex or *AGTR1* or *AGTR2* genotype.

Clinical Assessments and Antidepressant Treatment

Subjects completed a self-report questionnaire used in the NIMH Catchment Area program, 15 which assessed demographic factors and the presence or absence of several medical conditions, including hypertension, diabetes mellitus, and heart disease. In the depressed population, the clinician-scored Montgomery-Asberg Depression Rating Scale (MADRS)16 was used to measure depression severity.

Subjects were excluded if they had a diagnosis of dementia at enrollment. The majority of subjects had Mini Mental State Examination (MMSE)17 scores above 24; some severely depressed individuals had scores below 25. These subjects were followed through an acute three month treatment phase; if the scores remained below 25, they were removed from the study.

Depressed subjects were treated over the course of the study period according to the Duke Somatic Treatment Algorithm for Geriatric Depression.18 This algorithm mimics "real world" treatment options rather than adhering to a rigid clinical trial design, by providing a stepwise treatment approach while accounting for past treatments and depression severity. All marketed antidepressants are allowed, and there are provisions for lithium augmentation and electroconvulsive therapy.

MRI Acquisition

Subjects were imaged approximately two years apart (mean 727.1 days, SD = 53.9 days, range 462–892 days), using a 1.5 Tesla whole-body MRI system (Signa, GE Medical

Systems, Milwaukee, WI) with the standard head (volumetric) radiofrequency coil. A dual-echo fast spin-echo acquisition was obtained in the axial plane. The pulse sequence parameters are repetition time = 4000 ms, echo time = 30, 135 ms, 32 KHz (±16KHz) full imaging bandwidth, echo train length = 16, a 256 × 256 matrix, 3-mm section thickness, 1 excitation and a 20-cm field of view. The images were acquired in two separate acquisitions with a 3-mm gap between sections for each acquisition. The second acquisition was offset by 3 mm from the first so that the resulting data set consisted of contiguous sections with no gap.

Magnetic Resonance Image Analysis

The segmentation protocol has been previously described 19 and uses a modified version of MrX software (GE Corporate Research and Development, Schenectady, NY), originally modified by Brigham and Women's Hospital (Boston).20 This semi-automated method uses multiple MR contrasts to identify tissue classifications through a 'seeding' process wherein a trained analyst manually selected pixels in each tissue type to be identified. Lesion areas were selected based upon a set of explicit rules developed from neuroanatomical guidelines and consultation with a neuroradiologist. Both periventricular and deep white matter lesions were combined to provide a WML measure. Reliability was established by repeated measurements. Intraclass correlation coefficients were: left cerebral WMLs = 0.988, and right cerebral WMLs = 0.994.

Genotyping

SNP genotyping was performed by TaqMan, using 'Assays-on-Demand' SNP genotyping products (Applied Biosystems, Foster City, CA). For all assays, quality control measures were applied, including genotyping a series of blinded duplicate samples and Centre d'Etude du Polymorphism Humain (CEPH) controls. The genotypes of all duplicate samples had to match 100% in order for the assay to pass quality control. Further, we required that each assay achieve 95% efficiency (the genotypes of at least 95% of the samples could be called with certainty) before statistical analysis. PCR was performed on the ABI 9700 dual 384-well Geneamp PCR system and genotypes analyzed using an ABI Prism 7900HT Sequence Detection System (Applied Biosystems, Foster City, CA). Each reaction contained 2.7ng of total genomic DNA which had been extracted from whole blood using the Pure Gene Method by Gentra.

Analytic Plan

We performed initial screening comparisons of WML volume between the *AGTR1* (rs5186) and *AGTR2* (rs2148582) SNPs, as well as two SNPs in the gene coding for angiotensinogen (rs11568020 and rs2148582). As we found no signal for the angiotensinogen SNPs, we did not pursue further analyses. For the *AGTR1* A1166C polymorphism, we dichotomized subjects into A allele homozygotes and C allele carriers as others have used this strategy.21 For the x-linked *AGTR2* C3123A polymorphism, we examined men (A or C genotype) and women (A/A, A/C, or C/C) separately. There was no precedent to dichotomize women based on genotype, nor was one genotype under-represented.

We tested for group differences in demographic measures and unadjusted WML measures between diagnostic strata (depressed or nondepressed) and genotype groups. For dichotomous group comparisons, continuous variables were examined using two-tailed pooled t-tests or the Satterthwaite t-test for unequal variances. For the trichotomous *AGTR2* genotype groups in women, we used ANCOVA to test for differences in continuous variables. Chi-square tables were used for categorical variables, or Fisher's exact test for low cell counts.

To examine the relationship between the *AGTR1* and *AGTR2* polymorphisms with change in WML volume, general linear models were created using the PROC GLM function in SAS 9.1 (Cary, NC) and reduced to parsimonious models using backwards regression. The backwards regression was performed manually, removing independent variables or interaction terms one at a time based on which had the highest *p* value, then rerunning the model until the only covariates remaining were those that were significantly related to change in lesion volume at $p < 0.05$ or those a priori designated to be retained. We first examined *AGTR1* in all subjects. After reaching a final model for WML volume and *AGTR1*, we separated the sample into men and women, added *AGTR2* genotype and *AGTR2* interactions to the models, and continued the backwards regression in each sex.

For the first model, WML volume change was the dependent variable, while diagnostic strata and *AGTR1* genotype were independent variables to be retained. We additionally designated hypertension to be retained in the model as univariate analyses revealed difference in hypertension frequency between women with different *AGTR2* genotypes, requiring this variable for later *AGTR2* analyses. Other independent variables included baseline lesion volume, time between MRI scans, age, sex, and presence of diabetes and heart disease. We also included an interaction between *AGTR1* and diagnosis and an interaction between *AGTR1* and sex. We next split the sample based on sex, removed variables related to sex, and added *AGTR2* genotype and an *AGTR2* – hypertension interaction term. We planned to retain diagnostic strata, *AGTR1* and *AGTR2* genotypes in these models. After determining the final model for each sex, we tested for group differences between genotypes or interactions terms by examining adjusted predicted lesion volumes calculated using the least squares means (LSMEANS) procedure.

We validated our final models derived through backwards manual regression using bootstrapping.22 This involves repeatedly created random samples with replacement from the original sample where data for a given subject may be used more than once for each resample. We used PROC REG to perform automated backwards stepwise regression of the full model for each resample. This resampling was repeated five times for each model, and we examined each resampled model to determine how often variables that were significant in the manually-determined parsimonious model were present in the resampled models.

RESULTS

Sample Characteristics

The sample consisted of 137 depressed and 94 nondepressed older individuals (Table 1). The nondepressed strata had a significantly higher level of education, a greater

representation by women, and longer time between MRI scans, while the depressed strata included a higher percentage of subjects reporting hypertension.

The *AGTR1* A1166C polymorphism did not deviate from Hardy-Weinberg Equilibrium (HWE) in either depressed (χ^2 = 2.44, 1 *df*, *p* = 0.1185) or nondepressed subjects (χ^2 = 1.13, 1 *df*, *p* = 0.2869). There were no significant differences in A1166C allele frequency between diagnostic strata, nor were there significant differences in demographics between *AGTR1* A/A and C allele carrier cohorts (Supplemental Table S1). C allele carriers exhibited a significantly greater increase in WML volume over the study period (Table 2).

As the *AGTR2* gene is X-linked, we tested for deviation from HWE in women only; we found no deviation in the depressed population (χ^2 = 2.08, 1 *df*, *p* = 0.1489), however the nondepressed female population did exhibit deviation from HWE (χ^2 = 6.19, 1 *df*, *p* = 0.0129). There was no difference in *AGTR2* C3123A allele frequency between diagnostic strata, nor were there significant differences in demographics between genotypes (Supplemental Tables S2 and S3) except for hypertension being less frequent in C allele homozygous women (12.8%, 6/47) than in those carrying A alleles (A/C: 32.8%, 20/61; A/A: 34.0%, 16/47; χ^2 = 7.03, 2 *df*, p = 0.0297). There were no significant differences between AGTR2 genotypes in the unadjusted WML measures (Table 2).

Multivariate Analyses

The final model examining the relationship between the *AGTR1* A1166C polymorphism and change in WML volume is show in Table 3. When validated via bootstrapping, the significant variables in this model (*AGTR1* genotype, age, baseline WML volume, and the *AGTR1* – sex interaction) appeared in all of the bootstrapped models. The predicted values for the *AGTR1*-sex interaction demonstrated that men who were A/A homozygotes exhibited significantly less change in WML volume (0.6mL) when compared with male C allele carriers (2.5mL, p =0.0018) or female C allele carriers (1.8mL, p = 0.0141). The difference between male and female A/A homozygotes (1.4mL) was not statistically significant (P = 0.0996), nor was the difference between male and female C allele carriers (p = 0.2340)

We next added *AGTR2* genotype. In the female cohort, we found that neither *AGTR1* nor *AGTR2* genotype was significantly associated with change in WML volume, and the *AGTR2* by hypertension interaction term was removed from the model by backwards regression (Table 4). In the male cohort, *AGTR1* continued to be significantly associated with WML volume change, as was the hypertension by *AGTR2* interaction term (Table 4). This model was validated via bootstrapping, wherein *AGTR1*, age, baseline WML volume, and the *AGTR2* -hypertension interaction were present in 80% or more of the models. On examining the interaction term, the *AGTR2* C allele is characterized by less change in WML volume, but only in hypertensive men. The change in WML volume was significantly different at $p < 0.05$ between this group (C allele, HTN present: 0.1 mL) and the other three groups (C allele, HTN absent: 1.6 mL; A allele, HTN absent: 1.6 mL; A allele, HTN present: 2.6 mL). The differences between the other three groups did not reach statistical significance. There was no significant difference in mean number of antihypertensive medications used at study entry between hypertensive men with the C allele (1.7, SD=0.5, N=9) or the A allele (2.1,

SD=1.4, N=15; t=0.80, 22 df, p = 0.4322). Analyses of use of specific antihypertensives were not practical due to the wide range of medications.

DISCUSSION

The primary findings relate to the *AGTR1* and *AGTR2* polymorphisms in men. Men homozygous for the 1166A allele exhibit significantly less change in WML volume than do 1166C allele carriers. Also, men with the *AGTR2* C3123A polymorphism and hypertension exhibit significantly less change in WML than men without hypertension or hypertensive men with the A allele. These findings were not seen in women. We did not find any significant gene-gene or gene-diagnosis interactions.

The observed gender difference was not an a priori hypothesis. However, gender differences in blood pressure are well documented,23 and sex hormone influences on the RAS may be the cause.24 RAS gene polymorphisms may have differential gender-related effects on blood pressure 25 including effects on pulse pressure,26 which is itself is associated with WML severity,4 and men are more susceptible to blood pressure changes related to RAS polymorphisms.25, 26 Presumptively, we are observing a similar phenomenon in the relationship between these polymorphisms and cerebral hyperintensities.

The finding with the *AGTR1* A1166C polymorphism provides new information on the relationship between this polymorphism and cerebral hyperintensities. Although not clearly replicated in a large populations study examining multiple haplotypes,27 previous studies have associated the 1166C allele with increased risk of ischemic stroke,7, 8, 28 while others associate this polymorphism with cerebrovascular disease risk factors.7 Studies specifically examining WMLs do not present as clear a picture, reporting differences in which A1166C allele is associated with increased hyperintensity severity.21, 28 We did not find a significant relationship between the A1166C polymorphism and WML volume at either assessment period (Table 2), but did find that the genotypes exhibit different longitudinal changes in WML volume. This could reflect that the relationship between this polymorphism and WML change may be mediated by other environmental factors such as smoking or this polymorphism may not start affecting hyperintensity development until the time range examined in the study, beginning in the seventh decade of life. This cannot be determined in the current study as we did not include midlife adult subjects nor were all environmental factors measured. Importantly, this polymorphism has an effect on hyperintensity progression which is independent of hypertension. This finding is similar to previous observations that RAS polymorphisms, including the *AGTR1* A1166C polymorphism, increased the risk of stroke independently of hypertension.29

Our finding of an interaction between *AGTR2* genotype and hypertension raises the study limitation that hypertension was by subject self-report, and did not include blood pressure measures or current treatment. This is relevant as our finding may indicate that the 3123C allele is protective, but only in context of specific antihypertensive treatment, as not all antihypertensives have comparable protection against cerebral ischemia. This issue is less relevant for our analyses of *AGTR1*, as we did not find an association between this polymorphism and hypertension. Notably, others have observed an association between

antihypertensive response and RAS polymorphisms,26, 30 but it is not clear how this relationship may affect cerebrovascular risk. Objective measures of blood pressure, along with details of antihypertensive medication use over the study period would have strengthened this hypothesis.

Our findings related to the *AGTR2* polymorphism should be viewed in context of a limited sample size, where only nine subjects were male, hypertensive, and *AGTR2* A allele carriers. This highlights the difficulties of combining neuroimaging with genetic measures; a sample size sufficient for a neuroimaging study may be insufficient when examining multiple polymorphisms. We tested for power in this sample, comparing the 9 male subjects who were hypertensive and A allele carriers with the remaining 67 male subjects, and using an alpha of 0.05 calculated a power of 0.23. This is low, but as lower power is associated with greater risk of a Type II error or false negative rate, this does not alter the potential importance of a positive finding. It is important to note that our inability to associate C3123A with WML change in women may accurate but may be a false negative, as the *AGTR2* polymorphism was out of HWE in the female comparison cohort. Given these limitations, these *AGTR2* C3123A findings should be viewed cautiously. However, these limitations do not apply to our analyses of the AGTR1 polymorphism, which included greater numbers and utilized the entire sample rather than dichotomizing the sample by sex.

We have focused this discussion on the relationship between these polymorphisms, hypertension, and WMLs. However, our findings may have other explanations, as WML progression may be affected by a number of factors, including smoking history 31 and presence of diabetes, and in the process of regulating blood pressure, AII affects salt and fluid absorption and hormone secretion which may have independent effects. Moreover, AT_2 receptor stimulation potentiates the expression of neuroprotective factors,6 which raises the possibility that these polymorphisms may affect cerebral tissue resilience or susceptibility to small vessel ischemia. Finally, interactions between multiple other RAS polymorphisms may pose particular risk for cerebrovascular disease.32

As WML severity is associated with cognitive, motor, and psychiatric morbidity,1–3 it is important to better understand what factors contribute to WML development and progression and what factors may slow or prevent WML development. Although it is difficult to definitively demonstrate WMLs “cause” such deficits, some longitudinal studies have shown that greater temporal increases in hyperintensity volume are associated with increased cognitive deficits 33 and poorer long-term depression outcomes.13 WMLs are likely one risk factor among many for the development of such problems in later life, along with other biological, genetic, and psychosocial factors.

In conclusion, these RAS polymorphisms are associated with change in white matter hyperintensity volume in men. Further work is needed to determine how this relationship is modified by the use of specific antihypertensive medications, and if such interventions not only slow hyperintensity progression, but also affect important clinical outcomes. Such studies may require large samples to overcome sample size issues for specific genotype combinations.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Table 1

Demographic and neuroimaging differences between depressed and nondepressed subjects

	Depressed (N = 137)	Nondepressed (N = 94)	Df	Test statistic	p value
Age	69.3 (7.0)	69.9 (5.5)	224	0.79	0.4300
Education	14.3 (2.4)	15.6 (1.6)	229	4.78	< 0.0001
Sex (Female)	60.6% (83)	76.6% (72)	1	6.47	0.0109
Hypertension	36.2% (50)	17.2% (16)	1	10.4	0.0013
Heart Disease	15.2% (21)	8.6% (8)	1	-	0.1578
Diabetes	4.4% (6)	3.2% (3)	1	-	0.7415
MMSE	28.4 (1.9)	29.1 (1.2)	227	3.10	0.0022
MADRS	26.5 (7.6)	-	-	-	-
Age of Onset	43.4 (20.6)				
Time between MRI scans	715.1 (62.5)	744.0 (32.1)	228	4.52	< 0.0001
WML, BL	6.5 (11.0)	4.8 (6.8)	226	1.48	0.1407
WML, Y2	8.2 (13.1)	6.0 (8.7)	229	1.55	0.1227
WML, change	1.7 (3.5)	1.2 (2.4)	229	1.27	0.2064

Continuous measures presented as mean (SD); categorical presented as % (N). Age, education, and age of onset presented in years, time between MRIs in days, WML volumes in milliliters. MMSE and MADRS scores were scores at enrollment. Categorical variables analyzed using chi-square tests, except for heart disease and hypertension which used Fisher's exact test. All continuous measures analyzed using the Satterthwaite t-test due to unequal variances.

Table 2

Neuroimaging differences by *AGTR1* and *AGTR2* genotypes

AGTR1	***A/A* (N = 135)**		***C* allele carrier (N = 96)**	***df***	**Test statistic**	**p value**
WML, BL	5.0 (7.7)		6.9 (11.6)	153	1.40	0.1638
WML, Y2	6.0 (9.5)		9.2 (13.8)	157	1.96	0.0523
WML, change	0.9 (2.3)		2.2 (3.9)	142	2.89	0.0044
AGTR2: MEN	C allele (N = 33)		A allele (N = 43)	*df*	Test statistic	*p* value
WML, BL	5.0 (3.7)		7.1 (13.0)	50.8	1.00	0.3201
WML, Y2	5.6 (4.8)		9.0 (16.6)	50.9	1.28	0.2078
WML, change	0.6 (2.3)		1.9 (4.1)	68.9	1.76	0.0823
AGTR2: WOMEN	C/C (N=47)	A/C (N=61)	A/A (N=47)	*df*	Test statistic	*p* value
WML, BL	3.7 (1.7)	6.3 (12.3)	6.8 (9.1)	2,154	1.58	0.2085
WML, Y2	5.2 (4.1)	7.6 (13.5)	8.5 (11.8)	2,154	1.14	0.3238
WML, change	1.6 (2.9)	1.4 (2.8)	1.6 (3.3)	2,154	0.13	0.8821

Lesion volumes presented in milliliters as mean (SD) and analyzed used the Satterthwaite t-test due unequal variances for the *AGTR1* analyses and the *AGTR2* analyses in men. ANCOVA was used for *AGTR2* analyses in women as genotype was 3-tiered.

Mol Psychiatry. Author manuscript; available in PMC 2011 February 01.

Table 3

Final model examining WML volume change and *AGTR1* genotype

Variable	F value	p value
AGTR1	10.19	0.0016
Depression Diagnosis	0.29	0.5906
Age	4.14	0.0429
Baseline Lesion Volume	74.86	< 0.0001
Hypertension	0.20	0.6531
Sex	0.01	0.9101
AGTR1 – Sex Interaction	3.93	0.0488

Model variables determined through backwards regression. *AGTR1* genotype, depression diagnosis, and hypertension were designated as variables that had to remain in the model.

Table 4

Final models examining WML change and both *AGTR1* and *AGTR2*

	WML volume change: Men		WML volume change: Women	
Variable	**F value**	**p value**	**F value**	**p value**
AGTR1	15.21	0.0002	1.56	0.2144
AGTR2	6.43	0.0135	0.19	0.8271
Depression Diagnosis	0.16	0.6879	0.76	0.3841
Age	8.56	0.0047	2.78	0.0974
Baseline Lesion Volume	113.05	< 0.0001	19.58	< 0.0001
Hypertension	0.46	0.5010	-	-
AGTR2 – hypertension interaction	7.31	0.0087	-	-

Model variables determined through backwards regression. *AGTR1* genotype, AGTR2 genotype, and depression diagnosis designated as variables that had to remain in the model.