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The Association of Angiotensinogen (AGT M235T) Gene Polymorphism and Essential Hypertension in Thai Post-Menopausal Women

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ABSTRACT

Objectives: This study aims to determine the relationship between angiotensinogen (AGT) M235T polymorphism and hypertension among post-menopausal Thai women.

Materials and Methods: Case-control study was conducted. The study group was those who had hypertension or previously diagnosed and, the control were those who had no hypertension. Blood samples were taken for AGT M235T allelic characterization using allele specific oligonucleotides (ASO) PCR.

Results: Of 255 post-menopausal women, 128 had hypertension, regarded as "hypertension group", the other 127 without hypertension, regarded as "control group". The presence of AGT M235T polymorphism was 76.5% for homozygous mutation (73.4% for hypertension group and 79.5% for control group), 21.2% for heterozygous mutation (25.0% for hypertension group and 17.3% for control group, respectively) and 2.4% for homozygous wild-type (1.6% for hypertension group and 3.2% for control group, respectively). Distribution of MM, MT and TT genotypes was not significantly different between both group (p=0.251).

Conclusions: Interestingly, overall TT genotype was much higher than that of TM and MM in post-menopausal Thai women. AGT M235T polymorphism was not significantly associated with hypertension, though TT genotype tended to give a small risk. They may not serve as a good genetic marker for essential hypertension among Thai population.

Keywords: angiotensinogen (AGT) gene, AGT M235T polymorphism, essential hypertension, post-menopausal women

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การศึกษาความสัมพันธ์ระหว่างการเปลี่ยนแปลงของยีน angiotensinogen (AGT M235T) กับการเกิดโรคความดันโลหิตสูงในสตรีวัยหมดระดู

ธิติกาญจน์ เลิศหิรัญวงศ์, วีรวิทย์ ปิยะมงคล

บทคัดย่อ

วัตถุประสงค์: *เป็นการศึกษาความสัมพันธ์ระหว่างการเปลี่ยนแปลงของยีน angiotensinogen (AGT) กับความดันโลหิตสูงในสตรีไทยวัยหมดระดู*

วิธีการ: *เป็นการศึกษาแบบ case-control กลุ่มศึกษาคือกลุ่มที่มีความดันโลหิตสูงหรือเคยได้รับการวินิจฉัยว่าเป็นความดันโลหิตสูง และกลุ่มเปรียบเทียบคือกลุ่มที่ไม่มีความดันโลหิตสูง เก็บตัวอย่างเลือดเพื่อการวิเคราะห์คู่ยีน AGT M235T โดยใช้ปฏิกิริยาลูกโซ่*

ผลการศึกษา: *จากสตรีวัยหมดระดูจำนวน 255 คน 128 คน มีความดันโลหิตสูงจัดเป็นกลุ่มความดันโลหิตสูง อีก 127 คน ไม่มีความดันโลหิตสูงจัดเป็นกลุ่มควบคุม พบว่าการเปลี่ยนแปลงของยีน AGT M235T ร้อยละ 76.5 เป็นแบบ homozygous (ร้อยละ 73.4 สำหรับกลุ่มความดันโลหิตสูง และร้อยละ 79.5 สำหรับกลุ่มควบคุม) ร้อยละ 21.2 เป็นแบบ heterozygous (ร้อยละ 25.0 สำหรับกลุ่มความดันโลหิตสูง และร้อยละ 17.3 สำหรับกลุ่มควบคุม) และร้อยละ 2.4 สำหรับ homozygous wild-type (ร้อยละ 1.6 สำหรับกลุ่มความดันโลหิตสูง และร้อยละ 3.2 สำหรับกลุ่มควบคุม) การกระจายของยีน MM, MT และ TT ระหว่างสองกลุ่มไม่แตกต่างกันอย่างมีนัยสำคัญ (p=0.251)*

สรุป: *ข้อมูลที่น่าสนใจคือ พบอัลลีล TT มากกว่า TM และ MM ในสตรีไทยวัยหมดระดูอย่างมาก การเปลี่ยนแปลงของยีน AGT M235T ไม่สัมพันธ์กับความดันโลหิตสูงอย่างมีนัยสำคัญ แม้ว่าอัลลีล TT มีแนวโน้มที่จะเพิ่มความเสี่ยงเล็กน้อย การตรวจยีนนี้จึงอาจไม่ได้เป็นเครื่องบ่งชี้ทางพันธุกรรมสำหรับความดันโลหิตสูงในประชากรไทย*

คำสำคัญ: *ยีนแองจิโอเท็นซิโนเจน, เอจีที เอ็ม 235 ที โพลีมอร์ฟิซึม, โรคความดันโลหิตสูง, สตรีวัยหมดระดู*

Introduction

One of the major causes of death in women is cardiovascular disease, accounting for about 45%[1]. The risk factors of cardiovascular disease can be categorized into non-modifiable group i.e. age, genetic factor, or family history of cardiovascular disease and modifiable group such as lifestyle obesity, smoking[1, 2]. Of all causes, essential hypertension is the most vital risk for morbidity and mortality of cardiovascular disease[3, 4]. In particular, the risks in menopausal women are even more augmented, accompanying with aging, increased BMI, insulin levels, sex hormones deficiency and importantly, genetic encoding renin angiotensin system, adrenergic system, estrogen related genes and aromatase gene[4].

Several genes have been studied in the pathogenesis of essential hypertension, particularly angiotensin converting enzyme (angiotensinogen) gene polymorphism located within intron 16, including M235T and T174M polymorphism. In spite of contradictory results, M235T polymorphism is reported to be associated with essential hypertension the most. There are reports of possible involvement of the angiotensinogen (AGT) gene by the increasing plasma levels of angiotensinogen, the protein substrate of angiotensin I acted upon by renin, in hypertensive subjects and in children of hypertensive parents[5]. However, the definite roles in the hypertensive pathogenesis of these polymorphisms is still unclear and they are only a hypothetical supporter to clinical hypertension[6].

The renin-angiotensin-aldosterone system plays an essential role in the regulation of systemic blood pressure. AGT, angiotensin precursor synthesized and released from the liver, is a necessary component of the system[7]. Due to its function in regulating systemic blood pressure, changes in its gene sequences are likely to be responsible in the pathogenesis of hypertension or other cardiovascular diseases[8, 9]. Several AGT gene variants, including the rs4762 (previously known as p.T174M) and rs699 (known as p.M235T), are associated with susceptibility to various cardiovascular risks, including essential hypertension[10-14], coronary heart disease[15-18], and type 2 diabetes[18]. In addition, these variants are also associated with the severity of atherosclerosis[19].

The amino acid substitutions of AGT gene, MET235THR or M235T variant, contain M235 and T235 alleles. The genotype distribution is described as homozygous M235 (MM), heterozygous M235T (MT) and homozygous T235 (TT). Although, many studies specified that such gene-trait or gene-to-gene interactions contribute to events leading to complex disorders, this subject was not fully understood yet, especially in different parts of the world. In addition, results from several reports on the impacts of AGT gene variants or their interactions with such disease risk traits are still controversial, in particular the studies from various ethnic groups[12, 15, 18, 20, 21].

Therefore, this study decided to determine the relationship between AGT M235T polymorphism and the occurrence of essential hypertension among Thai menopausal women.

Materials and Methods

During September, 2013 to October, 2014, a case-control study was prospectively conducted at Maharaj Nakorn Chiang Mai Hospital, Faculty of Medicine, Chiang Mai University, Thailand following ethical approval by the Institute's Review Board. Post-menopausal women who visited the Menopause Clinic were counseled and asked to join the study with written informed consent. Post-menopausal women were counted as those who missed their menstrual periods for twelve months or longer.

The study group was those patients who were diagnosed for essential hypertension either on their first visit (new cases) or those previously diagnosed and treated for hypertension. The diagnosis of essential hypertension was based on 1) an examination at the Menopause Clinic with a systolic blood pressure (SBP) of 140 mmHg or higher and/or a diastolic blood pressure (DBP) of 90 mmHg or higher after resting for 15 minutes, at least two measurements of 15 minutes interval[22], 2) well documented medical records indicating that the patients had hypertension as the criteria defined above,

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3) the patients were treated for hypertension by internists, regardless blood pressure at the time of enrollment, and 4) no clinical evidence of secondary hypertension. The control group was those post-menopausal women who had no hypertension, defined previously, had no medical records of hypertension and attended the Menopausal Clinic at the same period.

Exclusion criteria involved women with last menstrual period of less than 12 months, and those who were not willing to join the study. All women in both groups were asked for information concerning risk factors of hypertension, including alcohol consumption, cigarette smoking and familial history of dyslipidemia, hypertension, diabetes mellitus and other medical conditions. Additionally, those with underlying diseases i.e. diabetes mellitus, dyslipidemia or other medical conditions were enquired for details and their medical records were thoroughly reviewed.

Two ml of blood samples were collected from both groups in 2% EDTA tubes and kept at -20°C until use. Genomic DNA was extracted from blood samples using QIAamp DNA Mini Kit (Qiagen® GmbH, Hilden, Germany). The PCR mixture consisted of 200 M of each primer, 0.2mM deoxynucleoside triphosphates (dNTPs) (Roche Diagnostics (Thailand) Ltd., Bangkok, Thailand), 1x GeneAmp Buffer (10x GeneAmp Buffer contains 100mM Tris-HCl pH 8.3, 500mM KCl, 15mM MgCl2) (GenePlus Co., Ltd., Bangkok, Thailand) and 1U AmpliTaq Gold (GenePlus Co., Ltd., Bangkok, Thailand) and was made up to a total volume of 25 µl with double distilled water.

Allele specific oligonucleotides (ASO) PCR techniques were used to characterize AGT M235T allelic distribution. AGT gene was amplified using common upstream primer (AGT-forward : 5' AGC AGA GAG AGG TGC CTT ACC T 3') and two downstream primers as AGT-M235: 5' TG GAA CTG TGG CTG CTC CCT GAT 3' and AGT-T235: 5' GAT GGA TCT CTG GCT GCT CCC AGA C 3'[23]. AGT M235 allele gives rise to a 118bp product, while AGT T235 allele gives rise to a 98bp product. PCR begins with primary denaturation step at 95°C for 10 min and thermal cycles were 95°C for 45 min, 60°C for 45 min, 72°C for 1 min for 35 cycles, then followed with final extension step at 72°C for 10 min. PCR products were then analyzed on a 2% agarose gel electrophoresis stained with ethidium bromide. PCR products were visualized using ultraviolet on a trans-illuminator. AGT M235 allele shows a 118bp fragment, while AGT T235 allele shows a 98bp fragment (Fig. 1). The results of AGT M235T alleles were collected for further statistical analysis. The odd ratio of AGT M235T gene polymorphism in the development of essential hypertension among post-menopausal women was the main outcome.

Statistical analysis: The statistical analysis was performed using SPSS version 21.0 (IBM Corp. Released 2012; IBM SPSS Statistics for Windows, Version 21.0. Armonk, NY: IBM Corp). In comparison for baseline characteristics, continuous data, demonstrated as means±SD, were compared using T-test if normal distribution was confirmed or Mann-Whitney-U test if the data were not normal distributed, whereas categorical variables, demonstrated as percentages, were compared using Chi-Square test. The adjusted odd ratios and 95% confident interval were used to compare various risk factors using logistic regression analysis. The difference was considered to be significant at $P < 0.05$.

Results

A total of 255 post-menopausal women were invited and enrolled into the study, none were excluded. One hundred and twenty eight subjects had essential hypertension, regarded as "hypertension group", the other 127 did not have evidence of hypertension, regarded as "control group". The baseline characteristics of both groups are shown in Table 1 and 2. The mean body weight and height of both groups were comparable; however, the mean age and BMI of the hypertension group were significantly higher than that of the control group (62.2±6.4 vs 59.4±6.0, $p < 0.001$ and 24.0±3.4 vs 23.1±2.8, $p = 0.022$, respectively). Other demographic data including underlying diseases, i.e. dyslipidemia and diabetes mellitus were not significantly different between the two groups. In addition, the risk factors based on familial history and life styles of both groups

were also comparable, as demonstrated in Table 2.

Of all 255 post-menopausal women, the presence of AGT M235T polymorphism was 76.5% for homozygous mutation (73.4% for hypertension group and 79.5% for control group), 21.2% for heterozygous mutation (25.0% for hypertension group and 17.3% for control group, respectively) and 2.4% for homozygous wild-type (1.6% for hypertension group and 3.2% for control group, respectively). The distribution of the MM, MT and TT genotypes was not significantly different between the study (hypertension) and the control group (p=0.251). Interestingly, the overall TT genotype of AGT M235T polymorphism was much higher than that of TM and MM (Table 2). The allele frequencies of AGT M235T polymorphism in all subjects were 12.9% (14.1% for hypertension group and 11.8% for control group, respectively) for M235 and 87.1% (85.9% for hypertension group and 88.2% for control group, respectively) for T235. There was no significant difference in the allele frequencies between the hypertension and control groups (p=0.499) (Table 3).

Based on logistic regression analysis (Table 4), advanced age and high BMI were significant risk factors for development of hypertension in post-menopausal women. Interestingly, M235T gene is not a significant risk factor; however, TT genotype had a tendency to increase such a risk (Odd ratio of 2.099 with 95%CI = 1.064-4.142 and p-value 0.032) (Table 4). The model for hypertension prediction was analyzed as "Log [OR: hypertension] = - 7.218 + 0.077 [Age in years] + 0.108 [BMI]".

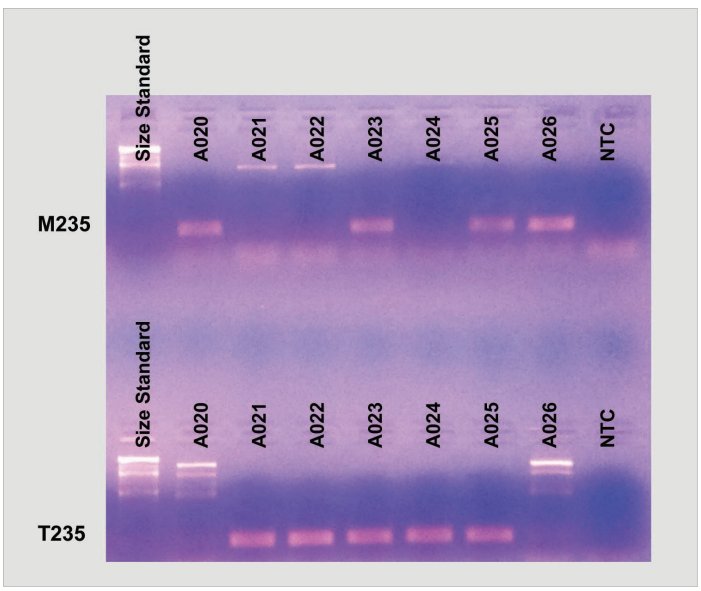

Fig. 1. Examples of analysis results using 2% agarose gel electrophoresis with ethidium bromide staining following PCR from genomic DNA extracted from peripheral blood of post-menopausal subjects. Allele specific oligonucleotides (ASO) PCR techniques were used to characterize AGT M235T allelic distribution. AGT M235 allele shows a 118bp fragment, while AGT T235 allele shows a 98bp fragment. Homozygous M235 (A020, A026), heterozygous M235/T235 (A023, A025) and homozygous T235 (A021, A022, A024) are demonstrated.

Table 1. Baseline characteristics of 255 post-menopausal women, 128 of the hypertension group, and 127 of the control group (continuous data).

Case	Hypertension Group (n=128)	Normal Control Group (n=127)	P
Age	62.2±6.4	59.4±6.0	< 0.001
BMI	24.0±3.4	23.1±2.8	0.022
Body weight (kg)	56.1±9.0	54.8±7.4	0.225
Height (cm)	152.9±5.6	154.14±5.4	0.074

* Student's T test

Table 2. Baseline characteristics of 255 post-menopausal women, 128 of the hypertension group, and 127 of the control group (categorical data).

	Hypertension group (n=128)	Control group (n=127)	P
Underlying disease: dyslipidemia	51 (39.84%)	42 (33.07%)	0.261
Underlying disease: diabetes mellitus	10 (7.81%)	4 (3.15%)	0.102
Familial history of hypertension	62 (48.44%)	58 (45.67%)	0.658
Familial history of dyslipidemia	27 (21.09%)	24 (18.90%)	0.661
Familial history of diabetes mellitus	29 (22.66%)	36 (28.35%)	0.297
Hormone replacement therapy	11 (8.6%)	9 (7.1%)	0.654
Life style: smoking	1 (0.78%)	0 (0.00%)	0.366
Life style: alcohol drinking	13 (10.2%)	15 (11.8%)	0.135
M235T Genotypes			
MM genotype (n, %)	2 (1.6%)	4 (3.2%)	Chi-square = 2.766
MT genotype (n, %)	32 (25.0%)	22 (17.3%)	p-value = 0.251
TT genotype (n, %)	94 (73.4%)	101 (79.5%)	

* Chi-Square test

Table 3. Two by two table shows AGT M235T polymorphism allelic distribution of the hypertension group and the control group of 255 post-menopausal women.

Alleles	Hypertension group (n=128)	Control group (n=127)	P
M235	36 (14.1%)	30 (11.8%)	Chi-square = 0.574
T235	220 (85.9%)	224 (88.2%)	p-value = 0.449
Total	256 (100.0%)	254 (100.0%)	

* Chi-square test, ** n=alleles

Table 4. Adjusted odd ratios for the risk of hypertension derived from logistic regression analysis of 255 post-menopausal women.

	p-value	Adjusted OR	95% Confident Interval for adjusted OR	
Age	0.001	1.080	1.035	1.128
BMI	0.013	1.115	1.023	1.214
Life style: alcohol drinking	0.340	2.977	0.317	27.946
Life style: smoking	0.306	4.175	0.270	64.598
Familial history of hypertension	0.228	0.690	0.378	1.261
Familial history of dyslipidemia	0.418	0.742	0.360	1.527
Familial history of diabetes mellitus	0.069	1.807	0.954	3.423
Underlying disease: dyslipidemia	0.445	0.804	0.459	1.408
Underlying disease: diabetes mellitus	0.118	0.360	0.100	1.294
T235	0.092	-	-	-
T235: TM	0.794	0.786	0.128	4.821
T235: TT	0.032	2.099	1.064	4.142

Discussion

Interestingly, in this study the frequency of TT genotype was much higher than that of MM and TM (76.5%, 2.4% and 21.2%, respectively) among post-menopausal women in northern Thailand, which is opposite to the other parts of the world. Our findings indicate that M235T polymorphism of angiotensinogen gene may not play an important role in the development of essential hypertension. Many studies on the association of the AGT M235T polymorphism and hypertension have reported in both western and eastern population[3, 24-28]. However, the data are still contradictory and there are not many data from this region of the world.

It is observed that essential hypertension is almost double in those with one or two hypertensive parents and several epidemiological studies point out that genetic factors account for a third of the variation in blood pressure in different populations[29]. Several genes have been connected; however none was obviously confirmed to be involved in pathogenesis of essential hypertension. One possible explanation is the interaction of multiple genes associated with renal sodium excretion which is necessary for homeostasis during low sodium intake and has become harmful with current high sodium intake habit. The subsequent volume expansion may induce hypertension.

AGT gene is one of the essential candidate genes, which has been studied extensively[3,12,14,30-32]. Pathogenesis of essential hypertension is complicated. However, the expression of AGT gene leads to an elevation of plasma levels of AGT, the protein substrate generating angiotensin I in response to renin[5], and accelerates the development of hypertension. M235T polymorphismwithin the AGT gene has been reported to be associated with primary hypertension. However, the results are still controversy. Most authors reported a significant effect of AGT M235T polymorphism to essential hypertension[3, 12, 32], whereas some failed to confirm such an evidence[30, 31], implying that different populations may have different AGT M235T polymorphism distribution. Theoretically, AGT gene may involve in the development of essential hypertension, however, several factors may influent the gene expression. For instance, the expression of the AGT gene in the liver is regulated by some specific cytokines, i.e. IL-1, IL-6 and TNF-a[33].

In this study, the frequency of the T235 allele was not statistically significant in associated with essential hypertensive (85.9% for hypertension group and 88.2%

for control group, respectively), Chi-square = 0.574, p-value = 0.449 (Table 3) which was different from those reported in Caucasian population. The variety in these results may be due to the difference in ethnicity. The results of this study suggest that there was no association between the M235T polymorphism in the gene encoding angiotensinogen in Thai population and essential hypertension.

Among the 128 post-menopausal Thai women with hypertension, 25.0 % were heterozygous (TM genotype) and 73.4% and 1.6% were homozygous (TT and MM genotypes); whereas among the healthy control post-menopausal Thai women, 17.3% were heterozygous (TM genotype) and 79.5% and 3.2% were homozygous (TT and MM genotypes), Chi-square = 2.766, p-value = 0.251 (Table 2). In the hypertension group, the T/M allele frequency ratio was 0.86/0.14, while it was 0.88/0.12 in the control group. Hardy Weinberg analysis was demonstrate as follow: $(p+q)^2 = p^2 + 2pq + q^2 : (0.13 + 0.87)^2 = (0.13)^2 + 2(0.13 \times 0.87) + (0.87)^2 = 1$.

The TT genotype and T allele frequencies of the AGT M235T polymorphism in the hypertension group were not statistically significant different from those in the control group. These results were different from those recently reported by Cheng JL who found the higher TT genotype and T235 allele frequencies of the AGT M235T polymorphism and an association to hypertension within Chinese population. In addition, the high frequencies of the T235 allele of AGT M235T polymorphism is associated with high cytokine concentrations (IL-1 and TNF-alpha) which can promote the transcription and expression of AGT gene, particularly in hypertensive patients with the 235TT genotype[33].

The frequency of the T235 allele was very high in this study. It seems that the T235 allele of the AGT M235T polymorphism is very common in the Southeast Asia populations. Moreover, there are evidence that the T235 allele confers no or minimal risk of hypertension with TT genotype. The findings in this study was consistent with previous reports by Ichihara et al[34] who studied in Japanese population and by Rotimi et al[28] who studied in the African-American population. Interestingly, the odd ratios for essential hypertension was not significantly different between the two groups in the univariate analysis (Table 2 and 3), but the TT genotype significantly, though minimally, increased the risk of hypertension in multivariate analysis after controlling other known risk factors (Table 4).

In this study, most demographic data including underlying diseases, i.e. dyslipidemia and diabetes mellitus, and risk factors based on familial history and life styles were not significantly different between the two groups. However, mean age and BMI of the hypertension group were significantly higher than that of the control group. The difference in these demographic characteristics may confound the statistical differences between the disease and control groups.

In conclusion, the T235 allele of the AGT M235T polymorphism was very high in post-menopausal Thai women. The AGT M235T polymorphism, TT, TM and MM genotypes, was not significantly associated with the risk of essential hypertension, though TT genotype tended to give a small risk. They may not serve as a good genetic marker for essential hypertension among Thai population.

Acknowledgments

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What is already known on this topic?

One of the major causes of death in women is cardiovascular disease, accounting for about 45%. Essential hypertension is the most vital risk for morbidity and mortality of cardiovascular disease. The renin-angiotensin-aldosterone system plays an essential role in the regulation of systemic blood pressure. Several AGT gene variants, including rs699 (known as p. M235T), are associated with susceptibility to various cardiovascular risks, including essential hypertension. The amino acid substitutions of AGT gene, MET235THR or M235T variant, contain M235 and T235 alleles. Results from several reports on the impacts of AGT gene variants or their interactions with such disease risk traits are still controversial, in particular the studies

from various ethnic groups.

What this study adds?

The T235 allele of the AGT M235T polymorphism was very high in post-menopausal Thai women. The AGT M235T polymorphism, TT, TM and MM genotypes, was not significantly associated with the risk of essential hypertension, though TT genotype tended to give a small risk. They may not serve as a good genetic marker for essential hypertension among Thai population.

Potential conflicts of interest

The authors declare no conflict of interest.

References

1. Shifren IS, Schiff I. Menopause. In: Berek JS, editor. Berek & Novak's Gynecology. 15 ed. Philadelphia: Wolters Kluwer | Lippincott Williams & Wilkins 2012;p. 1233-49.
2. Coylewright M, Reckelhoff JF, Ouyang P. Menopause and hypertension: an age-old debate. Hypertension 2008;51:952-9.
3. Caulfield M, Lavender P, Farrall M, Munroe P, Lawson M, Turner P, et al. Linkage of the angiotensinogen gene to essential hypertension. N Engl J Med 1994;330:1629-33.
4. Kimura S, Mullins JJ, Bunnemann B, Metzger R, Hilgenfeldt U, Zimmermann F, et al. High blood pressure in transgenic mice carrying the rat angiotensinogen gene. EMBO J 1992;11:821-7.
5. Watt GC, Harrap SB, Foy CJ, Holton DW, Edwards HV, Davidson HR, et al. Abnormalities of glucocorticoid metabolism and the renin-angiotensin system: a four-corners approach to the identification of genetic determinants of blood pressure. J Hypertens 1992;10:473-82.
6. Tamura K, Umemura S, Sumida Y, Nyui N, Kobayashi S, Ishigami T, et al. Effect of genetic deficiency of angiotensinogen on the renin-angiotensin system. Hypertension 1998;32:223-7.
7. Ambler SK, Brown RD. Genetic determinants of blood pressure regulation. J Cardiovasc Nurs 1999;13:59-77.
8. Schluter KD, Wenzel S. Angiotensin II: a hormone involved in and contributing to pro-hypertrophic cardiac networks and target of anti-hypertrophic cross-talks. Pharmacol Ther 2008;119:311-25.
9. Kumar R, Singh VP, Baker KM. The intracellular renin-angiotensin system: implications in cardiovascular remodeling. Curr Opin Nephrol Hypertens 2008;17:168-73.
10. Ragia G, Nikolaidis E, Tavridou A, Arvanitidis KI, Kanoni S, Dedoussis GV, et al. Renin-angiotensin-aldosterone system gene polymorphisms in coronary artery bypass graft surgery patients. J Renin Angiotensin Aldosterone Syst 2010;11:136-45.
11. Mohana VU, Swapna N, Surender RS, Vishnupriya S, Padma T. Gender-related association of AGT gene variants (M235T and T174M) with essential hypertension--a case-control study. Clin Exp Hypertens 2012;34:38-44.
12. Fang YJ, Deng HB, Thomas GN, Tzang CH, Li CX, Xu ZL, et al. Linkage of angiotensinogen gene polymorphisms with hypertension in a sibling study of Hong Kong Chinese. J Hypertens 2010;28:1203-9.
13. Xi B, Shen Y, Yan Y, Mi J. Association of polymorphisms in the AGT gene with essential hypertension in the Chinese population. J Renin Angiotensin Aldosterone Syst 2012;13:282-8.
14. Jeunemaitre X, Charru A, Chatellier G, Dumont C, Sassano P, Soubrier F, et al. M235T variant of the human angiotensinogen gene in unselected hypertensive patients. J Hypertens Suppl 1993;11:S80-S81.
15. Motawi T, Shaker O, Taha M, Sedrak H, Nabil M. Endothelial nitric oxide synthase and angiotensinogen gene polymorphism in coronary artery diseases in Egypt. Angiology 2011;62:191-7.
16. Katsuya T, Koike G, Yee TW, Sharpe N, Jackson R, Norton R, et al. Association of angiotensinogen gene T235 variant with increased risk of coronary heart disease. Lancet 1995;345:1600-3.
17. Goldenberg I, Moss AJ, Ryan D, McNitt S, Eberly SW, Zareba W. Polymorphism in the angiotensinogen gene, hypertension, and ethnic differences in the risk of recurrent coronary events. Hypertension 2006;48:693-9.
18. Mehri S, Koubaa N, Hammami S, Mahjoub S, Chaaba R, Nakbi A, et al. Genotypic interactions of renin-angiotensin system genes with diabetes type 2 in a Tunisian population. Life Sci 2010;87:49-54.
19. Lanz JR, Pereira AC, Lemos PA, Martinez E, Krieger JE. Angiotensinogen M235T polymorphism is associated with coronary artery disease severity. Clin Chim Acta 2005;362:176-81.
20. Ranjith N, Pegoraro RJ, Rom L, Lanning PA, Naidoo DP. Renin-angiotensin system and associated gene polymorphisms in myocardial infarction in young South African Indians. Cardiovasc J S Afr 2004;15:22-6.
21. Rodriguez-Perez JC, Rodriguez-Esparragon F, Hernandez-Perera O, Anabitarte A, Losada A, Medina A, 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.
22. Mancia G, De BG, Dominiczak A, Cifkova R, Fagard R, Germano G, et al. 2007 Guidelines for the management of arterial hypertension: The Task Force for the Management of Arterial Hypertension of the European Society of Hypertension (ESH) and of the European Society of Cardiology (ESC). Eur Heart J 2007;28:1462-536.
23. Morgan T, Craven C, Nelson L, Lalouel JM, Ward K.

Angiotensinogen T235 expression is elevated in decidual spiral arteries. J Clin Invest 1997;100:1406-15.
24. Kim YJ, Park MH, Park HS, Lee KS, Ha EH, Pang MG. Associations of polymorphisms of the angiotensinogen M235 polymorphism and angiotensin-converting-enzyme intron 16 insertion/deletion polymorphism with preeclampsia in Korean women. Eur J Obstet Gynecol Reprod Biol 2004;116:48-53.
25. Hata A, Namikawa C, Sasaki M, Sato K, Nakamura T, Tamura K, et al. Angiotensinogen as a risk factor for essential hypertension in Japan. J Clin Invest 1994;93:1285-7.
26. Brown MJ, Clayton D. Linkage of the angiotensin gene to essential hypertension. N Engl J Med 1994;331:1096-7.
27. Jeunemaitre X, Soubrier F, Kotelevtsev YV, Lifton RP, Williams CS, Charru A, et al. Molecular basis of human hypertension: role of angiotensinogen. Cell 1992;71:169-80.
28. Rotimi C, Morrison L, Cooper R, Oyejide C, Effiong E, Ladipo M, et al. Angiotensinogen gene in human hypertension. Lack of an association of the 235T allele among African Americans. Hypertension 1994;24:591-4.
29. Kupper N, Willemsen G, Riese H, Posthuma D, Boomsma DI, de Geus EJ. Heritability of daytime ambulatory blood pressure in an extended twin design. Hypertension 2005;45:80-5.
30. Mettimano M, Lanni A, Migneco A, Specchia ML, Romano-Spica V, Savi L. Angiotensin-related genes involved in essential hypertension: allelic distribution in an Italian population sample. Ital Heart J 2001;2:589-93.
31. Glavnik N, Petrovic D. M235T polymorphism of the angiotensinogen gene and insertion/deletion polymorphism of the angiotensin-1 converting enzyme gene in essential arterial hypertension in Caucasians. Folia Biol (Praha) 2007;53:69-70.
32. Lizanecz E, Pasztor ET, Mohacsi A, Papp Z, Edes I, Toth A. Mistyping of angiotensinogen M235T alleles. Hypertens Res 2006;29:197-201.
33. Cheng JL, Wang AL, Wan J. Association between the M235T polymorphism of the AGT gene and cytokines in patients with hypertension. Exp Ther Med 2012;3:509-12.
34. Ichihara S, Yokota M, Fujimura T, Kato S, Hirayama H, Tsunekawa A, et al. Lack of association between variants of the angiotensinogen gene and the risk of coronary artery disease in middle-aged Japanese men. Am Heart J 1997;134(2 Pt 1):260-5.