

Relation Between M235T Polymorphism of the Angiotensinogen Gene and Coronary Artery Disease in Taiwanese: An Angiography-Controlled Study

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Background: Polymorphisms of the renin-angiotensin-aldosterone system have been found in association with coronary artery disease (CAD). The M235T polymorphism is the most important angiotensinogen (AGT) genetic variant. The relationship between AGT gene polymorphisms and CAD has been investigated in only a few studies, however, with conflicting results. In most of these studies, not all participants underwent coronary angiography to determine the existence of coronary artery stenosis. In this study, we tested this relationship again in Taiwanese subjects who underwent coronary angiography.

Methods: This study enrolled 576 patients who underwent coronary angiography, including 362 patients with CAD (the CAD group) and 214 without CAD (the control group). The M235T angiotensinogen genotypes were determined by polymerase chain reaction and subsequent digestion of the products with Tth 111I.

Results: The frequencies of the variant M235T genotypes in the CAD group were MM 3.3%, MT 52.2%, and TT 44.5%, not significantly different from those in the control group (MM 3.7%, MT 49.5%, and TT 46.7%) ($p = 0.82$). In multivariate analysis regarding the presence of CAD with respect to the existence of polymorphism, the odds ratios were 0.85 (95% CI = 0.60-1.23, $p = 0.39$) for T235 homozygote, and 0.91 (95% CI = 0.69-1.20, $p = 0.49$) for T allele.

Conclusion: This study shows that the AGT M235T polymorphism is not associated with the presence of coronary artery disease in the Taiwanese population.

Key Words: Angiotensinogen • Coronary artery disease • Polymorphism • Angiography

INTRODUCTION

Numerous clinical data have shown that the renin-

angiotensin-aldosterone system (RAAS) plays an important role in coronary artery disease (CAD).¹⁻³ The plasma angiotensinogen (AGT) levels are correlated with blood pressure and left ventricular hypertrophy. Human AGT has a molecular weight of about 50,000 Da, and the AGT gene locates at 1q42-43 and contains 5 exons. The M235T polymorphism, which is known as the most important AGT polymorphism,⁴ is located on exon 2 of the AGT gene and encodes a threonine instead of a methionine at residual 235 of mature angiotensinogen. The T235 allele is associated with increased plasma AGT levels, with presumed feedback down-regulation of

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plasma renin levels.⁵⁻⁷ The M235T polymorphism is associated with a whole range of cardiovascular disorders, such as hypertension, left ventricular hypertrophy, restenosis after coronary angioplasty, mitral valve prolapse, and renal failure.⁸⁻¹² Recently, this M235T polymorphism has been found associated with CAD, and T allele increases the risk of myocardial infarction.^{1,13} However, some of these associations were disputed in subsequent investigations.^{1,13}

The major weak point of these studies is that not all subjects recruited underwent coronary angiography for a definite identification of CAD. Previous studies have defined normal control groups on the basis of no coronary artery disease history or normal electrocardiograms, however this may not absolutely be accurate as patients may be asymptomatic or have subclinical disease. Our inclusion of patients with angiographically normal coronary arteries clarifies this issue. In the current study, all subjects enrolled underwent coronary angiography and were classified into either the CAD or the control group. This study aimed to estimate the variations in the frequency of AGT M235T polymorphism in patients with normal coronary arteries and in those with coronary stenosis. We hypothesized that the AGT M235T polymorphism was associated with the presence of CAD.

METHODS

Patients

We enrolled 576 patients > 40 years old who underwent coronary angiography at our unit between January 2005 and May 2006. Patients presenting with cardiogenic shock were excluded from the study. All participants were unrelated Han Chinese living in Taiwan. The racially diverse Aborigines of the Taiwan Island were excluded. Angiograms were assessed independently by two experienced cardiologists. Patients were defined in the CAD group as long as there was a stenotic lesion of $\geq 50\%$ in any major coronary artery; however, patients with a coronary stenosis of $< 50\%$ were defined as the control group. Other factors, such as hypertension, diabetes, hypercholesterolemia, renal insufficiency, body mass index, smoking and drinking alcohol were recorded by history taking, analyzing previous medical records, reviewing current medications or by examining

the patients during hospitalization. In this study, hypercholesterolemia was defined as a serum total cholesterol level of 200 mg/dl or more. Current smoker was defined as more than one pack a day within the last 5 years. Current alcohol drinker was defined if alcohol consumption was more than 20 gm a day in the last 2 years. Renal insufficiency was defined as a serum creatinine level of more than 1.4 mg/dl. Informed consent was obtained from all patients, and the study was approved by our institutional research committee.

Genotyping of angiotensinogen

The genomic DNA was extracted with the QIAmp blood kit (QIAGEN Inc, Chatsworth, California, USA) from peripheral blood leukocytes with a standard protocol. Genotyping of AGT M235T polymorphism was performed according to the method described by Russ et al.¹⁷ with minor modification. Polymerase chain reaction (PCR) was used to amplify part of the exon 2 gene with primers designed to insert a half restriction endonuclease site into the product. Primers used were: sense-primer 5'CAGGGTGCTGTCCACACTGGACCCC3' and anti-sense primer 5'CCGTTTGTTCAGGGCCTGGCTCTCT3'. Amplification was obtained after the following steps were carried out: 5 minutes of denaturation at 95 °C, followed by 36 cycles of 1 minute at 95 °C, 1 minute at annealing temperature 58 °C, and 2 minutes of DNA synthesis at 72 °C, followed by 10 minutes at 72 °C. PCR products were digested with Tth 111I (Promega Co., Madison, Wisconsin, USA), resulting in a non-digested single 165-bp fragment corresponding to M235 allele and digested 141-bp and 24-bp fragments corresponding to T235 allele.

Statistical analysis

Differences in the genotype and allele frequencies between the two study groups (CAD and control groups) were evaluated with the chi-square test in a 3×2 and a 2×2 analysis, respectively. In addition, we calculated the odds ratio (OR) regarding the presence of CAD with respect to the existence of polymorphism. For continuous variables, results are presented as means \pm SD, and the differences between the 2 groups were evaluated with unpaired *t*-test. Categorical variables were presented using frequency counts, and intergroup comparisons were analyzed by the chi-square test. T allele was found to be

associated with plasma AGT levels, so T allele and T homozygote were used for analysis. Logistic regression methods were used to calculate OR to evaluate the relationship between the presence of AGT M235T gene polymorphism and the existence of CAD after adjusting for other coronary risk factors. Variables included in the analysis were hypertension, diabetes, current smoker, current alcohol drinker, hypercholesterolemia, renal insufficiency, and body mass index. OR was presented with a 95% confidence interval (CI). A two-tailed probability value of less than 0.05 was considered to be statistically significant. All statistical analyses were performed using SPSS 10.0 program (SPSS Inc., Chicago, Illinois).

RESULTS

Baseline characteristics

A total of 576 patients were enrolled in our study; 485 (84%) patients were referred with ischemic chest pain, 37 (6%) with valvular heart disease, 35 (6%) with dilated cardiomyopathy, 17 (3%) with congenital heart disease, and 2 with primary pulmonary hypertension. Coronary angiography identified 362 patients with CAD (the CAD group) and 214 without CAD (the control group). The baseline clinical characteristics of the two groups are shown in Table 1. Patients in the CAD group were older, predominantly male and had a higher prevalence of hypertension, diabetes, and smoking compared to the control group. There were no differences in body mass index, history of hyperlipidemia, renal insufficiency, or alcohol drinking.

Table 1. Demographic and clinical characteristics

Characteristics	CAD group	Control group	p value
	n = 362	n = 214	
Age, y	65.0 ± 11.4	60.0 ± 12.7	< 0.01
Male, %	76.0	63.1	< 0.01
Hypertension, %	51.9	39.3	< 0.01
Diabetes, %	30.4	15.9	< 0.01
Hypercholesterolemia, %	13.3	11.2	0.52
Body mass index, Kg/m ²	25.2 ± 3.9	25.6 ± 4.3	0.27
Current smoker, %	48.3	35.5	< 0.01
Current alcohol drinker, %	21.5	24.8	0.41
Renal insufficiency, %	6.6	6.1	0.86

CAD: coronary artery disease. Values are prevalence or mean ± SD.

Relationship between polymorphism and CAD

The frequencies of the variant M235T genotypes in the CAD group were MM 3.3%, MT 52.2%, and TT 44.5%, not significantly different from those in the control group (MM 3.7%, MT 49.5%, and TT 46.7%) (p = 0.82). The frequencies of the T235 homozygote and T allele were also not significantly different between the two groups (p = 0.73 and 0.79, respectively) (Table 2).

Multivariate analysis

In multivariate analysis, age, sex, hypertension, diabetes and smoking were all independent parameters associated with the presence of CAD (p < 0.01). The ORs were 1.04 for age, 1.89 for male, 1.48 for hypertension, 2.09 for diabetes, and 1.90 for smoking (all p < 0.05). However, the ORs were 0.93 for T235 homozygote and 0.96 for T allele (both p > 0.05). Even after other conventional CAD risk factors were adjusted, the ORs were 0.85 for T235 homozygote, and 0.91 for T allele (both p > 0.05). The presence of T235 homozygote or T allele was not associated with the existence of CAD in this study (Table 3).

Table 2. Genotype and allelic frequencies of the angiotensinogen M235T polymorphism in the CAD population

	MM (%)	MT (%)	TT (%)	M (%)	T (%)
Control group (n = 214)	8 (3.7)	106 (49.5)	100 (46.7)	28.5	71.5
CAD group (n = 362)	12 (3.3)	189 (52.2)	161 (44.5)	29.4	70.6
p value		0.82			0.79

CAD: coronary artery disease.

Table 3. Crude and adjusted odds ratios of T235 homozygote and T allele associated with coronary artery disease

	Unadjusted odds ratio (95% CI)	p value	Adjusted odds ratio (95% CI)	p value
T homozygote	0.93 (0.66-1.31)	0.68	0.85 (0.60-1.23)	0.39
T allele	0.96 (0.74-1.25)	0.74	0.91 (0.69-1.20)	0.49

These models were adjusted for the following risk factors: age, sex, smoke, hypertension, and diabetes. CI: confidence interval.

DISCUSSION

We provide evidence that in Taiwanese, the incidence of AGT M235T polymorphism in CAD patients is not different from that in controls. Unlike previous reports, our study only targeted patients undergoing coronary angiography.^{1,3,13,18} CAD is a multifactorial disease influenced by both environmental and genetic factors, namely the “gene-environment-disease” association. Furthermore, multivariate analysis showed that age, sex, hypertension, diabetes, and smoking were risk factors independently associated with the presence of CAD. No AGT genotype differences were found in hypertension, diabetes and male subgroup analysis.

In the Slovenic population, the presence of AGT T235 homozygote was associated with a 2-fold increase in myocardial infarction risk.¹⁵ In our study population, 72% of the CAD patients were noted to have conventional risk factors, showing that nearly one quarter of the CAD patients did not have these risk factors. We found that the AGT M235T polymorphism was not related to the presence of CAD. AGT is produced by the liver and cleaved to angiotensin I by renin, which is secreted by the kidney when blood pressure lowers. Angiotensin I then is converted to angiotensin II, which is a potent stimulus of myocardial fibrosis and hypertrophy, activates sympathetic nervous system, stimulates fibroblast proliferation, and stimulates vasoconstriction. Although the AGT T allele had a significant effect on the levels of AGT, the presence of AGT T allele was found to be related to only a small elevation in the concentration of AGT (about 7-11%).⁶ This may be a reason why many reports have shown conflicting results.

In the Spanish and New Zealand populations, T235 homozygote was associated with an increased risk of CAD.^{1,3} Nevertheless, the distribution of AGT genotype is an ethnic difference. Asians and Blacks have higher frequencies of T235 homozygote than the Caucasian population.⁶ We found that the AGT genotypes were MM in 3.7%, MT in 49.5%, and TT in 46.7% in our con-

trol group. The frequency of the T 235 homozygote in Taiwanese was higher than that reported in the Caucasian populations (47% vs. 20%, respectively). This finding can further explain the different results seen here compared to previous studies. Interestingly, our data suggested that T235 homozygote might have a protective role in developing CAD, with a risk reduction by 15%. However, to reach a definite conclusion, further studies with larger sample size will be required.

The identification of patients with microvascular disease was not complete, as only patients presenting with ischemic chest pain and found to have no fixed stenosis received methylergonovine coronary vasospasm test. Not all patients were screened. In our study, AGT M235T polymorphism analysis of patients who did receive methylergonovine coronary vasospasm testing did not show any significant difference compared to normal controls. Nonetheless, further studies correctly identifying the coronary vasospasm subgroup may give us additional information regarding the AGT M235T polymorphism distribution.

Study limitations

Patients undergoing coronary angiography study in our unit were enrolled consecutively into our study. Those with normal coronary arteries were defined as our control group. Thus, the distribution of AGT polymorphism was not compatible with the Hardy-Weinberg equilibrium.

CONCLUSION

This study shows that in Taiwanese, the presence of T235 homozygote of the AGT gene is not associated with the existence of CAD. Before a definite conclusion is drawn on these correlations, further studies with a larger study population are mandatory, and further studies on other gene polymorphisms are also necessary in patients with CAD.

REFERENCES

1. Katsuya T, Koike G, Yee TW, et al. Association of angiotensinogen gene T235 variant with increased risk of coronary heart disease. *Lancet* 1995;345:1600-3.
2. Brscic E, Bergerone S, Gagnor A, et al. Acute myocardial infarction in young adults: prognostic role of angiotensin-converting enzyme, angiotensin II type I receptor, apolipoprotein E, endothelial constitutive nitric oxide synthase, and glycoprotein IIIa genetic polymorphisms at medium-term follow-up. *Am Heart J* 2000;139:979-84.
3. Rodriguez-Perez JC, Rodriguez-Esparragon F, Hernandez-Perera O, et al. Association of angiotensinogen M235T and A (-6) G gene polymorphisms with coronary heart disease with independence of essential hypertension: the PROCAGENE study. *Prospective Cardiac Gene. J Am Coll Cardiol* 2001;37:1536-42.
4. Sato N, Katsuya T, Nakagawa T, et al. Nine polymorphisms of angiotensinogen gene in the susceptibility to essential hypertension. *Life Sciences* 2000;68:259-72.
5. Danser AHJ, Derkx FHM, Hense HW, et al. Angiotensinogen (M235T) and angiotensin-converting enzyme (I/D) polymorphisms in association with plasma rennin and prorenin levels. *J Hypertens* 1998;16:1879-83.
6. Staessen JA, Kuznetsova T, Wang JG, et al. M235T angiotensinogen gene polymorphism and cardiovascular renal risk. *J Hypertens* 1999;17:9-17.
7. Davis D, Liyou N, Lockwood D, et al. Angiotensinogen genotype, plasma protein and mRNA concentration in isolated systolic hypertension. *Clin Genet* 2002;61:363-8.
8. Jeunemaitre X, Soubrier F, Kotelevtsev YV, et al. Molecular basis of human hypertension: role of angiotensinogen. *Cell* 1992;71:169-80.
9. Gardemann A, Stricker J, Humme J, et al. Angiotensinogen T174M and M235T gene polymorphisms are associated with the extent of coronary atherosclerosis. *Atherosclerosis* 1999;145:309-14.
10. Volzke H, Hertwig S, Rettig R, et al. The angiotensinogen 235T variant is associated with an increased risk of restenosis after percutaneous transluminal coronary angioplasty. *Clin Sci* 2000;99:19-25.
11. Chou HT, Hung JS, Chen YT, et al. Association between angiotensinogen gene M235T polymorphism and mitral valve prolapse syndrome in Taiwan Chinese. *J Heart Valve Dis* 2002;11:830-6.
12. Tang W, Devereux RB, Rao DC, et al. Associations between angiotensinogen gene variants and left ventricular mass and function in the HyperGEN study. *Am Heart J* 2002;143:854-60.
13. Fernández-Arcás N, Dieguez-Lucena JL, Muñoz-Morán E, et al. Both alleles of the M235T polymorphism of the angiotensinogen gene can be a risk factor for myocardial infarction. *Clin Genet* 2001;60:52-7.
14. Frishberg Y, Becker-Cohen R, Halle D, et al. Genetic polymorphisms of the rennin-angiotensin system and the outcome of focal segmental glomerulosclerosis in children. *Kidney Int* 1998;54:1843-9.
15. Petrovič D, Zorc M, Kanič V, et al. Interaction between gene polymorphisms of rennin-angiotensin system and metabolic risk factors in premature myocardial infarction. *Angiology* 2001;52:247-52.
16. Losito A, Kalidas K, Santoni S, et al. Polymorphism of renin-angiotensin system genes in dialysis patients — association with cerebrovascular disease. *Nephrol Dial Transplant* 2002;17:2184-8.
17. Russ AP, Maerz W, Ruzicka V, et al. Rapid detection of the hypertension-associated met235-to-thr allele of the human angiotensinogen gene. *Hum Molec Genet* 1993;2:609-10.
18. Ko YL, Ko YS, Wang SM, et al. Angiotensinogen and angiotensin-I converting enzyme gene polymorphisms and the risk of coronary artery disease in Chinese. *Hum Genet* 1997;100:210-4.