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Research report

Neuromarkers of the common angiotensinogen polymorphism in healthy older adults: A comprehensive assessment of white matter integrity and cognition

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HIGHLIGHTS

- The *AGT* M268T SNP is associated with cardiovascular abnormalities.
- The 268T variant is a risk factor for reduced white matter in healthy adults.
- The superior longitudinal fasciculus and cingulum are vulnerable to 268T.
- Attention/processing speed and language are compromised among TT genotypes.
- White matter and cognition are impacted independent of hyperintensity burden.

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ABSTRACT

The common angiotensinogen (*AGT*) M268T polymorphism (rs699; historically referred to as M235T) has been identified as a significant risk factor for cerebrovascular pathologies, yet it is unclear if healthy older adults carrying the threonine amino acid variant have a greater risk for white matter damage in specific fiber tracts. Further, the impact of the threonine variant on cognitive function remains unknown. The present study utilized multiple indices of diffusion tensor imaging (DTI) and neuropsychological assessment to examine the integrity of specific white matter tracts and cognition between individuals with homozygous genotypes of M268T (MetMet $n = 27$, ThrThr $n = 27$). Differences in subcortical hyperintensity (SH) volume were also examined between groups. Results indicated that the threonine variant was associated with significantly reduced integrity in the superior longitudinal fasciculus (SLF) and the cingulate gyrus segment of the cingulum bundle (cingulum CG) compared to those with the methionine variant, and poorer cognitive performance on tests of attention/processing speed and language. Despite these associations, integrity of these tracts did not significantly mediate relationships between cognition and genetic status, and SH did not differ significantly between groups. Collectively our results suggest that the threonine variant of M268T is a significant risk factor for abnormalities in specific white matter tracts and cognitive domains in healthy older adults, independent of SH burden.

1. Introduction

Advanced age is the most common predictor of cerebrovascular disease (CVD) and is evident among the majority of individuals

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over the age of 65 [1,2]. CVD is a result of ischemic damage that results in lacunar infarcts and lesions that often aggregate in subcortical white matter of the vascular bed [3,4]. These lesions can be visualized *in vivo* as areas of high signal intensity on T2-weighted MRI [5] or hypointense signals on computed tomography (CT). The most common form of CVD is subcortical ischemic vascular disease, which represents a significant risk factor for vascular dementia [6] and [7]. While the etiological mechanisms of CVD are numerous and diverse, abnormalities in blood pressure are common antecedents of cerebral ischemia [8–11].

Blood pressure is endogenously regulated by localized renin-angiotensin system (RAS) activity across multiple organ tissues [12,13]. This biosynthetic pathway is initiated by an enzyme-substrate reaction between renin and angiotensinogen (AGT) that determines the synthesis rate of the main effector peptide, angiotensin II (AngII) [14]. When bound to the type 1 receptor, AngII causes constriction of the artery wall and a subsequent increase in blood pressure. Chronic elevations in circulating AngII have been associated with vascular factors such as hypertension and atherosclerosis, resulting in upregulation of the RAS and further damage to vessel structure [15–18]. Evidence suggests that genetic expression of the AngII precursor molecule, AGT, may explain a degree of inter-individual variation in AngII concentrations [19,20].

The M268T polymorphism, historically referred to as M235T, (rs699; p.Met268Thr) of the *AGT* gene is a missense mutation whereby a single nucleotide polymorphism in exon 2 (c.803T>C) is predicted to encode threonine instead of methionine at residue 268 of the peptide sequence [21,22]. The threonine variant of M268T has been associated with arterial hypertension, carotid atherosclerosis, coronary heart disease, ischemic heart disease, and ischemic stroke [20,22,23]. In addition to peripheral vascular damage, the 268T allele has been associated with progression of deep subcortical white matter lesions among community-dwelling elderly individuals [24] and increased lesion severity independent of arterial hypertension [25]. Not all studies have identified these relationships, however, as Paternoster et al. [26] revealed no significant relationship between 268T and white matter lesions in a meta-analysis of six studies. Differences in imaging methods may explain the variability in results, as most studies that did not report significant relationships utilized brain imaging techniques with lower sensitivity to white matter lesions compared to studies that reported significant results [27].

While previous investigations of M268T and brain integrity have focused on the development and progression of white matter lesions, evidence suggests that microstructural damage to myelinated axon fibers precedes lesion development [28]. Thus, tract-specific measures of fiber integrity may be more useful for assessing the impact of M268T in healthy older adults. Diffusion tensor imaging (DTI) is a non-invasive imaging technique that provides information about the integrity of white matter fibers by measuring directional properties of water diffusion on two-dimensional (2D) grayscale maps [29]. Scalar metrics such as fractional anisotropy (FA) and mean diffusivity (MD) can be calculated from the diffusion tensor to evaluate the directional restriction and rate of water movement along an axon fiber. Decreased FA and increased MD are indicative of white matter degeneration, likely due to demyelination and/or axonal loss [30].

In addition to scalar metrics, recent studies have shown that length-based diffusion tractography MRI (dtMRI) is sensitive to aging processes [31–36]. This technique combines DTI scalar metrics with traditional tractography methods [37] to computationally reconstruct fiber tract lines [34]. As a result, dtMRI captures subtle changes in white matter microstructure along the entire length of a fiber tract that may not reach a threshold to impact traditional scalar metrics [34,36]. Multimodal utilization of DTI scalar metrics and dtMRI may reveal independent patterns of white matter aging that are influenced by cerebrovascular dysfunction. Further, these methods can provide region-specific insight into the biological consequences of M268T on microstructural white matter integrity.

The purpose of this study was to examine the impact of the *AGT* M268T polymorphism on white matter integrity using DTI in two groups of 27 healthy older adults representing the MetMet (MM) and ThrThr (TT) genotypes. Given the known relationships between cardiovascular health and cognitive status [1,38–40], we additionally examined the impact of M268T on neuropsychological performance. In order to provide a comprehensive assessment of microstructural integrity, white matter was measured using dtMRI and DTI scalar metrics of FA and MD. As a secondary aim, genetic differences in subcortical hyperintensity (SH) volume were also investigated to allow for comparisons to previous research. Participants were grouped according to genotype so that only homozygous individuals with either the MM or TT genotypes were studied in order to preserve power and facilitate interpretation of study outcomes. We hypothesized that individuals with the threonine variant would exhibit poorer cognitive performance compared to those with the methionine variant, and that poor performance would be mediated by abnormalities in white matter microstructure.

2. Methods

2.1. Participants

Data were obtained from 54 healthy, mixed race, older adults (males $n = 22$, females $n = 32$) involved in larger study of cognitive aging. Participants were recruited from the local community using radio and print advertisements that advertised “healthy aging.” A small subset of participants was recruited from the Research Participant Registry of the Washington University Institute of Clinical and Translational Sciences (ICTS).

2.1.1. Inclusion criteria

English-speaking, between the ages of 50–85, and able to complete basic and instrumental activities of daily living (ADLs) according to the Lawton and Brody activities of daily living scale [41].

2.1.2. Exclusion criteria

History of medical or neurological disorder capable of influencing cognition (e.g., multiple sclerosis, thyroid disease, etc.), all Axis I and II psychological disorders with the exception of treated depression, history of significant head injury defined as a loss of consciousness >5 min, past or current substance abuse, treatment-dependent diabetes, a score <24 on the mini mental state examination (MMSE) [42], and contraindications for MRI (e.g., claustrophobia). Blood pressure was recorded as the average of three separate time points during the neuropsychological evaluation. Although we did not exclude individuals who met criteria for hypertension (systolic ≥ 140; diastolic ≥ 90), the frequency distribution of hypertension was examined between groups. A physician visually scanned all images to rule out gross radiological abnormalities (e.g., hydrocephalus) and those with abnormal scans were excluded from the study. Participants provided informed consent prior to completion of study procedures and were financially compensated for their time. All study procedures were approved by the institutional review board (IRB) of the corresponding institutions.

2.2. Genotyping

Saliva samples were collected during the initial neuropsychological evaluation using the Oragene DNA collection kit (DNA

Genotek, Ottawa, Canada) and shipped to Genetic Repositories Australia at Neuroscience Research Australia for processing. Genomic DNA was extracted from saliva samples using the Autopure LS nucleic acid purification system (QIAgen, Hilden, Germany).

The *AGT* genotypes at the rs699 polymorphism were determined using iPLEX Gold™ primer extension followed by mass spectrometry analysis on the Sequenom MassARRAY system (Sequenom, San Diego, CA) by the Australian Genome Research Facility (http://www.agrf.org.au/).

Genotyping results of the larger sample from which participants were extracted revealed an allele distribution that did not differ from that expected under Hardy-Weinberg equilibrium [X^2 (1, N = 103) = 1.17, p = 0.279]. The allele frequency for both Met and Thr alleles was 0.50. Observed genotype frequencies were MetMet = 0.28, MetThr = 0.45, ThrThr = 0.27. Homozygous subsets (n = 27 for each homozygous genotype) were selected from within this group for the present study.

2.3. Imaging acquisition

Participants were scanned in a head-only Magnetom Allegra 3T MRI, located at Washington University in St. Louis, MO (Siemens Healthcare, Erlangen, Germany). The Allegra is a high performance scanner with maximum strength gradients of 40 m/T/m in a 100 μs rise time and a slew rate of 400/T/m/s. Acquisition parameters were designed for whole-brain coverage with a high signal-to-noise ratio (SNR) to minimize scanner artifact. Head movement was restrained through specialized foam pads and by placing surgical tape across the forehead. Total scan time was <1 h. Quality control was maintained through daily quality assurance tests to ensure consistent MRI performance across subjects. The same scanner and processing software were used for the duration of the study. Head positioning was confirmed through a scout scan consisting of three orthogonal planes that was collected from each participant at the beginning of the scanning session.

Whole-brain scans were acquired using a T1-weighted magnetization-prepared rapid-acquisition gradient echo (MP-RAGE) sequence [43], a double-echo proton-density (PD)/T2-weighted turbo spin echo (TSE) sequence, and a T2-weighted fluid-attenuated inversion-recovery (FLAIR) TSE sequence [44]. Standard shimming was applied to adjust for magnetic inhomogeneities. A detailed description of this protocol can be found in Paul et al. [45].

2.4. Diffusion-weighted imaging (DWI) acquisition

Acquisition procedures included a single-shot multi-slice echo-planar tensor-encoded sequence with diffusion gradients applied in 31 non-collinear directions and 24 main directions (b = 996 s/mm^2). We used a "core" of tetrahedral-perpendicular directions [46] (b = 1412 and 680 s/mm^2) to maximize SNR and directional coverage, with 5 I_0 acquisitions ($b \approx 0$). Sixty-four contiguous slices were obtained per contrast with an acquisition matrix of 128 × 128 and a 256 × 256 mm field of view (FOV; isotropic 2.0 × 2.0 2.0 mm voxels). Using a full-Fourier transform, TR = 7.82 s and TE = 86.2 ms. Seventy-two total acquisitions were averaged over 2 scan repeats. Raw data were saved to a CD and backed up on the operating system and floating-point DWIs were reconstructed using a SunFire V880 computer server.

2.5. Neuroimaging analysis

Diffusion-weighted volumes were corrected for motion and eddy current artifacts by affine registration to the first baseline volume using FSL FLIRT with the mutual information cost [47]. The rotation induced by these registrations were used to correct the orientations of the gradient encoding vectors [48], and FSL BET (brain extraction tools; Oxford Centre for Functional MRI of the Brain (FMRIB), version 5.0) was used to extract brain tissue.

2.6. Quantitative diffusion tractography

Diffusion tensors were reconstructed using linear least squares and trilinear interpolation of the DWI signal [49]. Deterministic whole-brain streamline tractography was completed using the principal eigenvector with one randomly placed seed per voxel, second-order Runge–Kutta integration and the following thresholds: 35° angle, FA of 0.15, and a minimum fiber length of 10 mm.

To examine anatomical features of specific tracts, each subject's FA image was registered with the Johns Hopkins University (JHU) white matter atlas using affine registration and FSL FLIRT with the mutual information cost. The following tracts were selected due to their anatomical sensitivity to ischemic damage: superior longitudinal fasciculus (SLF) [50], inferior longitudinal fasciculus (ILF) [51], the cingulate gyrus segment of the cingulum bundle (cingulum CG) [52], inferior fronto-occipital fasciculus (IFOF) [53], corticospinal tract (CST) [54], and anterior thalamic radiation (ATR) [55]. Each tract was modeled separately by hemisphere. Streamlines were selected to be included in each bundle by comparing them to labels in the JHU atlas. Fibers were included in the bundle if at least 80% of the arc length was contained in the bundle mask. To increase specificity of fiber extraction, redundant fiber streamline within 0.8 mm of an existing tract were removed. The details of this culling algorithm are described in Zhang et al. [56].

Three quantitative tractography metrics were computed for each bundle: mean FA, mean MD, and fiber bundle length (FBL). The FBL was computed from the average length of the streamlines included in the bundle. To compute bundle FA and MD, the average value along each streamline was first computed by numerical integration, and the bundle average was computed from the per-streamline measures. FBLs were normalized by taking the average length of each bundle and dividing by intracranial volume (ICV). All FBL values were then corrected for head size using normalized algorithms described in previous work [34].

2.7. SH quantification

We used a semi-automated method in MANGO (Research Imaging Institute (RII), University of Texas Health Science Center—San Antonio (UTHSCSA)) to quantify SH. This method involved a series of standardized steps. First, FSL BET was used to remove non-brain tissues from the images in order to run directly through MANGO's plugin interface. FSL BET was also used to correct for magnetic inhomogeneities using the default parameters. Using the range of voxels associated with SH, images underwent an automated thresh-holding process that yielded a region of interest (ROI) map of SH voxels. A trained rater visually inspected SH maps and corrected any spurious results and/or manually traced any SH missed by the thresholding procedure. Raters were blinded to participant demographic information with high intra- and inter-rater reliability (r = 0.96 and r = 0.94, respectively). Total SH volume was quantified by summing the voxels across each brain slice and multiplying by a correction factor obtained from the scan parameters.

2.8. Neuropsychological assessment

Participants completed a comprehensive battery of neuropsychological tests within one month of the neuroimaging appointment. For this study we selected tests of attention/processing speed, language, and executive function as previous studies have associated cognitive dysfunction in these domains with cere-

Table 1
Demographic characteristics.

	Genotype		
Total sample ($N = 54$)[a]	MM ($n = 27$)	TT ($n = 27$)	p
Age (M, SD)	66.07 (7.33)	62.56 (9.09)	0.124
Years of Education	15.63 (2.66)	14.85 (2.67)	0.289
Gender (n) (Male, Female)	15, 12	7, 20	0.051
Race (% Caucasian)	92.6	40.7	0.000*
Hypertension (% Yes)	25.9	33.3	0.766
Pulse Pressure (M, SD)	53.08 (10.68)	53.32 (13.84)	0.944
Mean Arterial Pressure (M, SD)	95.22 (7.58)	97.29 (9.94)	0.394
BMI (M, SD)	25.16 (3.49)	27.10 (4.43)	0.081
Participants with imaging data ($N = 38$)	MM ($n = 22$)	TT ($n = 16$)	p
Age (M, SD)	66.09 (7.89)	59.88 (7.20)	0.018*
Years of Education	15.68 (2.59)	14.13 (2.36)	0.066
Gender (n) (Male, Female)	13, 9	3, 13	0.013*
Race (% Caucasian)	95.5	50.0	0.005*
Hypertension (% Yes)	27.3	37.5	0.503
Pulse Pressure (M, SD)	54.36 (9.88)	49.79 (14.0)	0.245
Mean Arterial Pressure (M, SD)	96.10 (7.11)	97.85 (11.13)	0.559
BMI (M, SD)	24.68 (3.29)	26.30 (4.90)	0.230

* Statistically significant $p < 0.05$.
[a] No significant differences were observed between TT or MM participants with and without imaging data for any of the demographic variables.

brovascular insult. The following tests were used to evaluate function in these domains.

2.8.1. Attention/processing speed

Coding and digit span subtests from the repeatable battery for the assessment of neuropsychological status (RBANS) [57], trail making test A (Trails A) [58], and trials 1 and 2 of the color word interference task (CWIT) [59]. Total correct was the primary outcome measure for both coding and digit span. Performance on Trails A and the CWIT was measured by time to completion.

2.8.2. Language

Word generation (animals) [1], and picture naming and semantic fluency (fruits and vegetables) from the RBANS. Total correct was the primary outcome measure for all three tests.

2.8.3. Executive function

Letter number sequencing (LNS) from the Wechsler adult intelligence scale-III [60], trail making test B (Trails B), and trials 3 and 4 from the CWIT. Total correct was the primary outcome measure for LNS and time to completion was the outcome measure for Trails B and the CWIT.

2.9. Statistical analyses

We compared participants with the methionine variant ($n = 27$) or the threonine variant ($n = 27$) to determine if there were any systematic differences in demographic and health-related variables. Because only a subset of participants underwent neuroimaging procedures ($n = 38$; MM $n = 22$, TT $n = 16$), group differences in demographic and medical variables were analyzed first across the entire sample and then only among participants with imaging data. Exploratory analyses of the data involved two independent samples t-tests for continuous demographic variables (age and years of education), and Fisher's exact tests for categorical variables (gender, race, and presence of hypertension). To rule out the possibility of confounding subclinical variables, we also examined group differences in vascular factors such as pulse pressure, mean arterial pressure, and body mass index (BMI) using independent samples t-tests.

Linear regression models were determined to assess the impact of genetic variations on each cognitive and imaging measure as response variables, where genotype and thus amino acid sequence was included as the independent variable, adjusting for covariates such as age, years of education, gender and race. Cognitive and imaging measures with p-values < 0.05 were retained for further mediation analysis. Cell sizes varied slightly between cognitive analyses and imaging analyses as imaging data were not obtained for all participants. The linear model assumptions were visually examined via diagnostic plots (Q–Q plots and residual plots) and diagnostic statistical values (Cook's distance < 1 and variance inflation factor < 4). The remedies for two assumption violation cases are described in the results.

Mediation models [61] were utilized to determine if white matter tract integrity mediated genetic relationships to cognitive performance, with cognitive measures as the outcome variables and imaging measures as the mediating variables, adjusting for the same set of covariates. The p-value for the indirect effect was calculated based on 1000 bootstraps to correct for potential deviation from the Gaussian distribution. All computation was carried out in R.

As a secondary aim, genetic differences in SH volume were examined using an independent samples t-test. Demographic variables were first analyzed to ensure consistency across genotypes. Although race differed significantly between groups (noted below), a one-way ANOVA revealed no significant differences in SH volume as a function of race and therefore race was not utilized as a covariate. Cell sizes were slightly reduced due to the limited prevalence of SH in this cohort (MM $n = 14$, TT $n = 15$).

3. Results

Demographic characteristics of the sample are provided in Table 1. Preliminary analyses for the entire sample ($N = 54$) revealed no significant group differences in vascular factors or demographic variables between the two genotypes with the exception of race ($p = 0.0001$, unadjusted). Hypertension also did not differ significantly between groups ($p = 0.766$). For individuals with imaging data, TT and MM groups differed significantly on age, sex, and ethnicity. A trend effect was also observed for group differences in years of education (Table 1). As such, each of these variables was included in the regression models examining imaging outcomes. Significant group differences were not observed on measures of

Table 2
Genetic impact on cognitive performance.

Cognitive test	TT vs MM[e]	*t*	*p*	Df	N (MM, TT)	Effect size
RBANS coding[a]	−7.270	−2.289	0.026*	48	54 (27, 27)	−0.513
Trails A[a]	2.745	0.663	0.511	47	53 (26, 27)	0.153
RBANS digit span[a]	−0.152	−0.189	0.851	48	54 (27, 27)	−0.043
CWIT Trial 1[b,c]	0.280	0.176	0.861	46	52 (27, 25)	0.049
CWIT Trial 2[b]	−0.339	−0.215	0.831	47	53 (27, 26)	−0.049
RBANS picture naming[a,d]	−0.205	−0.847	0.401	48	54 (27, 27)	−0.193
RBANS semantic fluency[a]	−3.797	−2.416	0.019*	48	54 (27, 27)	−0.541
Word generation FAS[a]	−2.309	−1.278	0.209	36	42 (18, 24)	−0.349
Word generation animals[a]	−3.713	−2.284	0.028*	36	42 (18, 24)	−0.614
Trails B[b]	24.495	1.236	0.222	48	54 (27, 27)	0.281
LNS[a]	−0.688	−0.842	0.404	48	54 (27, 27)	−0.192
CWIT Trial 3[b]	1.033	0.181	0.858	47	53 (27, 26)	0.041
CWIT Trial 4[b]	−4.476	−0.643	0.523	47	53 (27, 26)	−0.147
Maze task[b]	94.365	1.429	0.162	36	42 (18, 24)	0.389

Note: No significant differences were observed between TT or MM participants with and without imaging data on any of the cognitive measures (all *p*'s > 0.05).
[a] Number correct.
[b] Completion Time.
[c] One TT participant was removed from the analysis due to its Cook's distance larger than 1.
[d] Logistic regression showed the same and statistically non-significant trend as the linear regression.
[*] Statistically significant $p < 0.05$.

vascular health and therefore these variables were not included in the main analyses (Table 1).

The key assumptions were satisfied for all variables with the exception of two cognitive outcomes: RBANS picture naming and CWIT trial 1. For RBANS picture naming, individuals with the MM genotype scored one of two values (9 and 10) and individuals with the TT genotype scored one of four values (7, 8, 9, 10), thus violating the normality assumption. The majority of participants across both genetic groups scored the maximum value. We refitted an alternative regression model as a sensitivity analysis. Since scores for MM genotypes were limited to two values, we were only able to perform logistic regression using the response variable whether each participant scored the maximum value. Results of this analysis revealed similar trends and *p* values. For CWIT Trial 1, one participant showed a Cook's distance value larger than 1. After removing this participant from the analysis, the results were effectively unchanged (old *p* value = 0.802, new *p* value = 0.861). The values in Table 2 reflects the results after removing this participant from the analysis.

Results of the regression models revealed that TT status significantly predicted poorer performance on Coding ($t(48) = -2.29$, $p = 0.026$), and Semantic Fluency ($t(48) = -2.42$, $p = 0.019$) from the RBANS, and Animal Fluency ($t(36) = -2.28$, $p = 0.028$) (Table 2). TT status also predicted significantly lower white matter integrity in the left SLF on measures of FA and FBL, and in the left cingulum CG on measures of FA, MD, and FBL (Table 3). These significant associations showed medium effect sizes (>0.5), while all others showed small effect sizes (<0.5). Despite these associations, relationships between genetic status and cognitive performance were not significantly mediated by the imaging variables (Table 4). Of note, nearly 50% of RBANS coding performance was mediated by tract integrity of the left cingulum CG, yet this effect did not reach statistical significance (Fig. 1).

Secondary analysis of SH volume revealed no significant differences between groups ($t(27) = -0.238$, $p = 0.814$), therefore SH was not used as a mediating variable between genetic status and cognitive performance.

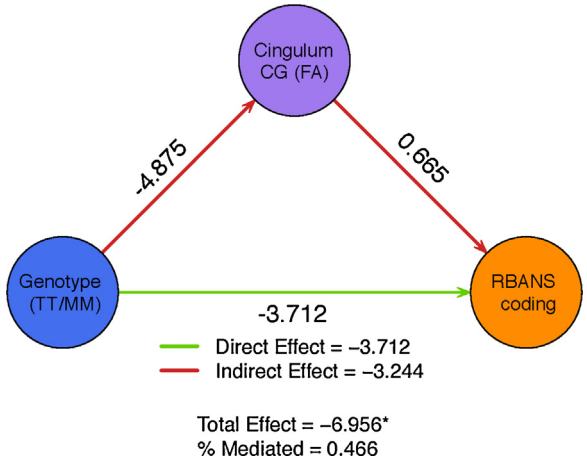

Fig. 1. Mediation effect between M268T and RBANS Coding by the Cingulum CG.

4. Discussion

This is the first study to examine the genetic impact of the *AGT* M268T polymorphism on white matter integrity and cognition among healthy older adults using tract-specific neuroimaging modalities and neuropsychological assessment. Results revealed that individuals with the homozygous TT genotype exhibited poorer performance on measures of attention/processing speed and language, and lower white matter integrity in the left SLF and cingulum CG compared to their MM counterparts. However, imaging metrics did not significantly mediate any relationship between genetic status and cognitive performance in this cohort, and SH volume did not differ significantly between groups. Collectively these results indicate a modest but direct effect of genetic differences in the *AGT* M268T polymorphism on specific domains of cognitive performance and white matter fiber tracts among healthy older adults.

Previous studies have indicated that the TT genotype is a significant risk factor for cardiovascular dysfunction and ischemic stroke in elderly populations [24,62–64]. As such, the majority of prior research examining the neurological impact of M268T has focused on white matter lesions as the primary neuromarker of brain health. While previous studies revealed positive associations between TT genotypes and white matter lesions [22,24,25], these findings were not observed in a meta-analysis by Paternoster et al. [26]. A goal of the present study was to utilize alternative imaging methods to evaluate the integrity of specific white matter tracts with genetic risk factors for compromised vasculature. Our study extends previous research by revealing a significant relationship between the TT genotype, and multiple measures of white matter tract integrity in the left SLF and cingulum CG.

Table 3
Genetic impact on neuroimaging variables.

	Left hemisphere				Right hemisphere			
Metric	TT vs MM	*t*	*p*	Effect size	TT vs MM	*t*	*p*	Effect size
SLF $N = 38$[a]								
FA	−2.198	−2.344	0.025*	−0.606	−1.999	−1.943	0.061	−0.507
MD	−0.003	−1.754	0.089	−0.460	−0.004	−1.601	0.119	−0.421
Length	−3.741	−2.039	0.049*	−0.531	−3.685	−1.879	0.069	−0.491
ILF $N = 38$[a]								
FA	−1.353	−1.251	0.220	−0.331	−0.477	−0.411	−0.684	−0.110
MD	−0.004	−1.525	0.137	−0.402	−0.002	−0.742	0.464	−0.198
Length	−2.902	−1.408	0.169	-0.372	−1.581	−0.706	0.485	−0.188
Cingulum CG $N = 37$[b]								
FA	−4.875	−2.657	0.012*	−0.708	0.133	0.102	0.919	0.028
MD	−0.008	−2.649	0.013*	−0.706	0.001	0.334	0.741	0.093
Length	−8.408	−2.704	0.011*	−0.720	0.828	0.345	0.732	0.096
IFOF $N = 37$[b]								
FA	−4.378	−1.750	0.090	−0.459	−4.088	−1.726	0.094	−0.453
MD	−0.008	−1.516	0.139	−0.399	−0.009	−1.743	0.091	−0.457
Length	−7.385	−1.560	0.129	−0.411	−8.197	−1.774	0.085	−0.465
CST $N = 37$[b]								
FA	−2.349	−1.012	0.319	−0.280	−2.143	−0.894	0.377	−0.248
MD	−0.003	−0.602	0.551	−0.167	0.002	−0.400	0.692	−0.111
Length	−2.400	−0.590	0.560	−0.164	−2.548	−0.581	0.566	−0.161
ATR $N = 37$[b]								
FA	−2.168	−1.339	0.190	−0.368	−0.856	−0.535	0.596	−0.149
MD	−0.005	−1.572	0.126	−0.430	0.001	0.344	0.733	0.096
Length	−4.160	−1.305	0.202	−0.359	−0.106	−0.032	0.975	−0.009

* Statistically significant $p < 0.05$.
[a] MM $n = 22$, TT $n = 16$.
[b] MM $n = 22$, TT $n = 15$.

Table 4
Mediation between M268T and cognition by the SLF and cingulum CG.

Mediator	Direct effect	Indirect effect	Total effect	% Mediated
RBANS coding[a]				
FA SLF	−7.692*	0.587	−7.105*	−0.083
Length SLF	−8.544*	1.439	−7.105*	−0.203
FA cingulum CG	−3.712	−3.244	−6.956*	0.466
MD cingulum CG	−5.387	−1.568	−6.956*	0.225
Length cingulum CG	−3.906	−3.050	−6.956*	0.439
RBANS semantic fluency[a]				
FA SLF	−4.528*	−0.859	−5.387*	0.160
Length SLF	−4.862*	−0.525	−5.387*	0.097
FA cingulum CG	−4.400*	−0.892	−5.292*	0.169
MD cingulum CG	−4.589*	−0.703	−5.292*	0.133
Length cingulum CG	−4.354*	−0.938	−5.292*	0.177
Word generation animals[a]				
FA SLF	−4.476	−0.300	−4.777*	0.063
Length SLF	−4.653	−0.124	−4.777*	0.026
FA cingulum CG	−4.067	−0.706	−4.773*	0.148
MD cingulum CG	−4.386	−0.387	−4.773*	0.081
Length cingulum CG	−3.999	−0.774	−4.773*	0.162

Note: All regions of interest are from the left hemisphere.
[a] Number correct.

Region-specific associations between M268T and white matter tracts are noteworthy. Work by Chen et al. [65] has demonstrated age-related reductions in FA of the SLF and cingulum as a function of reduced cerebral blood flow (CBF) in the default mode network (DMN). Vasculature within the DMN supplies blood to the SLF and cingulum and is particularly vulnerable to perfusion changes due to the localized rate of glucose and oxygen metabolism [66]. Although hypoperfusion is an established source of white matter lesions, the former study demonstrated that reduced cortical CBF is significantly associated with abnormalities in normal-appearing (i.e., non-lesioned) subcortical white matter [65]. These findings are consistent with previous research indicating that vascular pathology disrupts white matter microstructure prior to lesion development [67–70]. Consistent with this literature, results of the present study revealed no significant relationship between genetic status and SH, despite previous associations between the TT genotype and increased prevalence of white matter lesions [24,25]. It is possible that the 268T allele confers risk for early SLF and cingulum CG abnormalities due to regional vulnerability to perfusion changes. Longitudinal studies are needed that examine DTI metrics and SH conjunctively to determine if SLF and cingulum abnormalities represent early markers of CVD in individuals with the TT genotype.

An additional goal of this study was to determine the relationship between M268T and cognition given the established relationship between vascular deterioration and cognitive difficul-

ties [71–73]. Several studies have reported cognitive difficulties among individuals with cardiovascular conditions, independent of a vascular dementia diagnosis [74]. Age-related changes in cardiac output and carotid intima-media thickness also have been associated with poorer performance on tests of executive function among older individuals [74,75]. Other vascular factors including hypoperfusion, hypertension, and arterial stiffness have been associated with poor performance on tests of language, attention, processing speed, and executive function [76–78]. Because elevated AGT is associated with suboptimal vasculature, we hypothesized that M268T would contribute to cognitive dysfunction in these domains among older individuals. Results of the present study indicated that TT genotypes performed more poorly on measures of attention/processing speed and language, but not executive function. While the lack of genetic effect on executive function may be a result of low power, it is plausible that vascular risk associated with the 268T allele disrupts basic-level cognitive processes (e.g., attention/processing speed, language) prior to disturbances in executive networks [79]. This remains conjecture at this point and longitudinal studies are needed to assess the chronology of vascular-related cognitive changes in aging populations.

Although genetic differences in attention/processing speed and fluency were not significantly mediated by white matter integrity, it is worth noting that FA and FBL in the left cingulum CG did mediate nearly 50% of the relationship between M268T and Coding performance. Previous studies have shown that performance on coding tasks is significantly associated with tract integrity of the left cingulum CG in older adults [80], likely due to its anatomical proximity to the dorsal anterior cingulate cortex (dACC); a critical structure for attentional control [81]. The dACC is also an integral component of the DMN, and cognitive functions associated with this structure are vulnerable to early changes in circulation [82]. Based on the results of the present study, one possibility is that both cingulum CG integrity and attention/processing speed among TT genotypes are associated with perfusion changes in the DMN, but that specific cognitive alterations are mediated by additional neuropathological mechanisms that have not been identified. This could explain why the mediation analyses did not reach statistical significance despite the cingulate CG accounting for nearly half of the variance in coding performance between groups.

The mechanism by which the TT genotype of M268T imposes a risk for brain decrement is not fully understood. Evidence suggests that the threonine variant is associated with increased transcription of the *AGT* gene and a subsequent increase in circulating AngII, primarily in brain regions involved in cardiovascular regulation [83]. Genetic titration in mice has also revealed a causal relationship between the T allele, increased plasma AGT, and increased blood pressure [84], though this effect has been inconsistently observed in human populations [85,86].

The fact that hypertension did not differ significantly between genotypes in our study suggests an alternative pathway of pathology that may be related to functional variants that are in linkage disequilibrium with the 268 codon of *AGT* [87]. In particular, a functional variant in the proximal *AGT* promoter region has been associated with an increased risk for white matter lesions, independent of hypertension [88]. This variant encodes adenine instead of guanine six nucleotides upstream from the transcription site (-6A/G), and is believed to influence the interaction between at least one *trans*-acting nuclear factor and the *AGT* promoter [89]. Interactions in specific haplotypes (e.g., *AGT* promoter B-haplotype) have shown to increase basal AGT transcription in astrocytes, but not hepatocytes, suggesting an important role for localized RAS activity in the brain that is separate from the peripheral nervous system [90]. Because AGT transcription is an important determinant of AngII availability and CBF autoregulation [91,92], this molecular cascade may be responsible for genetic differences in AGT levels between MM and TT genotypes of M268T [93] that are not driven by alterations in the systemic RAS.

There are a few limitations to this study. The cell sizes for the MM and TT genotypes were small and may have limited statistical power to detect significant group differences in the outcome measures. As showed in Tables 2-3, the differences with small effect sizes (<0.5) did not reach statistical significance. Due to limited power, we are also unable to obtain statistically significant differences after p value adjustment (e.g., Bonferroni correction). This is particularly relevant for the imaging analyses, in which 16 individuals were carriers of the TT genotype. Relatedly, we are unable to comment on any dose response effect of the T allele, as heterozygous genotypes of M268T were excluded from this study. While we acknowledge the importance of investigating a dose response effect of the T allele, inclusion of heterozygotes would have required genetic model testing (dominant vs. recessive vs. additive) and further reduced power. Future studies consisting of larger cell sizes should implement the genetic modeling approach to determine the extent to which specific combinations of Thr and Met alleles represent a marker of "risk" versus "protection" in the aging brain. It is also worth noting that this cohort has been subject to previous genetic analyses and corrections for multiple testing have not been applied.

5. Conclusions

To our knowledge this is the first study to examine the impact of the *AGT* M268T polymorphism on white matter microstructure using a combination of DTI scalar metrics, FBL, and SH. Our study provides preliminary evidence that M268T may be a significant risk factor for white matter tract decrement and cognitive difficulties among healthy older adults, independent of genetic differences in hypertension and SH burden. Future studies consisting of larger cell sizes are needed to evaluate the stability of these findings in a larger sample of older individuals. Longitudinal examination of white matter tracts and cognitive performance will further elucidate intra-individual changes in brain integrity as a function of genetic variation in M268T. Examination of *AGT* promoter haplotypes will be an important design consideration for future studies investigating the mechanisms by which the 268T allele serves as a marker of risk for suboptimal brain health in aging populations.

Disclosure

There are no actual or potential conflicts of interest for any of the authors on this manuscript.

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