



Egyptian Society of Cardiology
The Egyptian Heart Journal

www.elsevier.com/locate/ehj
www.sciencedirect.com



CASE REPORT

Association between the Angiotensinogen (AGT) gene (M235T) polymorphism and Essential Hypertension in Egyptian patients



Marium M. Shamaa ^{a,*}, Hosny Fouad ^b, Medhat Haroun ^c, Mahmoud Hassanein ^d,
Mohamed Ayman Abdel Hay ^d

^a Dept. of Pharmacology, Faculty of Pharmacy, University of Pharos, Egypt

^b Dept. of Pharmacology, Faculty of Pharmacy, University of Alexandria, Egypt

^c Head of Biotechnology Dept., Institute of Graduate Studies and Research, University of Alexandria, Egypt

^d Dept. of Cardiology, Faculty of Medicine, University of Alexandria, Egypt

Received 26 August 2013; accepted 13 October 2013

Available online 22 November 2013

KEYWORDS

Essential hypertension;
AGT (M235T)
polymorphism;
Angiotensinogen gene

Abstract The pathogenesis of essential hypertension (EH) is affected by genetic and environmental factors. Mutations in hypertension-related genes can affect blood pressure (BP) via alteration of salt and water reabsorption by the nephron. The genes of the renin-angiotensin system (RAS) have been extensively studied because of the well documented role of this system in the control of BP. It has been previously shown that angiotensinogen (AGT) gene polymorphism could be associated with increased risk of EH. The current study evaluated the frequency of AGT (M235T) polymorphism in relation to EH in a group of Egyptian population. The study population included 83 hypertensive patients and 60 age and sex matched healthy control subjects. Restriction fragment length polymorphism-Polymerase chain reaction (RFLP-PCR) was used for the analysis of M235T polymorphism of AGT genes in peripheral blood samples of all patients and controls. The results revealed that there was a positive risk of developing EH when having the T allele whether in homozygous or heterozygous state. It was concluded that there was an association between AGT (M235T) gene polymorphism and the risk of developing EH.

© 2013 Production and hosting by Elsevier B.V. on behalf of Egyptian Society of Cardiology.

1. Introduction

Essential hypertension is a major risk factor for several cardiovascular diseases. It is a complex trait resulting from the interactions of multiple genetic and environmental factors. Moreover, not only genetic but also epigenetic inheritance plays a significant role. One can speculate that hypertension develops as a consequence of “errors” in the well-coordinated regulatory

* Corresponding author. Address: 6th AbuNawas St., in front of Arab Academy of Science and Technology, Miami, Alexandria, Egypt. Tel.: +20 35488324, mobile: +20 1227537121; fax: +20 33877149.

E-mail address: marium_ph2007@yahoo.com (M.M. Shamaa).

Peer review under responsibility of Egyptian Society of Cardiology.

systems of blood pressure.¹ This common disease-common variant concept suggests that the genetic heterogeneity underlying hypertension susceptibility could be relatively small. Therefore, it makes sense that variation in genes related to hypertension should be studied.² In recent years, the research is directed towards analysis of the disease on genetic level and explanation of the genetic bases of this disorder. Development of powerful and sensitive molecular techniques has resulted in widespread endeavors to dissect the genetic factors and their molecular defects accounting for hypertension in various populations. Today, the state of the art approach toward the diagnosis of primary hypertension involves analysis of the genetic bases for the disorder.³ The renin-angiotensin system (RAS) is a group of related hormones that act together to regulate BP by maintaining vascular tone and the balance of water and sodium. Angiotensinogen (AGT) is among the components that are involved in the activation/effector cascade of the RAS. It is suggested that AGT (M235T) polymorphism may be the functional genotype, as it affects the basal transcription rate of AGT, which could explain the association of the M235T genotypes with the plasma AGT concentration.⁴ Genetic polymorphisms in components of the RAS including AGT (M235T) are suggested to be associated with the pathogenesis of EH. However, obtained results are still debated.⁵

The analysis of RAS related genetic polymorphism of patients having EH has been carried out in Egyptian population on a limited scale. So, this study aimed to determine the association of genetic polymorphisms of the angiotensinogen (M235T) gene with EH in a cohort of genetically homogenous EH patients and normotensive controls (case-control study).

2. Materials and methods

The current study included 83 EH patients (55 male, 28 female) with mean age of 53.5 ± 7.4 years old. Also 60 healthy subjects (32 male, 28 female) matching in age and sex (51.3 ± 8.8 years old) were included as control group. The patients were selected from the outpatient clinic of the Cardiology Department, General Alexandria University Hospital during the period between 2009 and 2010. A written consent was obtained from all subjects accepting to participate in the study for both the clinical part and the genetic study. All subjects under study were subjected to thorough history taking and clinical examination with special emphasis on BP and BMI. Peripheral blood samples were collected from all subjects and routine investigations were done including FBS, lipid profile (total cholesterol, LDL – ch, HDL – ch and triglycerides) and renal function tests (serum urea and creatinine).

Essential hypertension was diagnosed in individuals with a systolic blood pressure (SBP) > 140 mmHg and/or a diastolic blood pressure (DBP) > 90 mmHg, for at least three consecutive blood pressure measurements. Blood pressure was measured using a mercury sphygmomanometer after a rest for at least 15 min in quiet condition and the pressure was determined as the average of the three measurements. Secondary forms of hypertension were excluded based on clinical history and laboratory investigations. The normotensive controls were healthy individuals with a negative history of hypertension and with a SBP < 140 mmHg and DBP < 90 mmHg measured on three separate occasions, also all study subjects were non diabetic with normal renal functions.

2.1. Genotyping

DNA samples were isolated from peripheral blood lymphocytes by the standard phenol extraction method. The AGT (M235T) polymorphisms was detected by RFLP PCR using the following primers: 5'-CCG TTT GTG CAG GGC CTG GCT CTC T-3' as forward primer and 5'-CAG GGT GCT GTC CAC ACT GGA CCC C-3' as reverse primer. Amplification resulted in a 165-bp product which was digested using restriction enzyme *Pseudomonas syringae* species (*PsyI*). Electrophoresis on a 3% (w/v) agarose gel containing 0.5 µg/ml ethidium bromide (Sigma–Aldrich, St. Louis, USA) and UV transillumination were used for analysis.

Statistical Analysis was carried out using SPSS version 15.0 software and the following tests were used for data analysis: Chi squared (χ^2) test, Monte Carlo Probability (MCP) and Fisher's Exact Probability (FEP) were used for analysis of categorical data, *T*-test was used for comparison between means, Odds ratio (OR) was used for the measurement of association.

3. Results

The results showed that the mean systolic and diastolic BP in hypertensive patients were (145.28 ± 7.0 and 91.2 ± 2.15 mmHg) while in normotensive subjects (control group) their corresponding values were (117.33 ± 4.27 and 79.17 ± 3.46 mmHg), respectively.

The results revealed that the frequency of the TT genotype was significantly ($\chi^2 = 53.784$, $P < 0.001$) increased in hypertensive patients. Furthermore, there was a positive risk of developing EH when having the TT genotype and the results were highly statistically significant (OR = 36.217, $P < 0.001$) for TT genotype compared to MM genotype (Tables 1 and 3).

Table 1 The frequency of AGT (M235T) polymorphic genotypes in normotensive subjects (control) and hypertensive patients.

	Control ($n = 60$)		Patients ($n = 83$)		χ^2 (p)
	No.	%	No.	%	
<i>AGT genotypes</i>					
MM	41	68.3	12	14.5	43.334 ^a (< 0.001)
MT	14	23.3	18	21.7	0.054 (0.816)
TT	5	8.3	53	63.9	44.530 ^a (< 0.001)
χ^2 (p)	53.784 ^a (< 0.001)				

χ^2 : Chi square test.

^a Statistically significant at $p \leq 0.05$.

Table 2 The frequency of AGT M and T alleles in normotensive subjects (control) and hypertensive patients.

	Control (<i>n</i> = 60)		Patients (<i>n</i> = 83)		χ^2 (<i>p</i>)
	Freq.	%	Freq.	%	
<i>AGT alleles</i>					
M	96	71.11	42	25.30	62.930 ^a (<0.001)
T	39	28.97	124	74.78	

 χ^2 : Chi square test.^a Statistically significant at $p \leq 0.05$.**Table 3** The risk of having AGT (M235T) polymorphism in relation to essential hypertension.

	Control (<i>n</i> = 60)		Patients (<i>n</i> = 83)		OR	95 % CI (LL–UL)	χ^2 (<i>p</i>)
	No.	%	No.	%			
<i>AGT genotypes</i>							
MM®	41	68.3	12	14.5	–	–	–
MT	14	23.3	18	21.7	4.393 ^a	1.700–11.353	9.869 ^a (0.002)
TT	5	8.3	53	63.9	36.217 ^a	11.816–111.008	53.920 ^a (<0.001)

 χ^2 : Chi square test.^a Statistically significant at $p \leq 0.05$.**Table 4** The risk of having T allele in relation to essential hypertension.

	Control (<i>n</i> = 60)		Patients (<i>n</i> = 83)		OR	95 % CI (LL–UL)	χ^2 (<i>p</i>)
	Freq. No.	%	Freq. No.	%			
<i>AGT alleles</i>							
M®	96	71.11	42	25.30	7.267 ^a	4.360–12.112	62.930 ^a (<0.001)
T	39	28.97	124	74.78			

 χ^2 : Chi square test.^a Statistically significant at $p \leq 0.05$.

The frequency of T allele was significantly ($\chi^2 = 62.930$, $P < 0.001$) increased in hypertensive patients. Furthermore, there was a positive risk of developing EH when having the T allele whether in homozygous or heterozygous state and the results were highly statistically significant (OR = 7.267, $P < 0.001$) (Tables 2 and 4) (see Fig. 1).

4. Discussion

Cardiovascular diseases are rapidly emerging as a major health concern in most developing countries, including Egypt, and hypertension is one of the major preventable causes of morbid-

ity and mortality from cardiovascular disease. Very recently, Arafa and Ez-elarab⁵ calculated the prevalence of prehypertension and hypertension based on the data of 6671 individuals from the EDHS 2008. The prevalence of prehypertension and hypertension in Egypt were 57.2% and 17.6%, respectively. Among Egyptian population, the responsiveness to anti-hypertensive drugs is very poor, thus Prediction of drug effect in a particular individual is a useful and important goal as the extent of blood pressure lowering by anti-hypertensive agents is difficult to predict for individual patients, even when evaluated in the context of biochemical or demographic information. Genetic predictors (mainly single nucleotide

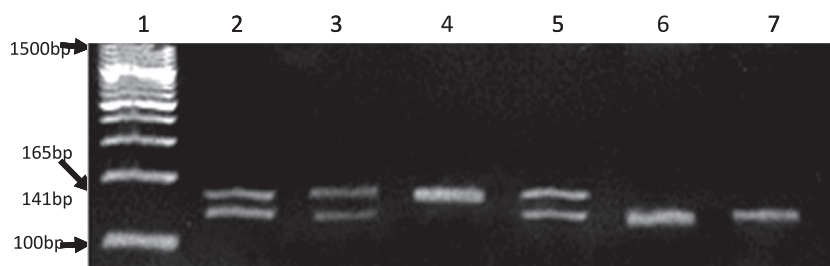


Figure 1 Agarose gel (3%) electrophoresis for RFLP digested PCR products by *Pst*I restriction enzyme. Lane 1, represents 100 bp ladder base pair marker. Lane (2,3,5) represent restricted fragments of PCR products of patients with MT genotype(141,165 bp). Lane 4, represents unrestricted PCR product of patient with MM genotype (165 bp). Lane 6,7, represent restricted PCR products of patients with TT genotype(141 bp).

polymorphisms, SNPs) have been the focus of several studies and are gaining much attention.⁶

The analysis of AGT (M235T) gene polymorphism of patients having EH has been carried out in Egyptian population on a limited scale. The potential role of the AGT gene in predisposition to hypertension is controversial. Since Jeunemaitre et al.⁷ and Hata et al.⁸ previously reported higher prevalence of the T235 allele among hypertensive than among normotensive subjects, a large number of studies have explored the relationship between AGT gene polymorphism and HT. In the current study, the percentage of the TT genotype was significantly more frequent in hypertensive patients than in the control group ($p < 0.001$). Moreover, the T allele was the risk allele for hypertension where the study subjects carrying T allele were at more than sevenfold higher risk for hypertension (OR = 7.267, $p < 0.001$). The results of this study were in accordance with studies that were conducted in the Malaysian subjects,⁹ Han Chinese population,¹⁰ in Uzbek males,¹¹ in South India,¹² Hong Kong Chinese population¹³ and in Tunisian individuals.¹⁴

On the contrary, there was no significant association between EH and AGT MT and TT genotypes in England,^{15,16} Caucasians,¹⁷ in the Mongolian population,^{18,19} Lebanese population²⁰ and in India.²¹ It has been suggested that the population heterogeneity in the association of AGT (M235T) polymorphism with essential hypertension may be due to significant variations of population backgrounds.²²

This study suggests the existence of a linkage between AGT gene polymorphism and the pathogenesis of essential hypertension. These findings might help to manipulate the therapeutic strategies in those cases as the potential for utility of genetic characterization of individual patients as a predictor of anti-hypertensive response has been realized.

5. Conclusion and recommendations

The present study provided an additional observation concerning AGT (M235T) polymorphic genotypes in a sample of Egyptian patients with EH. From this study it was concluded that:

1. The frequency of T allele was higher in hypertensive patients (75%) as compared to control subjects (29%). On the contrary, the M allele was less frequent in hypertensive patients (25%) as compared to control subjects (71%).
2. Homozygosity for the AGT T allele was a risk factor for EH. Moreover, the presence of AGT T allele, whether in homozygous or heterozygous state, was a risk factor for EH.

It is recommended that:

1. Future studies should investigate the influence of genetic polymorphisms of the AGT (M235T) polymorphic genotypes on response to anti-hypertensive therapy.
2. Future studies should evaluate the association of the AGT (M235T) polymorphic genotypes in other human diseases affected by alterations in the RAS.

Conflict of interest

None.

References

1. Kunes J, Zicha J. The interaction of genetic and environmental factors in the etiology of hypertension. *Physiol Res* 2009;**58**:S33–41.
2. Balam-ortiz E, Esquivel-villarreal A, Alfaro-ruiz L, Carrillo K, Elizalde A, Gil T, et al. Variants and haplotypes in angiotensinogen gene are associated with plasmatic angiotensinogen level in Mexican population. *Am J Med Sci* 2011;**342**:205–11.
3. Elzanaty F, Way A. Egypt Demographic and Health Survey 2008. Recommended citation. Ministry of Health El-Zanaty and Associates and Macro International. Chapter 17: Ad Health 2009; 66: 228–235.
4. Brand-Herrmann SM, Köpke K, Reichenberger F, Schmidt-Petersen K, Reineke T, Paul M, et al. Angiotensinogen promoter haplotypes are associated with blood pressure in untreated hypertensives. *J Hypertens* 2004;**22**:1289–97.
5. Unger T, Paulis L, Sica DA. Therapeutic perspectives in hypertension: novel means for renin–angiotensin–aldosterone system modulation and emerging device-based approaches. *Eur Heart J* 2011;**32**:2739–47.
6. Arafa NA, Ez-clarab HS. Epidemiology of prehypertension and hypertension among Egyptian adults. *Egypt J Commun Med* 2011;**29**:1–18.
7. Jeunemaitre X, Charu A, Chatellier G, Dumont C, Sassano P, Soubrier F, et al. M235T variant of the human angiotensinogen gene in unselected hypertensive patients. *J Hypertens* 1993;**11**:80–1.
8. Hata A, Namikaw T, Sasaki M, Sato K, Nokamura T, Tamura K, et al. Angiotensinogen as a risk factor for essential hypertension in Japan. *J Clin Invest* 1994;**93**:1285–7.
9. Say YH, Ling KH, Duraisamy G, Isaac S, Rosli R. Angiotensinogen M235T gene variants and its association with essential hypertension and plasma renin activity in Malaysian subjects: a case control study. *BMC Cardiovasc Disord* 2005;**5**:7–13.
10. Ji LD, Zhang LN, Shen P, Wang P, Zhang YM. Association of angiotensinogen gene M235T and angiotensin-converting enzyme gene I/D polymorphisms with essential hypertension in Han Chinese population: ameta-analysis. *J Hypertens* 2010;**28**:419–28.
11. Kurbanova D, Eliseyeva M. Genetic background of left ventricular hypertrophy in Uzbek hypertensive men. *Türk Kardiyol Dern arş* 2010;**38**:466–72.
12. Gopi Chand M, Srinath J, Rao RS, Lakkakula BV, Kumar S, Rao VR. Association between the M268T polymorphism in the angiotensinogen gene and essential hypertension in a South Indian population. *Biochem Genet* 2011;**49**:474–82.
13. 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.
14. Mehri S, Mahjoub S, Hammami S, Zaroui A, Frih A, Betbout F. Renin-angiotensin system polymorphisms in relation to hypertension status and obesity in a Tunisian population. *Mol Biol Rep* 2011;**23**:309–16.
15. 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;**30**:1624–33.
16. Hingorani AD, Sharma P, Jia H, Hopper R. Blood pressure and the M235T polymorphism of the angiotensinogen gene. *Hypertens* 1996;**28**:907–11.
17. Glavnik N, Petrovič 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.
18. Bautista LE, Vargas CI, Oróstegui M, Gamarra G. Population-based case-control study of renin–angiotensin system genes polymorphisms and hypertension among Hispanics. *Hypertens Res* 2008;**31**:401–8.

19. Ying CQ, Wang YH, Wu ZL, Fang MW, Wang J, Li YS, et al. Association of the renin gene polymorphism, three angiotensinogen gene polymorphisms and the haplotypes with essential hypertension in the Mongolian population. *Clin Exp Hypertens* 2010;**32**:293–300.
20. Saab YB, Gard PR, Overall ADJ. The association of hypertension with renin–angiotensin system gene polymorphisms in the Lebanese population. *J Renin–Angiotensin–Aldosterone Syst* 2011;**12**:588–94.
21. 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.
22. Ortiz EB, Villarreal AE, Hernandez DH, et al. Hypercontrols in genotype-phenotype analysis reveal ancestral haplotypes associated with essential hypertension. *J Am Heart Assoc Inc* 2012;**59**:847–53.