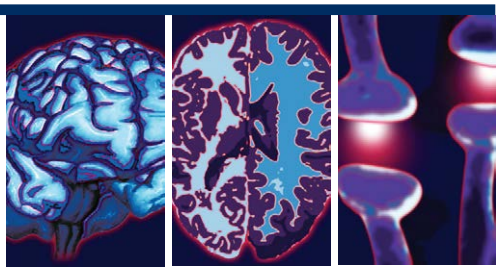

The Disparity of Angiotensinogen M235T Polymorphism in Patients with Major Depressive Disorder and Hypertension

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ABSTRACT

Objective:

This study aimed to investigate variations in angiotensinogen (AGT) M235T polymorphism among patients with major depressive disorder (MDD) and hypertension.

Methods:

This study recruited 101 patients with MDD, 100 with hypertension, 35 with both MDD and hypertension, and 572 community controls.

Results:

The frequency of MM genotype of AGT M235T polymorphism was highest in patients with both MDD and hypertension, second highest in patients with MDD, third highest in patients with hypertension and lowest in control participants. The same trend was seen for the frequency of M235 allele and M genotype of AGT M235T polymorphism. In addition, patients with both MDD and hypertension had a 2.027 times risk of having an M genotype of AGT M235T polymorphism, and patients with only MDD had a 1.712 times risk, and patients only with hypertension had a 1.242 times risk.

Conclusion:

The AGT M235T polymorphism may have a pleiotropic effect in depression and hypertension. The depressive symptoms, hypertension medication and age of onset should be considered in the clinical setting. A population-based study should be needed for further clarification to the results in future studies.

Keywords

Major depressive disorder, Hypertension, Angiotensinogen (AGT) M235T polymorphism

Introduction

Major depressive disorder (MDD) is one of the most common mental disorders worldwide, with a prevalence of 2.1-7.6% [1,2]. The prevalence of MDD in Taiwan increased from 0.167% in 1996 to 1.724% in 2003, but is lower than that in western countries [3]. Nevertheless, hypertension is associated with at least 7.6

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million deaths per year worldwide; it accounts for 13.5%of all deaths and is the leading risk factor for cardiovascular disease [4]. A cross-sectional study from 17 countries showed that 40.8% had hypertension [5]. The prevalence of hypertension in Taiwan was 23.2% during 1993-1996, and decreased to 17.6% during 2005-2008. In 2013–2014, the prevalence increased to 25.6% [6].

Several studies have shown an association between MDD and hypertension [7-12]. Two studies found that the risk of hypertension is increased 5-10 years after the occurrence of MDD [9,10]. There has been one large population-based cohort study, which involved 1 million individuals registered in the Taiwanese National Health Insurance System [12]. An association was found between MDD and a subsequent diagnosis of hypertension, with an odds ratio (OR) of 1.22. Another study [13] which used administrative healthcare data in Stockholm County, Sweden, showed that the age-adjusted OR for depression in persons with hypertension was 1.293. Our previous study showed that hypertension is a possible vulnerability marker for depression in patients with end-stage renal disease [14]. Based on the above studies, there appears to be an association between depression and hypertension. However, the under lying mechanism remains unclear.

Past studies have shown that the renin–angiotensin system (RAS) regulates blood pressure, cardiovascular homeostasis and vascular tone [15] and that increased RAS activity may increase the relative risk of depression [16]. The activity of the RAS is reflected by angiotensinogen (AGT), angiotensin I (AI), angiotensin II (AII) and angiotensin-converting enzyme (ACE). Initial step of AII synthesis is renin on AGT to produce AI. AI is subsequently converted to AII by ACE. In a previous study [16] associations were found between four RAS-associated gene polymorphisms [ACE insertion/deletion (I/D);AGT M235T; angiotensin receptor type I (AT1R) A1166C; and angiotensin receptor type II (AT2R) C3213A] and brain RAS activity.

Given the association between RAS and hypertension, genes that encode RAS proteins may be candidates for hypertension and cardiovascular and cerebrovascular diseases. AGT is associated with hypertension, carotid atherosclerosis, and cardiovascular and cerebrovascular diseases, although findings have been inconsistent [17-20]. Other studies have shown that AGT M235T polymorphism (the substitution of threonine for methionine at amino acid residue 235, rs699) is associated with hypertension in different populations [21-23].

However, antihypertensive medication, such as beta blockers, can also cause depressive symptoms [24]. In contrast, reduction of AII activity by ACE inhibitors, such as captopril, can produce antidepressant-like effects in experimental animals and humans [16,25]. It may represent that there was association between depression and RAS.

AGT M235T polymorphism encodes the substitution of methionine by threonine at residue 235 of the AGT protein, increasing plasma AGT levels in 235T homozygotes [26]. In animal study [27], a low level of AGT in the brain led to anxiety-like behavior accompanied by a depression-like state. Regarding the association between AGT M235T polymorphism and depression, López-León et al. showed a significant relationship between AGT M235T polymorphism and depressive symptoms in men [28]. However, Firouzabadi et al. showed no such association in an Iranian population [29]. Based on above reasons, it may be concluded that the etiology of hypertension and depression shares AGT M235T polymorphism. No study has explored the role of AGT M235T in combined hypertension and MDD. So we focused on AGT M235T polymorphism in this study. Hence, the association of AGT M235T between both diseases would be investigated.

Methods

■ Participants and procedures

The study was approved by the Institutional Review Board of Kaohsiung Armed Forces General Hospital in Southern Taiwan. After detailed explanation, written informed consent was obtained from all participants.

All participants were selected by age and gender frequency matching in a teaching hospital. All patients with MDD were interviewed face-to-face by a senior psychiatrist, and fulfilled the criteria of MDD on the basis of the Mini International Neuropsychiatric Interview (MINI) [30] for DSM-IV criteria. The hypertensive patients were consecutively recruited from the cardiovascular outpatient department for our previous study [31]. Hypertension was defined blood pressure ≥ 140/90 mm Hg or the use of antihypertensive medication [32,33]. The patients with MDD

and those with hypertension were recruited between March 2001 and December 2010. Control participants were selected by stratified random household sampling from the general population of Tainan City, and these individuals were invited to attend a health-screening program [34].

■ Demographic characteristics

Total of 808 participants were recruited and allocated to four groups: 35 patients with both MDD and hypertension; 101 with MDD; 100 with hypertension; and 572 controls. The demographic characteristics of the four groups are compared in **Table 1**. On χ^2 and ANOVA testing, the differences in mean age ($p = 0.140$) and gender ($p = 0.561$) among the four groups were not statistically significant **(Table 1)**, which showed that all participants were completed frequency matching by age and gender.

■ Genotyping

All participants underwent venous blood collection for DNA extraction. Genomic DNA was isolated by phenol-chloroform extraction from peripheral whole blood drawn into tubes containing potassium EDTA [35]. DNA extraction was performed using the Nucleospin blood kit (Macherey-Nagel, Germany). The DNA was eluted in a clean 1.5 mL microcentrifuge tube with 100 µL elution buffer.

Genomic DNA was amplified by polymerase chain reaction (PCR) with oligonucleotide primers that were specific to the AGT gene sequences:5'-CCGTTTGTGCAGGGCCTGGCTCTCT-3' and 5'-CAGGGTGCTGTCCACACTGGACCCC-3'. The PCR products were digested with restriction enzyme Tth111 I at 65°C overnight. The PCR-restricted fragment length polymorphism fragments of the homozygotes of M allele of AGT M235T polymorphism were non-digested bands of 165 bp, and the fragments of homozygotes of the T allele were two digested bands of 140 and 25 bp. The heterozygotes had PCR products of 165, 145 and 25 bp. In addition, 10% of the samples were selected for direct sequencing to confirm consistency with PCR-restricted fragment length polymorphism results.

Statistical analysis

Data were analyzed using SPSS for Windows version 21.0 software package. Hardy–Weinberg equilibrium proportions of AGTM235T polymorphism were tested in four groups. Baseline characteristics were compared using univariate ANOVA test or χ^2 test. All variables were analyzed using primary descriptive statistics. Multinomial logistic regression was used to explore the possible related factor of MDD and hypertension.

Results

■ Difference in the AGT M235T polymorphism among patients with MDD, patients with hypertension and controls

In each group, AGT M235T genotype distribution was within the Hardy–Weinberg equilibrium (patients with both MDD and hypertension: $\chi^2 = 0.13$, $df = 1$, patients with MDD: $\chi^2 = 0.07$, $df = 1$, patients with hypertension: $\chi^2 = 0.79$, $df = 1$, community controls: $\chi^2 = 0.62$, $df = 1$). Among the four groups, there was a significantly different distribution in the genotype of AGTM235T polymorphism ($\chi^2 = 29.237$, df = 2, $p<0.001$) **(Table 2)**, with MM genotype most common in patients with both MDD and hypertension, second most common in patients with MDD, third most common in hypertensive patients and least common in control participants. The AGT M235 allele showed the same trend, and there was a statistically significant difference in AGT M235T allelic frequency among four groups ($\chi^2=24.768$; $p<0.001$) **(Table 2)**. Comparison of M genotype versus non-M genotype (TT)

Table 1: Demographic data on the four groups participating in the study.

Variable	MDD and hypertension n = 35 n (%)	MDD n = 101 n (%)	Hypertension n=100 n (%)ss	Control n =572 n (%)	P* value
Age (years) Mean (SD)	67.54 (14.84)	63.83 (11.20)	68.55 (10.57)	65.84 (16.11)	0.140
Gender					0.561
Male (%)	17 (48.6%)	45 (44.6%)	50 (50.0%)	298 (52.1%)	
Female (%)	18 (51.4%)	56 (55.4%)	50 (50.0%)	274 (47.9%)	

*The *P* values for the percentages of each gender refer to χ^2 tests of differences between the MDD group, hypertension group and control group. For age, the *P* value refers to the ANOVA test of differences among the MDD group, hypertension group and control group.

distribution in four groups showed that M genotype (MM and MT)was significantly more frequent in patients with both MDD and hypertension, second most frequent in patients with MDD, third most common in hypertensive patients and least frequent in controls (χ^2 = 19.028, p<0.001) (Table 2).

■ Relative risk of M genotype of AGT M235T polymorphism among three groups

We explored the relative risk of having M genotype (MM and MT) of AGT M235T polymorphism among three groups (patients with both MDD and hypertension, only MDD, hypertension alone) in comparison with the control group (Table 3). Relative to the control group, patients with both MDD and hypertension had a 2.027 times risk of having M genotype (MM and MT)patients with only MDD had a 1.712 times risk; and patients with hypertension alone had a 1.242 times risk.

■ Multinomial logistic regression analysis for the possible association of M genotype (MM and MT) of AGT M235T polymorphism with MDD and hypertension

After logistic regression analysis by adjusted for gender and age (Table 4).we found that individuals with MM genotype(MM and MT) of AGT M235T polymorphism had a greater risk of having both MDD and hypertension (OR: 11.633) than those with TT genotype. Participants with MT genotype also had a greater risk of having both MDD and hypertension (OR: 2.442) than those with TT genotype. Furthermore, we found that individuals with MM genotype\m had a greater risk of having MDD (OR: 5.961) than those with TT genotype. Participants with MT genotype also had a greater risk of having MDD (OR:

Table 2: Genotype distribution and allelic frequency of AGT M235T in the four groups.

	AGT M235T	MDD and hypertension n = 35 n (%)	MDD n = 101 n (%)	Hypertension n=100 n (%)	Control n =572 n (%)	χ^2 test	P value
Genotype	**MM**	3 (8.6%)	5 (5.0%)	1 (1.0%)	6 (1.0%)	29.237†	<0.001*
	MT	13 (37.1%)	34 (33.7%)	27 (27.0%)	123 (21.5%)		
	TT	19 (54.3%)	62 (61.4%)	72 (72.0%)	443 (77.4%)		
Allele	**M235**	19 (27.1%)	44 (21.8%)	29 (14.5%)	135 (11.8%)	24.768‡	<0.001*
	T235	51 (72.9%)	158 (78.2%)	171 (85.5%)	1009 (88.2%)		
Genotype	**Non-M** (TT)	19 (54.3%)	62 (61.4%)	72 (72.0%)	443 (77.4%)	19.028‡	<0.001*
	M (MT & MM)	16 (45.7%)	39 (38.6%)	28 (28.0%)	129 (22.6%)		

† = $\chi^2_{.95}$ > 5.99 in χ^2 test (df = 2). ‡= $\chi^2_{.95}$ > 3.84 in χ^2 test (df = 1).
* = p < 0.05 in χ^2 test.

Table 3: Relative risk of M genotype (MM and MT) of AGT M235T polymorphism among three groups (patients with both MDD and hypertension, patients with MDD, patients with hypertension) in comparison with the control group.

		Hypertension	
		−	+
Major depression	−	1	1.242
	+	1.712	2.027

Table 4: Polychotomous logistic regression analysis comparing patients with both MDD and hypertension, patients with MDD and patients with hypertension in relative to controls.

Group	Variables	P	OR	P	OR
		Unadjusted		Adjusted	
MDD and hypertension	MM/TT	0.001	11.658	0.001	11.633
	MT/TT	0.016	2.464	0.017	2.442
MDD	MM/TT	0.004	5.954	0.004	5.961
	MT/TT	0.004	1.975	0.003	2.013
Hypertension	MM/TT	0.982	1.025	0.980	1.027
	MT/TT	0.225	1.351	0.252	1.329

2.013) than those with TT genotype. However, participants with MM (OR: 1.027, p = 0.980) or MT (OR: 1.329, p = 0.252) genotype both had no significant greater risk of having hypertension than those with TT genotype.

Discussion

Given the age and gender frequency matching allowed us to exclude the possible influence of age and gender on MDD and hypertension in order to focus on the effect of the AGT M235T polymorphism in MDD and hypertension. Even small subgroups in MM genotype were found in our study, AGT M235T genotype distribution was within the Hardy–Weinberg equilibrium in each group. For prevent the possibility of data distortion induced by small subgroups in MM genotype, we re-divided the AGT M235T genotype to M genotype (MM and MT) and non-M genotype (TT). Relative to the control group, patients with MDD alone had a 1.712 times risk of having the M genotype (MM and MT) of the AGT M235T polymorphism; patients with only hypertension had a 1.242 times risk; and patients with both MDD and hypertension had a 2.027 times risk.

We found a trend in the distribution of the AGT M235 allele in four groups. Subsequently, participants with more AGT M235 alleles had a higher risk of both MDD and hypertension, or MDD alone. It therefore appears that the AGT M235 allele may have a dose-related effect on the risk of having both MDD and hypertension, or MDD alone. However, when we compared the hypertensive patients with the controls, we did not have similar finding **(Table 4)**. We also explored the distribution of the AGT M235T genotype polymorphism between patients with both MDD and hypertension and patients with only MDD. The M genotype (MM and MT) of the AGT M235T polymorphism was more common in patients with both MDD and hypertension than in patients with only MDD (45.7% vs. 38.6%). However, the difference between the groups was not significant (χ^2 = 0.544, p = 0.461). On multi-nominal logistic regression analysis, there was also no significant association between the AGT M235T genotype polymorphism and risk of hypertension in patients with MDD (including 35 patients with both MDD and hypertension; 101 with MDD) (MM/TT: p = 0.303; MT/TT: p = 0.444, adjusted for gender and age, data was not showed). Therefore, we can conclude that participants with more M alleles of the AGT M235T polymorphism did not have a higher risk of hypertension; whether or not they also had MDD.

Participants with the AGT T235 allele showed elevated levels of plasma AGT, and AGT T235 allele has been shown to be positively associated with hypertension in a previous study [36]. Another study found that the frequency of the 235T allele was higher among hypertensive than normotensive individuals (93 vs. 85%; p = 0.015) and the OR for association with the 235T allele (vs. 235M) in hypertensive individuals was 2.20 [37]. In the polynominal logistic regression analysis, AGT M235T genotype distribution had no significant association with hypertension **(Table 4)**. These different findings may be due to several factors. First, ethnic difference may lead to different allelic frequencies. Participants were mostly of European, Mulatto and African descent in the study of Pereira et al. [36], but participants in our study were Taiwanese. In the study of Wang et al., the participants were Taiwanese [37], but they recruited only 96 healthy controls, whereas our control group comprised 572 participants. Their lower number of controls may cause the lower power; it would have led to a reduced likelihood of positive finding. Second, other gene polymorphisms, including ACE gene insertion/deletion (I/D), AT1R gene A1166C, and AT2R gene C3213A, which were not included in our study also have associations with the RAS, which is an important regulator of blood pressure.

Regarding the association of AGT M235T polymorphism with hypertension, a statistically significant association with hypertension was identified for the TT versus MM genotype (OR 1.54, 95% CI 1.16-2.03, p = 0.002) in a previous study [38]. In that study of the AGT M235T polymorphism, the frequency of T allele was 75.2% in the hypertensive group and 72.7% in controls. In addition, the prevalence of MM/MT/TT for the hypertensive and control groups was 6.9%, 35.8%, 57.3% (MM/MT/TT in hypertensive group) and 7.9%, 38.8%, 53.3% (MM/MT/TT in control group), respectively. The genotype distribution differed between their results and our study, which may have contributed to the negative finding in our study. However, in another study from Taiwan [37], the frequencies of MM, MT, and TT genotypes in hypertensive (1, 13 and 86%) and normotensive participants (0.30 and 70%; p =

0.008) was similar to our study. On the other hand, there were different AGT M235T allelic frequency distribution either hypertension patients or control group in two past studies [37,39] which were both selected from Taiwan. However, the prevalence of the AGT M235T (rs699) M allele in Southern Han Chinese from NCBI resource (https://www.ncbi.nlm.nih.gov/variation/tools/1000genomes/) was 12.86%, similar to that in our control group (11.8%) **(Table 2)**.

With regard to the effect of AGT M235T on depression, a significant relationship between the AGT M235T polymorphism and Centre of Epidemiological Studies Depression Scale (CES-D) scores in men was reported in a cohort study [28]. The authors found that individuals with the TT genotype had severer of depressive symptoms than those with the M allele, which seems to contradict our study. In our study, M genotype (MM and MT) of the AGT M235T polymorphism was significantly more common in patients with both MDD and hypertension, second most common in patients with MDD, third most common in hypertensive patients and least common in controls ($\chi^2 = 19.028$, $p<0.001$). However, the cases in the previous study were from the normal population with evaluated depressive symptoms, rather than patients with true depressive disorder. Therefore, it could not be concluded that TT genotype is more common in patients with MDD. In addition, their study was conducted within the Rotterdam Study, a cohort study in the Netherlands. Ethnic difference may have led to differences in allelic frequency. In the study of López-León *et al.* [28], AGT M235T allelic frequency distribution was different from that in our study, another study from Eastern Taiwan [37], another study in Taiwanese population [39] and our past study [40].

Regarding the presence of the T allele of the AGT gene was associated with increased AGT level in a previous study [41]. This increased AGT level may enhance the feedback on the hypothalamic–pituitary–adrenal axis mediated by RAS modulation and lead to depressive symptoms. So the T allele of AGT gene may lead depressive symptoms in their study, this result was not consistent with our findings. But in an experimental animal study [27], a low AGT level in the brain led to anxiety-like behavior accompanied by a depression-like state, which was consistent with our results. But finding the initial SNP is not the same as finding the underlying biology, more studies are needed to explore the true effect of the AGT M235T gene polymorphism on AGT level. AGT is a liver protein that interacts with renin to produce AI. Therefore, the AGT level may not be the most important factor in the RAS associated with depression. Other RAS-related factors, such as the level of AI, AII and ACE, may have an influence on depression. And past study [42] showed that several RAS related genes had their roles in hypertension, we also need to consider their possible impact on depression in the future. Although our study was not genome-wide association study and did not contain all candidate gene related RAS, but the gene AGTM235T we selected was based on past studies [21-23,28,40,43].

This study had several limitations. First, hypertension and MDD are both high-prevalence diseases. Large study samples, including case and control groups, are necessary to help us to understand the true inter-relationship between MDD and hypertension. Second, we had controlled the factors of age and gender, but early onset was noted in some MDD cases, which may affect the results. Third, life style like alcohol, tobacco use and life stress which may confound the results between hypertension and MDD. Fourth, although our control group was selected from Tainan which is located in Southern Taiwan [44], that had showed its community representation. The case group was also selected from Southern Taiwan. However, this study still could not avoid Berkson's and collider bias [45]. Fifth, we did not record the disease duration and onset of MDD and hypertension. So we can't analysis the association between the AGT M235T gene polymorphism and disease duration and onset and clarify whether hypertension causes MDD or MDD causes hypertension. Sixth, we did not record the information of antidepressants intake. So we can't analysis the differences between differences in antidepressant intake between Western and Taiwanese populations. Seventh, we did not check the AGT level in our cases. So we can't explore the true effect of the AGT M235T gene polymorphism on AGT level.

In summary, we can conclude that the AGT M235T polymorphism may have a pleiotropic effect in depression and hypertension. A population-based study should be needed for further clarification to the results in future studies. On the other hand, we hope to examine

depressive symptoms by questionnaire, to understand the effect of AGT M235T gene polymorphism on depressive symptoms in the future. We can also collect information about antihypertensive medications use to explore the effect of antihypertensive medications (such as ACE inhibitors or AII receptor blockers) on depressive symptoms in hypertensive patients.

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Declaration of Interest

All authors have no conflict of interest to declare.

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