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AGTR1 gene variation: association with depression and frontotemporal morphology

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Abstract

The renin-angiotensin system (RAS) is implicated in the response to physiological and psychosocial stressors however its role in stress-related psychiatric disorders is poorly understood. We examined if variation in *AGTR1*, the gene coding for the type 1 angiotensin II receptor (AT_1R), is associated with a diagnosis of depression and differences in white matter hyperintensities and frontotemporal brain volumes. 257 depressed and 116 nondepressed elderly Caucasian subjects completed clinical assessments and provided blood samples for genotyping. We utilized a haplotype-tagging single nucleotide polymorphism (htSNP) analysis to test for variation in *AGTR1*. 1.5T MRI data for measurement of hyperintense lesions were available on 281 subjects, while 70 subjects completed 3T MRI allowing for measurements of the hippocampus and dorsolateral prefrontal cortex (dlPFC). Two htSNPs exhibited statistically significant frequency differences between diagnostic cohorts: rs10935724 and rs12721331. Although hyperintense lesion volume did not significantly differ by any htSNP, dlPFC and hippocampus volume differed significantly for several htSNPs. Intriguingly, for those htSNPs differing significantly for both dlPFC and hippocampus volume, the variant associated with smaller dlPFC volume was associated with larger hippocampal volume. This supports that genetic variation in *AGTR1* is associated with depression and differences in frontotemporal morphology.

Keywords

Geriatrics; renin-angiotensin system; MRI; genetic polymorphisms; hippocampus; amygdala; prefrontal cortex

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1. Introduction

There has been significant progress in elucidating the neural circuitry contributing to the pathogenesis of Major Depressive Disorder (MDD). Through a variety of approaches, this work has been partially integrated with molecular hypotheses of MDD, examining how variation in monoamine systems, the hypothalamic-pituitary-adrenal (HPA) axis, and neurotrophic function may influence the structure and function of frontal, cingulate, and temporal regions. However, we need to look beyond these systems to identify other influences on neural circuitry which will inform us of the pathophysiology of MDD and guide potential new therapeutic approaches (Krishnan and Nestler, 2010). The renin-angiotensin system (RAS) is such a candidate system.

Although the systemic RAS is best known for regulation of fluid homeostasis and blood pressure, there is also a brain-specific RAS that plays a crucial role in the response to physiological and psychosocial stressors (Saavedra et al., 2011). Increased RAS activity, mediated by angiotensin II, directly influences many molecular systems implicated in MDD, including: 1) stimulation of the HPA axis by stimulating the production and release of corticotrophin releasing factor (CRF) (Aguilera et al., 1995); 2) inhibition of neurogenesis and neuroplasticity (Castren, 2005; De Bundel et al., 2008); 3) increase in expression of proinflammatory cytokines (Miller et al., 2009; Lanz et al., 2010); and 4) regulation of monoamine neurotransmitters, particularly norepinephrine (Gelband et al., 1997). Thus RAS activity is activated by a variety of stressors and in turn it influences other systems implicated in the pathogenesis of MDD. Moreover, animal studies have demonstrated that RAS-blocking drugs reduce depressive behavior across several paradigms (Giardina and Ebert, 1989; Martin et al., 1990; Gard et al., 1999; Gard, 2002; Nayak and Patil, 2008), with effects comparable to those observed with tricyclic antidepressants (Gard et al., 1999; Nayak and Patil, 2008).

Despite this theoretical link between the RAS and MDD, this potential relationship has not been widely examined. There have been scattered studies examining relationships between MDD and genetic variation in several RAS elements, including genes coding for angiotensin converting enzyme (*ACE*; chromosome 17), angiotensinogen (*AGT*; chromosome 1), and angotensin-II AT_1 receptors (AT_1R) (*AGTR1*; chromosome 3). Examination of specific, isolated polymorphisms in these genes has not resulted in replicable associations with MDD (Arinami et al., 1996; Baghai et al., 2006; Lopez-Leon et al., 2006; Saab et al., 2007; Lopez-Leon et al., 2008; Sparks et al., 2009), although studies examining broader variation in these genes are more encouraging (Baghai et al., 2006). Despite AT_1R being the primary receptor for angiotensin-II, *AGTR1* is not widely studied, with mixed results from studies examining the common *AGTR1* A-to-C substitution at nucleotide 1166 (rs5186; A1166C) polymorphism in MDD (Saab et al., 2007; Taylor et al., 2010). In another study, individuals homozygous for the C allele of rs5186 had a better 4-week antidepressant response (Bondy et al., 2005), although this differs with a study of elderly, outpatient unipolar depressed subjects, wherein C1166 allele homozygous individuals exhibited a poorer antidepressant response over a longer period (Kondo et al., 2007). These differences may be related to heterogeneity in the clinical phenotypes across studies.

In contrast to examining genetic variation in heterogeneous clinical diagnoses, a more advantageous approach may be to examine the relationship between genetic variants and neuroimaging-based intermediate phenotypes (Rasetti and Weinberger, 2011). There are few studies examining how genetic variation in the RAS is associated with brain structure or function, and these are limited to isolated reports examining the *ACE* insertion/deletion (I/D) polymorphism. This polymorphism is located in intron 16 of the *ACE* gene and the D variant is associated with increased ACE expression and activity (Rigat et al., 1990),

resulting in increased angiotensin-II (Brown et al., 1998). In studies examining populations with cognitive disorders, the D allele is associated with reduced gray matter volumes of frontal gyri (Zhang et al., 2011) but larger volumes of the hippocampus and amygdala (Sleegers et al., 2005). A similar pattern was observed in remitted geriatric depression, with the D variant being associated with smaller frontal gyri volumes and larger temporal gyri volumes (Hou et al., 2010). Thus it appears that increased ACE activity and subsequently increased angiotensin-II is associated with smaller frontal but larger temporal gray matter volumes.

In older cohorts, there is a series of studies examining the associations between RAS variants with leukoaraiosis or hyperintense lesions, often referred to as white matter lesions (WMLs). WMLs are related to hypertension and cerebral ischemia and may promote or perpetuate geriatric depression.(Alexopoulos et al., 1997; Krishnan et al., 2004) Given associations between WMLs and vascular risk factors, it is unsurprising that polymorphisms in *ACE* (Sierra et al., 2002; Szolnoki et al., 2004), *AGT* (Takami et al., 2000; Van Rijn et al., 2007), and *AGTR1* (Takami et al., 2000; Henskens et al., 2005; Taylor et al., 2010; Taylor et al., 2011) are associated with greater WML severity. Thus the RAS not only is involved in the response to various stressors as described above, but its broader effect on vascular function may contribute to vulnerability to ischemia.

In this study we utilized a haplotype-tagging single nucleotide polymorphism (htSNP) analysis to examine the relationship between variation in the *AGTR1* gene, clinical diagnosis of MDD, and neuroimaging findings in a cohort of elderly subjects. Working under the hypothesis that increased RAS activity may contribute to the pathogenesis of MDD, we hypothesized that *AGTR1* htSNP variants would be associated with a clinical diagnosis of MDD. Based on our past work (Taylor et al., 2010), we also hypothesized that *AGTR1* variants would be associated with volumetric differences of WMLs. Finally, based on literature examining the ACE I/D polymorphism (Sleegers et al., 2005; Hou et al., 2010; Zhang et al., 2011), we hypothesized that *AGTR1* variants would be associated with smaller dorsolateral prefrontal cortex (dlPFC) but larger hippocampal volumes.

2. Materials and Methods

2.1 Participants and clinical assessment

Participants were age 60 or over and were enrolled in the Conte Center for the Neuroscience of Depression in Late Life at Duke University Medical Center (DUMC). All depressed participants met DSM-IV criteria for Major Depressive Disorder as diagnosed using the Diagnostic Interview Schedule (Robins et al., 1981) and confirmed via a clinical evaluation by a geriatric psychiatrist. Exclusion criteria for study entry included: 1) another major psychiatric illness, including bipolar disorder, schizophrenia, or dementia; 2) history of alcohol or drug abuse or dependence; 3) primary neurologic illness, including dementia; 4) metal in the body precluding magnetic resonance imaging (MRI) and 5) Mini Mental State Exam score less than 25. Depressed participants were primarily recruited from clinical referrals and limited advertisements.

Nondepressed participants were community-dwelling Conte Center participants recruited primarily through advertisements and from the Aging Center Subject Registry at Duke University. Eligible participants met similar entry criteria, but additionally had no evidence of a current or past psychiatric disorder based on the Diagnostic Interview Schedule.

Given past work demonstrating differences in *AGTR1* allele frequency in individuals of different racial ancestry (Hindorff et al., 2002), we limited the current study to Caucasian participants. Notably, neuroimaging data were not available for all study participants.

However, all subjects of Caucasian decent who had both *AGTR1* genetic data and neuroimaging data were included in these analyses. The study protocol was approved by the Duke University Medical Center Institutional Review Board. All participants provided written informed consent before beginning study procedures.

All subjects were interviewed to obtain demographic information and Mini-Mental State Exam (MMSE) (Folstein et al., 1975) testing was performed as a cognitive screen. Depressed participants had depression severity measured with the Montgomery-Asberg Depression Rating Scale (MADRS) (Montgomery and Asberg, 1979).

2.2 Genetic Analyses

DNA was extracted from whole blood using the Gentra PureGene system by Qiagen (Valencia, CA, USA). We used LD Select (Carlson et al., 2004) to identify ten htSNPs with a minor allele frequency of at least 0.10 and LD of $r^2 < 0.64$ in the CEU population from the HapMap project (www.hapmap.org) to provide genetic coverage of the full *AGTR1* gene. For all assays, quality control procedures were applied, including genotyping a series of blinded duplicate samples. The genotypes of all duplicate samples had to match 100% for the assay to pass quality control. Further, we required that each assay achieve 95% efficiency before statistical analyses. We tested for deviations from Hardy-Weinberg Equilibrium (HWE) for all htSNPs separately in the depressed and non-depressed cohorts using exact tests implemented in the Genetic Data Analysis program (Zaykin et al., 1995). For all analyses, htSNP genotypes were dichotomized by combining minor allele homozygotes with heterozygotes.

2.3 MRI Acquisition

Examination of neuroimaging data required 1.5 T MRI data for measurement of WML volume and 3 T MRI data for measurement of hippocampal and dlPFC volume. Use of the 1.5T WML measures allowed for a comparison with our previously study examining the association between the AGTR1 A1166C SNP and WML volumes (Taylor et al., 2010). Use of the 3T data allowed measurement of both the dlPFC and hippocampus with good measurement reliability.

1.5T MRI acquisition was performed on a whole-body system (SIGNA, GE Medical Systems, Milwaukee, WI) using the standard head (volumetric) radiofrequency coil. The previously published imaging protocol included a dual-echo fast spin echo acquisition for proton density (PD) and T2-weighted images (Payne et al., 2002). 3 Tesla cranial MRI was performed using the 8-channel parallel imaging head coil on a whole-body MRI system (Trio, Siemens Medical Systems, Malvern, PA). The imaging protocol has been previously published (Chang et al., 2011) and included the acquisition of PD, T1-weighted, T2-weighted and fluid-attenuated inversion recovery (FLAIR) images.

2.4 MRI Processing

Methods for processing 1.5T MRI data for WML volume measurement involved a previously published, supervised, semi-automated method that used the multiple MR contrasts available to identify different tissues classification, including WML classification (Payne et al., 2002). This allowed summation of WML volume in the cerebrum, a measure which included both deep WMLs and periventricular lesions. Processing of 3T MRI data included a previously published automated 4-channel tissue segmentation (Chang et al., 2011). The algorithm used a variation of the fully automated Expectation Maximization Segmentation (EMS) method (Van Leemput et al., 1999; Van Leemput et al., 2001; Van Leemput et al., 2003) which assigns probabilities of tissue assignment derived in an iterative process using the intensity distributions of the different tissues from each of the input image

contrasts. This tissue segmentation process provides a measure of total cerebral volume (excluding cerebellum and brain stem) which is used as a proxy for total brain volume.

The detailed methods for measuring dlPFC volume have been previously published (Chang et al., 2011). For this study, the dlPFC is defined as consisting of the superior frontal gyri and middle frontal gyri, which is a broader definition of the dlPFC than other definitions proposed using cytoarchitectonic observations (Sanches et al., 2009). The dlPFC's posterior boundary is delineated by a coronal plane passing through the anterior extent of the inner surface of the genu. Because of the segmentation process, reliability was measured separately for dlPFC white and gray matter, however to reduce the number of comparisons, for this study we only examined total dlPFC volume in each hemisphere. Methods for delineating the hippocampus have also been previously published (Qiu et al., 2009); this method measures only the head and body of the hippocampus.

Reliability was established for all image analysts, consisting of blinded processing of MRI scans separated by at least a week. Intraclass correlation coefficients (ICCs) were calculated separately in each hemisphere: left WML = 0.99: right WML = 0.99; left dlPFC white matter = 0.97, right dlPFC white matter = 0.98; left dlPFC gray matter = 0.90, right dlPFC gray matter = 0.87; left hippocampus = 0.99; right hippocampus = 0.98.

2.5 Statistical Analysis

Analyses were conducted using SAS 9.2 (Cary, NC, USA). For all analyses, htSNP genotypes were dichotomized by combining minor allele homozygous subjects with heterozygous subjects. Initial comparisons between diagnostic cohorts used chi-square tests for categorical variables and pooled, two-tailed t-tests for continuous variables.

Logistic regression models were developed to test for differences in htSNP allele frequency between diagnostic cohorts. In these models, the presence or absence of a diagnosis of MDD was the dependent variable, while htSNP genotype, sex, and age were independent variables. Generalized linear models were subsequently developed to test for associations between htSNP genotype and neuroimaging measures. In these models, the neuroimaging measures were the dependent variables, with independent variables including htSNP genotype, diagnostic cohort, sex, and age. Total cerebral volume was also included as an independent variable for models examining hippocampal and dlPFC volumes.

We planned to examine results before and after adjusting for multiple comparisons. We used spectral decomposition to determine the "effective" number of htSNPs based on the residual LD among the htSNPs in our data set (Li and Ji, 2005). The effective number of independent SNPs is eight, resulting in an adjusted alpha of 0.00625.

3. Results

3.1 Sample Characteristics

The sample consisted of 257 depressed (DEP) and 116 nondepressed (ND) elderly individuals with an age range between 60 and 96 years. There was no difference in sex representation between these diagnostic cohorts (DEP: 64.6% women, 166/257; ND: 71.5% women, 83/116; 1 df, $\chi^2 = 1.74$, $p = 0.1865$), however the depressed cohort was younger than the nondepressed cohort (DEP: 70.8y (7.1); ND: 72.7y (6.7); 371 df, $t = 2.48$, $p = 0.0137$). The depressed cohort additionally exhibited a significantly lower MMSE score (DEP: 28.1 (2.2); ND: 29.2 (1.0); 371 df, $t = 6.34$, $p < 0.0001$). At enrollment, the DEP cohort was symptomatic (mean MADRS score = 26.1 (7.8), range 18-54).

3.2 *AGTR1* Genotype Frequency

We tested for HWE for all ten *AGTR1* htSNPs. Two htSNPs deviated from HWE in the depressed cohort: rs10935724 (HWE p value = 0.0031) and rs718858 (HWE p = 0.0103). These htSNPs did not significantly deviate from HWE in the nondepressed cohort (rs10935724 HWE p = 0.7616; rs718858 HWE p = 0.4813). The data from both of these SNPs were reviewed for potential technical explanations for the HWE deviations and no obvious technical difficulties could be identified. Since deviations from HWE occurring in cases but not controls, as observed with these two SNPs, could represent a potential true association with case status, we elected to keep these two htSNPs in subsequent analyses. The remaining eight htSNPs did not significantly deviate from HWE in either cohort. Some participants were missing some htSNP data. The htSNPs with the most missing data were rs2638363 and rs1492103 with a failure rate of 4.0% (N = 358).

After controlling for age and sex, two of the htSNPs (rs10935724 and rs12721331) exhibited statistically significant frequency differences between these diagnostic cohorts (Table 1). For rs10935724, the AA genotype was more common in the depressed cohort. This resulted in AA carriers having an increased odds of being depressed, with an odds ratio of 1.58 (95% CI: 1.003 – 2.474). For rs12721331, the TT genotype was more common in the depressed cohort, so TT homozygous individuals had increased odds of depression (OR = 2.32; 95% CI: 1.243 – 4.336).

3.3 *AGTR1* Associations with MRI Measures

We initially examined the relationship between WML volumes and *AGTR1* htSNP genotypes. As we have previously reported (Taylor et al., 2005), the depressed cohort (N = 181) exhibited significantly greater WML volumes than did the nondepressed cohort (N = 111) (DEP = 6.9ml (9.7); ND = 4.8ml (6.4); Satterthwaite t = 2.05, 290df, p = 0.0412). These models examined WML volumes as the dependent variable, with independent variables including htSNP genotype, age, sex, and diagnostic cohort. In these models, no htSNP was significantly associated with WML volumes at or below a p-value of 0.05 (data not shown), however WML volume continued to be significantly different between diagnostic cohorts in all models at a threshold of $p < 0.05$.

Next we examined associations between htSNP genotype and volumes of the hippocampus (N = 70) and dlPFC (N= 62) after controlling for age, sex, diagnostic cohort, and total cerebral volume. Left hemisphere hippocampal volume did not significantly differ between any htSNP genotype, however right hemisphere hippocampal volume significantly differed between four htSNP genotypes (Table 2). Similarly, dlPFC volumes were significantly different between three htSNP genotypes, two associated with left hemispheric volumes and one with right. Intriguingly, two htSNPs exhibited associations both with left hemisphere dlPFC volumes and right hemisphere hippocampal volumes. In both instances, the genotype associated with *larger* right hippocampal volume was associated with *smaller* left dlPFC volume.

4. Discussion

In this study examining the *AGTR1* gene, we found that two out of ten htSNPs exhibited frequency differences between the depressed and nondepressed cohorts. When we examined the MRI data, we found no support for our hypothesis that WML volume would differ as a function of genotype. However, we did find that dlPFC and right hemisphere hippocampus volume differed significantly between several htSNP genotypes. Intriguingly, for the two htSNP analyses which exhibited relationships with both dlPFC and hippocampus volume, we observed that the variant associated with larger hippocampal volume was associated with smaller dlPFC volume.

Interpreting htSNP data in this context can be challenging. Rather than genotyping a known, functional SNP, htSNPs are selected to capture variation across the gene and so htSNPs themselves may not have functional effects. The majority of these htSNPs are in non-coding intronic regions; however they flank the first 4 exonic sequences and may capture variation in exonic sequences which influence the *AGTR1* transcript variant. There are also reports associating several of these variants (the rs2638363 A allele, the rs10935724 A allele, and the rs12695902 G allele) with clinical findings concordant with increased RAS activity, such as essential hypertension, pro-atherogenic responses, and heart failure (Ruano et al., 2005; Bahrami et al., 2008; Nie et al., 2010). Thus these htSNPs may tag functional variants.

This is one of the first studies to examine if genetic variation in *AGTR1* is associated with a diagnosis of MDD. *AGTR1* codes for AT_1Rs, which are the primary receptors for angiotensin-II and expressed in crucial regions involved in mood regulation, including the amygdala, hippocampus, and cingulate cortex (Tsutsumi and Saavedra, 1991; Johren and Saavedra, 1996). AT_1Rs are also present on hypothalamic paraventricular nuclei CRH-releasing neurons (Aguilera et al., 1995; Armando et al., 2007) and the locus coeruleus (Tsutsumi and Saavedra, 1991; Lenkei et al., 1996) where they regulate central norepinephrine activity (Macova et al., 2009). In the current study, we found two SNPs were associated with MDD in an elderly population – one in intron 1 and one in intron 2. Interestingly, rs12721331 was also identified as a potential risk allele in a genome-wide association study, although it did not survive corrections for multiple comparisons (Bosker et al., 2011). These htSNPs are distant from the previously studied functional A1166C (rs5186) polymorphism, which is located in exon 5 and associated with increased receptor responsiveness to angiotensin-II (Spiering et al., 2000).

In contrast to our prior studies examining the relationship between the *AGTR1* A1166C polymorphism (rs5186) and WMLs (Taylor et al., 2010; Taylor et al., 2011), the current study did not find an association between any htSNP and WML volume. This argues against a potential role for *AGTR1* variation in contributing to hyperintense lesion development, at least for variants occurring outside of exon 5 where rs5186 is located.

We did find associations between several htSNPs and measures of the hippocampus and dlPFC, where there appears to be a similar association between genotype and frontotemporal morphology as observed for the *ACE* I/D polymorphism. In the studies of the *ACE* I/D polymorphism, the D variant is associated with increased ACE activity, increased conversion to angiotensin-II, smaller frontal volumes, and larger temporal volumes (Brown et al., 1998; Sleegers et al., 2005; Zhang et al., 2011). Our neuroanatomical findings are similar and two variants were associated both with smaller dlPFC volume and larger hippocampal volume. Although we cannot demonstrate the functional significance of those SNPs, one of them - the rs2638363 A allele variant – was previously associated with heart failure (Bahrami et al., 2008), a condition related to increased RAS activity. Thus we hypothesize that genetic variants associated with increased RAS activity may also be associated with smaller frontal but larger hippocampal volumes.

This relationship with hippocampal volumes is somewhat at odds with previous reports and meta-analyses demonstrating that MDD is generally associated with smaller hippocampal volume (Koolschijn et al., 2009; Kempton et al., 2011). As glucocorticoid levels are generally increased in MDD and exposure to high levels of glucocorticoids may injure hippocampal neurons, the relationship between MDD and smaller hippocampal volumes are often hypothesized to be related to increased HPA axis activity, although recent work has not found such a relationship (Gerritsen et al., 2011). Central RAS activation promotes CRH release and HPA axis activity, so by extension, this hypothesis would predict variants associated with greater RAS activity would be associated with smaller hippocampal volume.

However this relationship is complex, as AT_1Rs are expressed in the hippocampus (Tsutsumi and Saavedra, 1991), and the hippocampus in turn plays an important role in regulating the HPA axis (Herman et al., 2005; Pruessner et al., 2010). Based on our genetic association findings, we hypothesize that increased AT_1R activity may contribute to differences in hippocampal volume based on the mutual role of the RAS and hippocampus in the stress response. How these relationships fit with the broader pathophysiology of depressive and anxiety disorders is less clear. This issue reflects the study limitation of being unable to demonstrate the relationship between the htSNPs and RAS activity and broader challenges in linking structure and putative function in brain imaging studies.

4.1 Strengths and Limitations

This study has the strengths of looking at broad variation in *AGTR1* while also using reliable methods to look at frontotemporal brain volumes. It also has limitations, the primary being that when we controlled for multiple comparisons, none of our findings achieved statistical significance at the adjusted alpha level. Thus these results should be considered to be hypothesis-generating rather than definitive associations. Other limitations included differences in sample size across the analyses with relatively small sample sizes for the 3T MRI samples. Additionally, our segmentation method for measuring WML volume does not distinguish between periventricular and deep white matter lesions. As WMLs occurring in these different regions may have different clinical correlations (Grool et al., 2011), this is a limitation of our methods.

We should also acknowledge that we hypothesize the relationship between the RAS, depression, and neuroimaging changes may be related to RAS responses to various physiological or psychosocial stressors, but none of these measures were available. Similarly, although all depressed participants were symptomatic at study entry, many of them were taking antidepressants, which could have affected some volumetric measures; data on antidepressant use were not available for analysis. Finally, these findings may not generalize to a younger adult population.

5. Conclusion

This study supports that variants of the *AGTR1* gene may be associated both with diagnosis of MDD and alterations in frontotemporal morphology in older adults. Given the multiple comparisons made in this study, these findings should be considered preliminary and hypothesis-generating. Further work is needed to examine how functional *AGTR1* variants may be associated not only with depression and structural brain differences, but also with altered frontotemporal circuit function. Broader investigation of the RAS's role in the pathogenesis of MDD and other stress-related psychiatric disorders is warranted.

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Table 1

AGTR1 htSNP genotype frequency differences by diagnostic cohort

htSNP	Location	SNP	Depressed	Nondepressed	Wald χ^2	P value
rs2638363	Intron 1	A/G	GG: 68.3% (168) AA / AG: 31.7% (78)	GG: 66.1% (74) AA / AG: 33.9% (38)	0.1460	0.7024
rs10935724	Intron 1	A/C	AA: 52.0% (128) CC / AC: 48.0% (118)	AA: 41.2% (47) CC / AC: 58.8% (67)	3.89	0.0487
rs1492103	Intron 2	C/T	TT: 69.6% (172) CC / CT: 30.4% (75)	TT: 69.4% (77) CC / CT: 30.6% (34)	0.00	0.9727
rs718858	Intron 2	C/T	CC / CT:27.8% (68) TT:72.2% (177)	CC / CT: 35.1% (40) TT: 64.9% (74)	1.96	0.1621
rs12721331	Intron 2	C/T	TT: 89.9% (222) CC / CT: 10.1% (25)	TT: 79.8% (91) CC / CT: 20.2% (23)	6.98	0.0082
rs2675511	Intron 2	A/G	GG / AG: 47.4% (117) AA: 52.6% (130)	GG / AG: 43.9% (50) AA: 56.1% (64)	0.33	0.5678
rs389566	Intron 2	A/T	AA / AT:46.4% (117) TT: 53.6% (135)	AA / AT:46.1% (53) TT: 53.9% (62)	0.00	0.9585
rs12695902	Intron 3	A/G	GG / AG: 16.5% (41) AA: 83.5% (208)	GG / AG: 21.7% (25) AA: 78.3% (90)	1.51	0.2190
rs385338	Intron 3	C/G	CC / CG: 33.9% (83) GG: 66.1% (162)	CC / CG: 33.3% (38) GG: 66.7% (76)	0.05	0.8317
rs5182	Exon 5	C/T	CC / CT: 78.9% (198) TT: 21.1% (53)	CC / CT: 79.1% (91) TT: 20.9% (240)	0.00	1.0000

Data presented as % (N) for each diagnostic group. Statistical results presented from logistic regression models predicting diagnostic cohort as the dependent variable, including age and sex as independent covariates.

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Table 2

AGTR1 htSNP associations with dlPFC and hippocampus volumes

htSNP	Region	Variant 1	Variant 2	F value	P value
rs2638363		GG	AA / AG		
		N = 43	N = 19		
	dlPFC, left	51.85 (8.26)	48.49 (6.52)	4.13	0.0466
		N = 50	N = 20		
	HPC, right	3.73 (0.52)	3.97 (0.48)	4.03	0.0488
rs1492103		TT	CC / CT		
		N = 45	N = 17		
	dlPFC, left	51.94 (8.10)	47.77 (6.72)	6.29	0.0148
		N = 51	N = 19		
	HPC, right	3.73 (0.52)	4.00 (0.45)	4.68	0.0342
rs12721331		TT	CC / CT		
		N = 62	N = 8		
	HPC, right	3.85 (0.54)	3.45 (0.51)	5.76	0.0192
rs2675511		AA	GG / AG		
		N = 43	N = 27		
	HPC, right	3.71 (0.50)	3.95 (0.51)	4.21	0.0443
rs12695902		AA	GG / AG		
		N = 54	N = 8		
	dlPFC, right	51.89 (9.85)	44.84 (8.94)	6.42	0.0139

Data presented in milliliters, adjusted mean (standard deviation). Models controlled for age, sex, diagnostic cohort, and total cerebral volume. Models examining hippocampal volumes had 64 df; models examining dlPFC volumes had 56 df.