Renin Angiotensin System Gene Polymorphisms and Cerebral Blood Flow Regulation

The MOBILIZE Boston Study

Ihab Hajjar, MD, MS; Farzaneh Sorond, MD, PhD; Yi-Hsiang Hsu, ScD; Andrew Galica, BS; L. Adrienne Cupples, PhD; Lewis A. Lipsitz, MD

Background and Purpose—Our objective was to investigate the associations between polymorphisms in representative genes of the renin angiotensin system with measures of cerebral blood flow regulation in older adults.

Methods—Participants in this analysis were white subjects (n=335) in the MOBILIZE Boston study (Maintenance of Balance, Independent Living, Intellect, and Zest in the Elderly of Boston), an observational study of community-dwelling elders who underwent transcranial Doppler while sitting and standing and during hypercapnea and hypocapnea. Autoregulation phenotype was the change in cerebrovascular resistance from sit to stand. Vasoreactivity phenotype was the slope of the change in cerebrovascular conductance versus change in end-tidal CO₂. A total of 33 tagged single nucleotide polymorphisms were selected in the angiotensinogen gene, the angiotensin converting enzyme gene, and the angiotensin receptor gene. Regression analyses adjusted for age, gender, body mass index, mean arterial blood pressure, stroke, and use of antihypertensives were conducted for each single nucleotide polymorphism and outcome. Bonferroni corrections were used to adjust P values for multiple testing.

Results—In the angiotensinogen gene, only the rs699 single nucleotide polymorphism was associated with vasoreactivity after Bonferroni correction (P=0.00028). Homozygous carriers of the CC genotype of this single nucleotide polymorphism had lower vasoreactivity compared with the CT or TT genotypes. There were no significant associations with autoregulation measures. None of the single nucleotide polymorphisms in the other genes were associated with our phenotypes.

Conclusion—This analysis suggests that the angiotensinogen gene may be involved in vasoreactivity independent of blood pressure. Larger studies are needed to confirm the role of this gene in cerebrovascular health and aging. (Stroke. 2010; 41:635-640.)

Key Words: cerebral blood flow ■ cerebral hemodynamics ■ gene regulation ■ renin angiotensin

Both hypertension and aging lead to alterations in cerebral blood flow regulation.¹ These alterations may be associated with stroke² and cognitive impairment.³ Recent evidence suggest that angiotensin II plays an integral role in the regulation of cerebral blood flow.⁴ The mechanisms are not fully understood but are likely to be mediated by the endothelial response to angiotensin II in the brain.⁵ In particular, angiotensin II affects the endothelial production of nitric oxide, and inhibiting the angiotensin receptors is associated with improved cerebral autoregulation,⁶ reduction in cerebral blood flow decline after middle cerebral artery (MCA) occlusion,⁷ and normalization of endothelial nitric oxide production.⁸ Endothelial nitric oxide production is a key factor in the cerebrovascular response to CO₂.⁹ The latter can be clinically assessed by transcranial Doppler (TCD) technology and is a measure of cerebrovascular health and function. Further, renin angiotensin system gene polymorphisms are associated with angiotensin II levels and measures of systemic blood pressure regulation¹⁰ and baroreflex sensitivity.¹¹ Therefore, we hypothesized that polymorphisms in genes of the renin angiotensin system are also associated with autoregulation and the cerebrovascular response to changes in CO₂.

Multiple genes in this system have been linked with various aging and vascular phenotypes. Of those, the angiotensigen gene (AGT), the angiotensin converting enzyme (ACE) gene, and the angiotensin receptor gene are the most widely studied. AGT controls angiotensin II activity by promoting the transcription of the angiotensigen protein. ACE controls angiotensin II activity by promoting the tran-
scription of the ACE protein, the enzyme that metabolizes angiotensin I to angiotensin II. Angiotensin receptor gene controls the transcription of the receptor that binds angiotensin II to produce its vascular effects. Although these genes have been linked to various vascular phenotypes, no study has investigated the association of these genes to cerebrovascular function and regulation. Because blood flow regulation and vascular reactivity are heritable traits (h² is ≥60%), a genetic association study is justifiable. Autoregulation and cerebral vasoreactivity (VR) to carbon dioxide are rarely collected in large cohort studies. The MOBILIZE Boston study is a population-based prospective observational study with genetic data to measure cerebrovascular function using TCD in elderly persons.

Therefore, our objective was to investigate the associations between polymorphisms in the 3 representative genes of the renin angiotensin system and measures of cerebral blood flow regulation in the MOBILIZE Boston study.

Methods

Study Design

MOBILIZE Boston is a population-based prospective observational study funded through a National Institute on Aging program project grant. The details on the design and recruitment are described previously.13,14 The institutional review board at Hebrew SeniorLife approved this study, and each participant provided written informed consent.

Participants

The recruitment process included a door-to-door population-based recruitment of a probability sample.13,14 Eligibility criteria included a minimum age of 70 years, ability to speak and understand English, and plans to be living in the recruitment area for ≥2 years. Exclusion criteria included cognitive impairment defined as a Mini-Mental State Examination score of <18,15 hearing or visual impairment that interfered with communication, having a terminal illness, and inability to walk 20 feet without assistance. Of the 4319 persons who were age eligible, 1616 agreed to be screened, 765 were eligible and enrolled, and 686 (545 white) agreed to have DNA collected and were genotyped.14 Participants’ assessments included anthropometric and blood pressure measurement, health habits, medical history (self-reported stroke, diabetes, heart disease, congestive heart failure, hypertension, other medical diagnoses), medication inventory, and functional and cognitive evaluations (Mini-Mental State Examination; Trail Making Test), and cholesterol measurements.14

TCD Procedure and Measures of Cerebral Blood Flow Regulation

Subjects were instrumented for heart rate (ECG) and beat-to-beat arterial pressure monitoring (blood pressure; Finapres, Ohmeda Monitoring Systems) as described previously.14 End-tidal CO₂ was measured using a Vacumed CO₂ Analyzer attached to a nasal cannula. TCD ultrasonography (MultiDop X4; DWL-Transcranial Doppler Systems Inc.) was used to measure MCA mean blood flow velocity during a sit-to-stand protocol, as described previously.16 The MCA signal was identified according to the criteria of Aaslid et al and recorded at a depth of 50 to 60 mm. A Mueller–Moll probe fixation device was used to stabilize the Doppler probe at the temporal bone window for the duration of the study. The envelope of the velocity waveform, derived from a fast-Fourier analysis of the Doppler frequency signal, was digitized at 500 Hz, displayed simultaneously with the blood pressure, ECG, and end-tidal CO₂ signals, and stored for later off-line analysis. TCD procedures were conducted by one dedicated TCD technician for the MOBILIZE Boston study. The correlations between 2 TCD measurements on 21 subjects collected 6 months apart were excellent, with R² correlation coefficients of 0.79 and 0.83, and intraclass correlation coefficients of 0.92 and 0.95, respectively.

CO₂ Breathing Protocol to Measure VR

Blood flow velocity in the MCA was measured continuously while subjects inspired a gas mixture of 8% CO₂, 21% O₂, and balance nitrogen for 2 minutes and then mildly hyperventilated to an end-tidal CO₂ of ~25 mm Hg for 2 minutes. CO₂ itself may also affect blood pressure, and the degree of change is dependent on baseline blood flow velocity.18 Therefore, we used cerebrovascular conductance (cerebral blood flow/mean arterial blood pressure) and the percentage change from baseline as our measure for VR.19,20 Hence, VR was calculated as the slope of the percentage change in cerebrovascular conductance versus the change in end-tidal carbon dioxide.20 This measure is more reflective of change in the vascular response to end-tidal CO₂.21

Sit-to-Stand Protocol to Measure Cerebral Autoregulation

The active sit-to-stand procedure is described previously in detail.22 After instrumentation, subjects sat in a straight-backed chair with their legs elevated at 90° in front of them on a stool. For each of 2 active stands, subjects rested in the sitting position for 5 minutes, then stood upright for 1 minute. The initiation of standing was timed from the moment both feet touched the floor. Blood flow velocity was continuously measured during the final 1 minute of sitting and 1 minute of standing. To ensure that a sufficient stimulus was applied, a blood pressure drop of ≥10 mm Hg during the stand event was required when calculating the outcome measures. Cerebrovascular resistance (CVR) was calculated from the ratio of blood pressure/cerebral blood flow velocity. Autoregulation was quantified by the difference between CVR sitting and standing (del-CVR = CVR_sit – CVR_Stand).

Gene and Single Nucleotide Polymorphism Selection

The following genes in the renin angiotensin system were included: AGT, ACE1, and angiotensin II receptor 1. To capture the variation in these candidate genes, we selected tagged single nucleotide polymorphisms (SNPs) within each gene. These Tag SNPs captured most of the genetic information in a region through linkage disequilibrium. Nonredundant Tag SNPs were selected for pairwise correlation (r²) ≥ 0.80 of HapMap II in the white population using Haploview. We selected Tag SNPs that covered the entire gene region as well as its 10-kb 5’ upstream and 10-kb 3’ downstream regions. We added empirical SNPs in the target genes that have been reported previously in the literature to be related to other vascular phenotypes. We identified 33 Tag and empirical SNPs in the selected genes based on our criteria. Table 1 provides the list of genes and selected SNPs, P values for the tests for Hardy–Weinberg equilibrium, and the minor allele frequency in the MOBILIZE Boston study.

Genotyping

Genotyping was conducted at the Harvard Medical School-Partners Healthcare Center for Genetics and Genomics using the Sequenom iPLEX SNP genotyping. Multiplex polymerase chain reaction assays are designed using Sequenom SpectroDESIGNER software (version 3.0.0.3) by inputting sequences containing the SNP site and 100 bp of flanking sequence on either side of the SNP. Quality control was conducted using a subsample (5%) of duplicate genotyping to identify any discordance in the results.

Statistical Analyses

Hardy–Weinberg equilibrium was examined for each SNP using the Fisher exact test. We used multiple regression analyses to test the association between cerebral blood flow phenotypes and each SNP in the selected genes. The additive genetic model was used for the main analyses. Covariate selection was based on clinical and previous
Del-CVR

Only 3 polymorphisms in the AGT gene and one in the ACE gene were associated with the autoregulation phenotype after adjusting for covariates. However, after adjusting for multiple testing, none of these associations were significant (Table 2).

Vasoreactivity

Four polymorphisms in the AGT gene were associated with the cerebral VR phenotypes. However, only one SNP remained significant after Bonferroni correction (\(P=0.00027\)) and permutation adjustment (\(P=0.03\). Table 2 shows the nominal and adjusted \(P\) values for these associations. As shown in the Figure, carriers of the CC genotype (112 [33%]) of rs699) had lower VR compared with either the CT or TT genotypes or the combined CT/TT genotype groups (223 [67%]). Because the differences in VR may be related to other factors, we compared clinical, biochemical, and physiological factors in the CC genotype to the CT or TT genotype. As shown in Table 3, there were no significant differences in these factors between the 2 groups, suggesting that the association is unlikely to be related to nongenetic factors.

None of the other SNPs in the AGT, ACE, or angiotensin receptor genes were significantly associated with VR or del-CVR. The full list of SNP–phenotype associations is provided in the online supplement (supplemental Table III). Further, none of the haplotypes were associated with either TCD data of all the SNPs except for ACE_rs12451328 (\(P=0.0026\); supplemental Table I, available online at http://stroke.ahajournals.org). There was also no association between demographic or vascular factors and the autoregulation phenotype. In contrast, lower VR was associated with older age, female sex, higher mean arterial blood pressure, smoking, and higher cholesterol levels (supplemental Table II).

Results

Study Sample and Characteristics

Of the 545 enrolled white participants, all had successful genotyping data. Of those, 210 (39%) did not have cerebral blood flow data secondary to inability to insonate the MCA or poor data quality. Therefore, this analysis was completed on 335 white participants (77.8±0.3 years of age; body mass index, 26.8±0.2; women, 54%; stroke, 9%; receiving antihypertensives, 62%; blood pressure, 127.4±0.8/69.6±0.4 mm Hg). Those without a Doppler insonation window were older (79.3±0.4; \(P=0.03\)), more likely to be women (75%; \(P<0.001\)), and had higher systolic blood pressure (132.8±1.7; \(P=0.001\)). There were no differences in the genotype distributions between subjects with and without evidence for potential confounding in the gene–cerebral blood flow relation. In addition to demographics and body mass index, stroke affects the TCD measures and may be associated with renin angiotensin gene polymorphisms.23 Both genes and TCD measures are associated with blood pressure, and hence, this was included in the model.24 Antihypertensives affect blood pressure and may interact with renin angiotensin genes in their blood pressure effect. Because of our sample size, we limited covariates to age, gender, mean blood pressure during the TCD procedure, body mass index, previous stroke, and use of antihypertensive medications.

To adjust for the single-point significance level for multiple testing with correct type-I error rate, we used a very conservative Bonferroni correction method. We investigated 2 phenotypes (VR and del-CVR) and 33 SNPs. Therefore, we considered a value of \(P=0.00075\) to be “analysis-wide” significant. We also calculated empirical \(P\) values based on global random 10,000 permutations tests as described previously.23 All reported \(P\) values are from 2-sided tests. To minimize population admixture, we limited our analysis to white participants. Finally, in the SNPs for which we identify an association with our outcome, we explored the possibility that it will interact with ACE inhibitors (ACEINH). We provide these results in the online supplement.

Table 1. Selected Renin Angiotensin System Genes/SNPs, Minor Allele Frequencies, and Their Hardy–Weinberg Equilibrium in the White Participants of MOBILIZE Boston

<table>
<thead>
<tr>
<th>Gene SnP</th>
<th>HWE</th>
<th>MAF</th>
<th>Gene SnP</th>
<th>HWE</th>
<th>MAF</th>
</tr>
</thead>
<tbody>
<tr>
<td>ACE_rs4293</td>
<td>0.7919</td>
<td>0.24</td>
<td>AGT_rs4762</td>
<td>0.324</td>
<td>0.01</td>
</tr>
<tr>
<td>ACE_rs4295</td>
<td>0.7782</td>
<td>0.18</td>
<td>AGT_rs11122576</td>
<td>0.1811</td>
<td>0.11</td>
</tr>
<tr>
<td>ACE_rs4309</td>
<td>0.5607</td>
<td>0.14</td>
<td>AGT_rs11122577</td>
<td>0.539</td>
<td>0.02</td>
</tr>
<tr>
<td>ACE_rs4311</td>
<td>0.2304</td>
<td>0.26</td>
<td>AGT_rs2004776</td>
<td>0.447</td>
<td>0.04</td>
</tr>
<tr>
<td>ACE_rs4329</td>
<td>0.9069</td>
<td>0.18</td>
<td>AGT_rs3889728</td>
<td>0.6523</td>
<td>0.04</td>
</tr>
<tr>
<td>ACE_rs4341</td>
<td>0.8569</td>
<td>0.18</td>
<td>AGT_rs4028824</td>
<td>0.3227</td>
<td>0.004</td>
</tr>
<tr>
<td>ACE_rs4357</td>
<td>0.8622</td>
<td>0.01</td>
<td>AGTR1_rs4681157</td>
<td>0.9429</td>
<td>0.18</td>
</tr>
<tr>
<td>ACE_rs4461142</td>
<td>0.3841</td>
<td>0.23</td>
<td>AGTR1_rs2933250</td>
<td>0.173</td>
<td>0.05</td>
</tr>
<tr>
<td>ACE_rs4459610</td>
<td>0.492</td>
<td>0.16</td>
<td>AGTR1_rs2638363</td>
<td>0.6138</td>
<td>0.05</td>
</tr>
<tr>
<td>ACE_rs8066276</td>
<td>0.3407</td>
<td>0.13</td>
<td>AGTR1_rs10935724</td>
<td>0.8723</td>
<td>0.10</td>
</tr>
<tr>
<td>ACE_rs12451328</td>
<td>0.7243</td>
<td>0.18</td>
<td>AGTR1_rs4681444</td>
<td>0.4035</td>
<td>0.04</td>
</tr>
<tr>
<td>AGT_rs7079</td>
<td>0.653</td>
<td>0.15</td>
<td>AGTR1_rs1492100</td>
<td>0.0764</td>
<td>0.05</td>
</tr>
<tr>
<td>AGT_rs2478523</td>
<td>0.7171</td>
<td>0.14</td>
<td>AGTR1_rs1492099</td>
<td>0.9518</td>
<td>0.03</td>
</tr>
<tr>
<td>AGT_rs2478545</td>
<td>0.127</td>
<td>0.05</td>
<td>AGTR1_rs389566</td>
<td>0.6865</td>
<td>0.10</td>
</tr>
<tr>
<td>AGT_rs2478544</td>
<td>0.2938</td>
<td>0.03</td>
<td>AGTR1_rs385338</td>
<td>0.7642</td>
<td>0.04</td>
</tr>
<tr>
<td>AGT_rs687360</td>
<td>0.1706</td>
<td>0.10</td>
<td>AGTR1_rs5182</td>
<td>0.8081</td>
<td>0.18</td>
</tr>
<tr>
<td>AGT_rs699</td>
<td>0.1368</td>
<td>0.16</td>
<td>AGTR1_rs5186</td>
<td>0.2399</td>
<td>0.08</td>
</tr>
</tbody>
</table>

HWE indicates Hardy–Weinberg Equilibrium; MAF, Minor Allele Frequencies.
phenotype. Finally, ACEINH were not associated with VR and did not interact with the rs699 SNP in the AGT gene (supplemental Tables IV and V).

Discussion

This study provides preliminary evidence that a polymorphism in the AGT gene is independently associated with cerebral VR in white elderly persons. Homozygous carriers of the CC genotype of the rs699 SNPs have lower cerebral CO₂ VR compared with the other genotypes.

To our knowledge, this is the first study to provide evidence that renin angiotensin system genes are also involved in cerebral VR. Previous evidence suggests a genetic role of this system in brain health and diseases such as stroke, depression, and cognitive impairment.²⁶,²⁷ This study adds evidence that this system may also be involved in VR, which is linked with aging outcomes such as stroke² and dementia.²⁸

CO₂-dependent VR is mediated in part by the endothelium and is related to changes in nitric oxide.²⁹,³⁰ Changes in end-tidal CO₂ are associated with fast changes in pH, which modulate the effect of nitric oxide synthase leading to changes in nitric oxide production.³¹ In addition, ATP-dependent K⁺ channel activation may mediate CO₂-induced nitric oxide activity in the pial arterioles.³² Angiotensin II modulates nitric oxide production³³ and affects K⁺ chan-
nels.4 Our finding that a polymorphism in the AGT gene is associated with VR lends further support to the role of angiotensin II in the endothelium and cerebrovascular response to CO2, possibly by affecting nitric oxide production and potassium channels. This role is further supported by the fact that in animal models, hypercapnia has been associated with increased angiotensin II levels.5,6

Within the AGT gene, only one SNP was associated with VR: rs4699 (also termed M235T).7 This polymorphism has been reported previously to be associated with angiotensin II levels.8 Carriers of the CC genotypes have lower angiotensin II levels, which is likely to be associated with lower type 2 angiotensin II receptor activation and decreased endothelial-mediated vasodilatation. The fact that the heterozygous CT genotype did not have an intermediate level between CC and TT genotypes suggests that the genetic model for VR is a dominant one, whereas having T/C or T/T is associated with higher VR.

The lack of interaction between ACEINH and this SNP in AGT gene is likely related to our small sample size, especially those on ACEINH (n=87). In addition, the genotype that is likely associated with low angiotensin II levels is also associated with lower VR. Therefore, it is unlikely to find in a cross-sectional study a relationship between drugs that lower angiotensin II levels and VR. To test this hypothesis, a longitudinal study design is needed in which VR is measured before and after exposure to ACEINH. Such a design will allow us to test whether the genotype with the lower level or higher level of renin angiotensin system is more likely to demonstrate an ACEINH by gene interaction.

We did not identify a genetic association with autoregulation in our sample. The robustness of autoregulation in the face of aging and vascular changes9 may explain the lack of a significant association between our genetic polymorphisms and autoregulation phenotype. This may also be attributable to the relatively small sample size of our study.

One important limitation of this study is the relatively small sample size. Collecting cerebral blood flow data are limited by the time, cost, and ability to insonate the MCA. We were not able to obtain cerebral blood flow data in 39% of those enrolled in the MOBILIZE Boston study. This TCD failure rate is lower than previous population studies.2 Although we found that those without TCD had higher vascular risk, there was no association with the AGT_rs4699 SNP, suggesting that the potential bias from TCD measurement failure is likely to have a small impact on our results. Although a larger study is necessary to confirm and replicate our findings, such a study will be extremely expensive and resource intensive. Our study is also cross-sectional, raising the possibility that there is selective dropout of those with impaired cerebral blood flow before reaching older age. Finally, population admixture is a concern in any genetic association study, especially in vascular phenotypes in which racial differences are critical. We limited our analysis to white participants to decrease the effect of admixture on our findings. However, this limits the generalizability of our findings to non-whites. We also did not investigate other genes in the renin angiotensin system pathway, such as the renin gene, that may have an impact on cerebrovascular function.

Conclusion

This study suggests that a polymorphism in the AGT gene known to be involved in blood pressure control is also associated with cerebral VR. This association is independent of blood pressure and stroke. Although it maybe difficult to achieve, larger studies are needed to confirm the role of renin angiotensin system genes in abnormal cerebral blood flow regulation and its clinical consequences.

Acknowledgments

We acknowledge the MOBILIZE Boston research team and study participants for the contribution of their time, effort, and dedication.

Sources of Funding

MOBILIZE Boston was funded by a program project grant from the National Institute on Aging to L.A.L. (AG004390). I.H. was supported by grant 1 K23 AG030057 from the National Institute on Aging. L.A.C. was supported by grant P01 AG04390 from the National Institute on Aging. L.A.L. holds the Irving and Edyth S. Usen chair in geriatric medicine at Hebrew SeniorLife. He was also supported by grants AG004390, AG08812, AG025037, and AG005134 from the National Institute on Aging.

The sponsor did not participate in the design and conduct of the study, collection, management, analysis, and interpretation of the data, nor preparation, review, or approval of this article.

Disclosures

None.

References


Renin Angiotensin System Gene Polymorphisms and Cerebral Blood Flow Regulation: The MOBILIZE Boston Study
Ihab Hajjar, Farzaneh Sorond, Yi-Hsiang Hsu, Andrew Galica, L. Adrienne Cupples and Lewis A. Lipsitz

Stroke. 2010;41:635-640; originally published online February 25, 2010; doi: 10.1161/STROKEAHA.109.572669

Stroke is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
Copyright © 2010 American Heart Association, Inc. All rights reserved.
Print ISSN: 0039-2499. Online ISSN: 1524-4628

The online version of this article, along with updated information and services, is located on the World Wide Web at:
http://stroke.ahajournals.org/content/41/4/635