Journal of Medical Sciences

ISSN 1682-4474

science alert

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Research Paper

J. Med. Sci., 14 (4): 168-173
15th May, 2014
DOI: 10.3923/jms.2014.168.173

Relationship between Angiotensinogen gene T174M Polymorphism and Essential Hypertension in a Sample of Algerian Population: Case Control Study

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Essential hypertension is a multifactorial complex trait. It affects around one billion people worldwide. Many genes have been incriminated in its onset. This study aimed to determine the association of the AGT T174M gene variant with essential hypertension in a sample of Algerian population of the Oran city. In this study, we involved 350 subjects, 180 hypertensives and 170 normotensives. Consents were obtained from all the participated subjects. Polymerase Chain Reaction (PCR) combined with Restrictive Fragment Length Polymorphism (RFLP) was used to detect the T174M variant of angiotensinogen (AGT) gene. Blood pressure, body height and weight, fasting blood glucose and serum lipid were measured in all subjects. The genotypic and allelic distribution of the T174M variant of the AGT gene did not differ in hypertensives and normotensives group (OR = 1.05; 95% CI [0.583-1.932]; $\chi^2 = 5.298$; $p>0.05$; $\chi^2 = 5.692$; $p>0.05$), where the frequency of genotypes in the patient with essential hypertension was TT: 64.4%; TM: 20.5%; MM: 15% vs. TT: 55.8%; TM: 19.4%; MM: 24.7% for the controls. The allelic frequency was 0.7 vs. 0.66 for the T allele and 0.26 vs. 0.34 for the mutant allele in hypertensives and controls, respectively. This study shows that the T174M variant of the AGT gene is not associated with essential hypertension in this sample of Algerian population of the Oran city.

Key words: Renin angiotensin system, angiotensinogen gene, T174M gene polymorphism, essential hypertension, Algerian population

JMS (ISSN 1682-4474) is an International, peer-reviewed scientific journal that publishes original article in experimental & clinical medicine and related disciplines such as molecular biology, biochemistry, genetics, biophysics, bio-and medical technology. JMS is issued eight times per year on paper and in electronic format.

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INTRODUCTION

Hypertension is a chronic elevation of blood pressure, defined by systolic/diastolic blood pressure (SBP/DBP) above 140/90 mmHg. It affects 20- 30% of the population worldwide and will alarmingly rise to 1.5 billion by 2020 (Kearney *et al*., 2005). It is thought to be a multifactorial disorder causing severe damage to human health. It is estimated that over 95% of adult hypertension is of the Essential Hypertension (EH) type (Dosh, 2002). The pathogenesis of EH is determined by both genetic and environmental factors, such as obesity, dietary salt intake, alcohol consumption, stress and in which genetic contributes up to 30-50% (Newhouse *et al*., 2005). Genome-wide association studies have identified more than 100 quantitative trait loci attributed to essential hypertension across the genome, especially in chromosomes 1, 2, 3, 17 and 18 (Cowley, 2006). Most of these candidate genes are involved directly or indirectly in the regulation of the blood pressure, especially the genes of the renin angiotensin aldosterone system (Kato, 2002; Matsubara, 2000). Some studies have found a possible association between the genes encoding for the components of the RAAS such as the angiotensinogen (AGT) (Procopciuc *et al*., 2002; Say *et al*., 2005) the Angiotensin Converting Enzyme (ACE), Angiotensin II Type 1 Receptor (AT1R) and essential hypertension.

The Angiotensin Gene (AGT) is located on chromosome 1 q42-q43 (Isa *et al*., 1990). It comprises five exons and four introns (Gaillard *et al*., 1989). From the identified fifteen molecular variants, only three have so far been reported to have a possible genetic association with hypertension (Jeunemaitre *et al*., 1992). One of these variants encodes threonine instead of methionine at position 235(T235) (Jeunemaitre *et al*., 1992, 1993), the others encode methionine instead of threonine at position 174. Association studies of the AGT T174M have yielded conflicting results. Some of them have reported a possible association (Say *et al*., 2005; Jeunemaitre *et al*., 1992, 1993; Caulfield *et al*., 1994; Lee *et al*., 1996; Glavnik and Petrovic, 2007; Vasku *et al*., 2002; Corvol and Jeunemaitre, 1997; Zhu *et al*., 2003; Hata *et al*., 1994) while it did not in other studies (Rutledge *et al*., 1994; Mustafina *et al*., 2002; Rotimi *et al*., 1994).

In this study, we aim to investigate the possible association of the AGT T174M gene polymorphism and essential hypertension in Algerian population from the city of Oran where little is known about the genetic background of this prevalent disease.

MATERIALS AND METHODS

Study population: A case control study comprised a total of 350 subjects from the city of Oran in Algeria. After giving an informed consent, 170 normotensive subjects were selected from local blood donors and 180 hypertensive patients from the local health center.

All hypertensive patients included in the study were diagnosed as suffering from primary hypertension. Hypertensives defined as having an elevated systolic blood pressure SBD≥140 mmHg and sustained diastolic blood pressure DBP≥90 mmHg or who were currently receiving antihypertensive therapy. The blood pressure BP was measured with a Sphygmomanometer (KDM CE 0123), where the person is in sitting position, feet placed on the floor, left arm relaxed and placed on the table at heart level and hand palm up. The person should have an empty bladder and should not have had moderate or intensive physical activity, smoked or drank alcohol during the previous 30 min. For analysis, the last BP measurement was considered, as long as the difference between them was not larger than 5 mmHg. In case of larger differences, BP was measured two further times, with 3 min intervals and the last measurement was considered. Any subjects with possibility of a secondary hypertension and with diabetes type 2 were excluded. Hypertensive subjects whose parents both had hypertension were considered to have a positive family history of hypertension. Normotensives were defined as those with a blood pressure of less than 140/90 mmHg. Both groups with subjects under the influence of estrogen, thyroid and cortisol hormones were excluded. Height and weight of subjects were obtained by using the weighing scale. The Body Mass Index (BMI) of subjects was calculated as weight (kg)/height (m^2).

Sample collection and biochemical analysis: Four to five milliliters of blood samples were collected from the peripheral blood leukocytes into an EDTA K3 tube (FL medical, Italy). Plasma was separated from the blood by centrifugation method and stored at -20°C for further analysis. Plasma samples were analysed to determine the level of triglycerides (TG), high density lipoprotein cholesterol (HDL-C) and total cholesterol (TC). Low density lipoprotein cholesterol (LDL-C) was calculated by Friedewald formula.

Genotyping methods: DNA samples were isolated from peripheral blood lymphocytes using DNA extraction Kit (Strategene Inc., Canada) and quantified following spectrophotometric analysis. DNA fragments including

the T174M polymorphism of AGT gene were amplified by polymerase chain reaction and digested with *NcoI*. PCR with forward primers (5'TGGCACCCTGGCCTCTCTCT ATCT3') and reverse primer (5'CAGCCTGCATGAACCT GTCAATCT3') was performed in a volume of 25 μL following method described by Caulfield *et al*. (1994). The reaction mixture contained 500 ng DNA, 0, 2 μM of each of two primers, 0.2 mM of dNTP, 67 mM Tris-HCl (pH = 8.8), 16 mM $(NH_4)_2SO_4$, 0.01%Tween 20, 1.5 mM $MgCl_2$ and 1.25 U of Taq DNA polymerase (Ozyme, France).

DNA amplification was performed in thermal cycler (Master cycler, Eppendorf). Initial denaturation at 94°C for 5 min was followed by 30 cycles of denaturation at 94°C for 1 min, annealing at 65°C for 40 sec and chain elongation at 72°C for 1 min followed by final extension at 72°C for 10 min. PCR products were digested with 1 U of *NcoI* enzyme for 2 h at 37°C and electrophoresed on a 2% agarose gel with ethidium bromide staining.

Statistical analysis: All genotype groups obeyed the Hardy-Weinberg equilibrium. Data analysis was done with the help of an (SPSS Inc., Chicago, Illinois, USA) version 21.0. Clinical characteristics of all the subjects are expressed as Means±SD. Continuous variables were compared between the groups by using two-tailed student's t-test. Allele frequencies were calculated from genotype frequencies and were compared using chi-squared (χ^2) statistics. The p-value <0.05 was considered statistically significant.

RESULTS

Baseline characteristics: To examine the possible association of the AGT T174M variant with hypertension in a sample of Algerian population of Oran city, a total of 350 individuals comprising 180 hypertensives (44.4% women; 55.5% men) and 170 controls (47.05% women; 52.94% men) were enrolled in this study. The mean age of patients was 50.14±2.1 versus 47.02±3.2 in the control group and it was statistically different between the two groups (p>0.0001). The present study showed a positive association between hypertensives and controls with respect to age, SBP, DBP, BMI, family history of hypertension (p>0.0001).

In contrast, there was no statistical significant differences between case and controls in term of fasting blood glucose, HDL-C, LDL-C, TG (p>0.05). The baseline characteristics of all the subjects enrolled in this study are shown in Table 1.

Genotypes and allele frequencies: According to Table 2, the prevalence of AGT T174M variant in all subjects was 60.2% for homozygous wild type (64.4% for hypertensives and 55.8% for normotensives) and 20% for heterozygous mutation (20.5% for hypertensives and 19.4% for normotensives), 19.4% for homozygous mutation (15% for hypertensives and 24.7% for normotensives). The allele frequencies and genotype distribution of the T174M variant were in the Hardy-Weinberg equilibrium. In the present study, the genotype distribution of the Algerian population did not significantly differ between case and control subjects (Table 2) (OR = 1.05; 95% CI [0.583-1.932]; $\chi^2 = 5.298$; p>0.05; TT: 64.4%; TM: 20.5%; MM 15% vs. TT 55.88%; TM 19.41; MM: 24.70) neither in allele frequency for T allele (0.74 vs. 0.66) and for M allele (0.26 vs. 0.34) in hypertensives and controls, respectively. The effect of gender on HTN was also considered in this study in respect of the AGT gene. Table 3 shows the genotype and allelic distributions of the T174M variant among males and females in case and controls. There was not a significant difference between the prevalence of the M allele of hypertensives and normotensives group in female and male ($\chi^2 = 3.745$, p>0.05).

Table 1: Baseline characteristics of all the subjects

Parameters	HTN (n = 180)	NT (n = 170)	p
Gender M/F	80/100	90/80	NS
Age (years)	50.14±2.1	47.02±3.2	>0.0001
BMI (kg m^{-2})	28.29±2.38	25.55±3.41	>0.0001
SBP (mmHg)	134.04±11.21	119.06±7.86	>0.0001
DBP (mmHg)	80.97±9.28	69.82±7.11	>0.0001
Family history of hypertension	120 (66.6%)	30 (17.64%)	>0.01
FBG (mmol L^{-1})	3.4±0.70	3.1±0.80	NS
TC (mmol L^{-1})	5.2±0.80	5.4±0.20	NS
LDL-C (mmol L^{-1})	2.9±0.03	2.7±0.05	NS
HDL-C (mmol L^{-1})	1.2±0.02	1.3±0.04	NS
TG (mmol L^{-1})	1.4±0.40	1.4±0.50	NS

Values are given as Mean±SD, HT: Hypertensives, SBP, DBP: Systolic and diastolic blood pressures, MAP: Mean arterial pressure, FBG: Fasting blood glucose, TG: Triglycerides, TC: Total cholesterol, HDL-C: High density lipoprotein cholesterol, LDL-C: Low density lipoprotein cholesterol, NS: Non significant

Table 2: Distribution of AGT genotype and allele frequencies in case and control population

		Genotype distribution (%)			Allele frequency	
Population	n	TT	TM	MM	T	M
Hypertensives	180	116 (64.4%)	37(20.5%)	27(15%)	0.74	0.26
Normotensives	170	95(55.8%)	33(19.4%)	42(24.7%)	0.66	0.34
χ^2 value		5.298			4.124	
p-value		>0.05			>0.05	

Table 3: Genotypic distribution in male and female subjects

	Hypertensives		Normotensives		Total	
Genotypes	Male	Female	Male	Female	Male	Female
MM	10	17	32	10	42	27
MT	14	23	18	15	32	38
TT	56	60	40	55	96	115
Total	80	100	90	80	170	180
χ^2 value		3.745				
p-value		>0.05				

DISCUSSION

The detection of the association between a single nucleotide polymorphism and a complex trait such as essential hypertension is a controversial method. Nevertheless, it can be a useful to give a better understanding to the genetic etiology of such multifactorial human disease (Agachan *et al*., 2003).

The Renin Angiotensin Aldosterone System (RAAS) is an important system in regulating blood pressure and electrolyte balance (Niu *et al*., 1999). RAAS gene variants have been widely studied to determine the genetic susceptibility to HTN (Schmidt *et al*., 1995). The AGT gene is an important gene of this system; it has been implicated with essential hypertension in both Utah and French Caucasians since the findings of Jeunemaitre *et al*. (1993), they have reported that subjects carrying M235T genotypes with or without the T174M variant were more associated with hypertensives than with controls. Since then, many controversial studies have been carried out to investigate the eventual relationship between T174M, M235T genotypes and essential hypertension (Tiret *et al*., 1995; Chiang *et al*., 1997). These controversial findings prompted us to study for the first time, the polymorphism of the AGT gene in Algerian hypertensive population of the Oran city.

In this candidate gene study, we tried to evaluate the possible association between T174M variant and essential hypertension. Our results did not show significant differences in the genotypic and allelic frequencies between hypertensive and control subjects (OR = 1.05; 95% CI [0.583-1.932]; $\chi^2 = 5.298$; $p>0.05$) and thus it did not confirm the association between the 174M allele and essential hypertension. These findings joined previous studies done in different populations such as in Africans/African-Americans and some Asians (Glavnik and Petrovic, 2007; Vasku *et al*., 2002; Rotimi *et al*., 1994; Agachan *et al*., 2003) while other studies have shown a significant positive association of the T174M and M235T polymorphism with HTN (Say *et al*., 2005, Jeunemaitre *et al*., 1992, 1993; Lee *et al*., 1996; Corvol and Jeunemaitre, 1997; Niu *et al*., 1999; Tiret *et al*., 1995; Chiang *et al*., 1997; Caulfield *et al*., 2003; Nakajima *et al*., 2004; Charita *et al*., 2012; Mohana *et al*., 2012; Chand *et al*., 2011; Martinez *et al*., 2002). However, a positive association does not prove necessarily a causal relationship; it can only provide important information regarding the clinical importance of a genetic marker.

In addition, we have noticed a high risk for individuals with a positive family history of HTN besides the higher frequency of the T allele in hypertensives compared to controls 0.7 vs. 0.66 (Table 2). Martinez *et al*. (2002) have found that threonines at position 174 and 235 of the angiotensinogen polypeptide chain are more related to familial history of hypertension in a Spanish-Mediterranean population than the 174M allele. These findings and other ones (Rotimi *et al*., 1997; Iso *et al*., 2000) are in conjunction with our results.

Furthermore, our data did not show any association with respect to gender and other confounding factors. However, Mohana *et al*.(2012) have found that the 174M allele was more prevalent among female hypertensives than among female controls (0.20 vs. 0.12; $p = 0.059$) while examining 279 hypertensive patients and 200 normotensive subjects. The discrepancies may be due to some bias such as methodological sampling and interaction with environmental factors which can contribute to the negative association (Mustafina *et al*., 2002).

Although our negative result could be taken to imply the absence of association between the AGT T174M and essential hypertension within the Algerian population but some limitations must be taken in account such as the nature of the study which is retrospective case-control one like other ones (Jeunemaitre *et al*., 1992; Caulfield *et al*., 1994; Hata *et al*., 1994; Ward *et al*., 1993; Arngrimsson *et al*., 1993) this kind of study provides an imperfect insight into causal process. In addition to the randomized control design (no age-sex matched controls were used) so that the controls were relatively younger than case subjects.

However, further studies with large sample size are necessary to confirm or to infirm the possible association between AGT T174M variant and essential hypertension and to check the eventual role of other polymorphisms of the RAAS genes in relation to HTN in the Algerian population. Nevertheless, there is still some big challenges in finding answers to the missing heritability problem of essential hypertension by a better focusing on epigenetic aspects, a better understanding of the interactions between gene-gene and gene-environment.

CONCLUSION

This study shows that T174M variant is not associated with essential hypertension in Algerian population of Oran city. However, this study may be improved with further studies with well-designed larger sample size subjects involving other polymorphisms in RAAS genes in relation with essential hypertension.

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