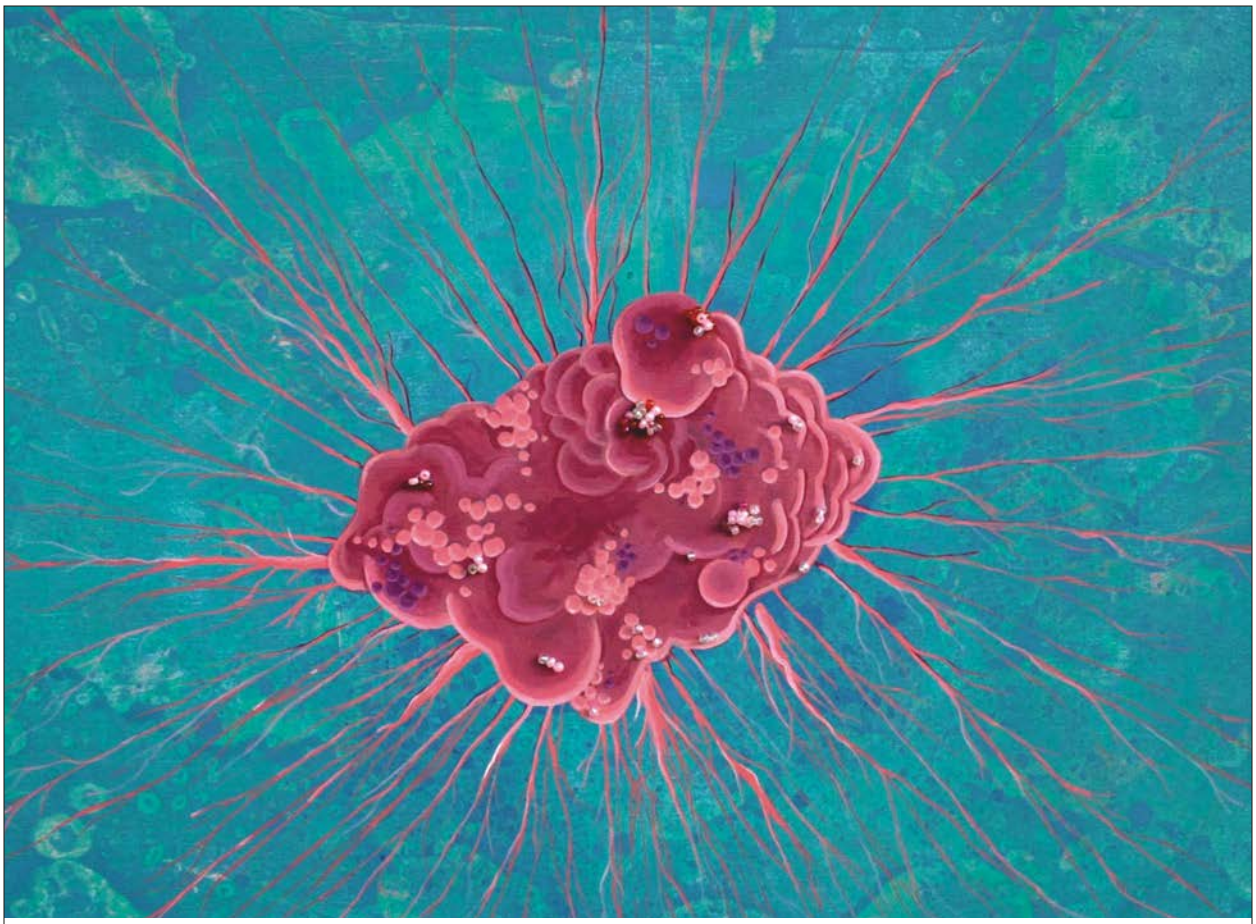

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Association of Angiotensin Converting Enzyme Insertion/Deletion and Angiotensinogen T235 Polymorphisms with Risk of Essential Hypertension in Egyptian Patients

[1]Naglaa Raafat AbdRaboh, [1]Neveen Salah El Din Hemimi, [1]Manal Louis Louka and [2]Samah El Bakry

[1]Department of Medical Biochemistry and Molecular Biology, Ain Shams Faculty of Medicine, Cairo, Egypt
[2]Department of Internal Medicine, Ain Shams Faculty of Medicine, Cairo, Egypt

Corresponding Author: Naglaa Raafat AbdRaboh, Department of Medical Biochemistry and Molecular Biology, Ain Shams Faculty of Medicine, Cairo, Egypt Tel: +971/508778149, +971/042610228

ABSTRACT

Polymorphism of rennin angiotensin system has been proved to be implicated in the pathogenesis of several multigenetic diseases including hypertension. To investigate the linkage of insertion/deletion (I/D) polymorphism of Angiotensin Converting Enzyme (ACE) and Met to Thr variant at position 235 of Angiotensinogen (AGT) to the incidence of essential hypertension in a population sample from Egypt. Samples of blood were collected from 203 subjects with matched age and sex (110 hypertensive and 93 normal controls). The polymorphisms of ACE and AGT were investigated by polymerase chain reaction techniques. AGT polymorphism showed significantly increased incidence of hypertension in TT genotype as compared to (MT and MM) genotypes (OR = 2.2, 95%CI 1.0-5.1, $p<0.05$). Similarly the study of ACE polymorphism declared a minor risk for hypertension in DD compared to (ID and II) genotypes (OR = 1.2, 95% CI 0.7-2.2). No significant association was found between ACE and AGT polymorphism and traditional metabolic risk factors of hypertension and no synergism was found between ACE and AGT genes to be involved in development of hypertension. TT genotype of angiotensinogen polymorphism is a potential risk factor for hypertension in Egyptian subjects.

Key words: Hypertension, rennin-angiotensin system, polymorphism

INTRODUCTION

Hypertension is a worldwide public health problem because of its high prevalence, affecting 20-30% of adult population and associated with increased risk of morbidity and mortality (Benetos *et al.*, 2005). Essential hypertension is a multi-factorial disease resulting from complex interaction between several genes with each other and with environmental factors such as obesity (Canoy *et al.*, 2004), smoking (Szszch *et al.*, 2004), dietary salt intake (Beeks *et al.*, 2004), alcohol consumption (Berlin, 2005) and stress (Elser and Parati, 2004).

There is evidence suggesting that several genes may contribute to 30% of the variation of blood pressure. However the number of genes involved, the model of interaction of other genes, or environmental risk factors is not completely known (Marteau *et al.*, 2005; Tripathi *et al.*, 2006).

Several studies focused on cluster of genes coding for proteins that regulate blood pressure as Rennin Angiotensin System (RAS) which plays an important role in regulation of blood pressure

and sodium homeostasis. A series of RAS gene polymorphisms were described to have significant influence on the rate of gene transcription, including angiotensinogen (AGT), angiotensin converting enzyme (ACE), angiotensin II type I receptor (AGTR1) and rennin. They have been extensively investigated as genetic determinants of essential hypertension and related pathologies (Jiang *et al.*, 2009; Freitas *et al.*, 2008; Cardoso *et al.*, 2008; Hai-dong *et al.*, 2010). The ACE I/D polymorphic locus, identified in a non-coding sequence, is more likely to serve as a genetic marker because of its linkage disequilibrium with putative disease causing locus located nearby Subjects having ACE deletion (D) allele have been shown to have increased ACE serum and tissue activity which determines the level of vasoactive peptide angiotensin II (Van Der Kleij *et al.*, 2002; Pop *et al.*, 2007), while the T235 AGT variant has been associated with elevated angiotensinogen level (Miller and Scholey, 2004). The plasma concentration of AGT is correlated with blood pressure (Menard *et al.*, 1991). The variant of AGT encoding threonine rather than methionine at position 235(M235T) has been reported to be associated with hypertension in many populations (Jeunemaitre *et al.*, 1993). A further study by Caprioli *et al.* (2008) proved a role of synergistic ACE I//D and endothelin receptor polymorphism in salt sensitive hypertension. Mehri *et al.* (2011) recorded that both TT of AGT and DD of ACE significantly increased the risk for essential hypertension especially in obese patients. These finding has been opposed by some investigators (Caulfield *et al.*, 1994). Up till now there are contradictory results in studies investigating the relation of frequency of these genetic polymorphisms and increased incidence of hypertension in various populations (Mondry *et al.*, 2005). Allele frequency of different genes may vary according to population or ethnicity and to our knowledge no much work correlated these gene variants to hypertension in Egypt, so we aimed in the current study to investigate the relation between variants of I/D ACE gene and M235T AGT gene polymorphism and the presence of essential hypertension in Egyptian people. Moreover the combined effect of ACE and AGT gene polymorphism on blood pressure and their interplay with metabolic risk factors of patients was analyzed.

MATERIALS AND METHODS

Patients: This case-control study included 203 individuals (110 hypertensive and 93 normotensive subjects, 54 females and 149 males) after giving an informed consent. The patients were selected from the Out-patient clinics of Internal medicine Department, faculty of medicine, Ain Shams University. Hypertensive subjects were defined according to Joint National Committee (JNC-7) classification system for hypertension (Chobanian, 2003). We included patients with stage I hypertension (those with Systolic Blood Pressure (SBP) of greater than 140- 145 mm Hg and with a Diastolic Pressure (DBP) of greater than 90-95 mmHg); or are currently taking at least one antihypertensive medication.

All individuals were subjected to complete history taking, thorough clinical examination by a physician. As well, anthropometric data were taken, blood pressure was measured and Body Mass Index (BMI) was calculated.

Venous blood samples were collected from each subject in two separate test tubes; one was used for colorimetric analysis of serum urea, creatinine, Total Cholesterol (TC), HDL, LDL, Triacylglycerols (TAGs) and fasting glucose using DiaSys Commercial kits (Diagnostic System, GMBH, D65558 Holzheim, Germany).

The other test tube contained venous blood samples collected on EDTA and were subjected to DNA extraction. Genomic DNA was extracted from white blood cell pellets by salting out extraction method (Josef *et al.*, 2002) using wizard genomic DNA extraction kit from Promega. Red blood cell

lysis was done by using red cell lysis buffer (20 mM tris-CL pH 7.6) followed by centrifugation. Nuclei lysis was carried by cell lysis buffer (10 mM Tris-CL pH 8.0, 1 mM EDTA pH 8.0, 0.1% (w/v) SDS) and proteinase K (20 mg mL^{-1}) followed by centrifugation. Protein was precipitated by protein precipitation solution (60 mL of 5 M potassium acetate, 11.5 mL of glacial acetic acid, 28.5 mL of water) followed by centrifugation. Finally DNA was precipitated by isopropanol and then ethanol 70% and rehydrated in TE buffer (pH 7.6) and stored in -20°C. The DNA purity and concentration were determined by spectrophotometer measurement of absorbance at 260 and 280 nm.

Polymerase chain reaction: To determine the frequencies of the I/D polymorphisms of the ACE gene in all samples (n = 203) and homo-/heterozygote of the M235T AGT gene in 123 samples, Polymerase Chain Reaction (PCR) was done (Marre *et al.*, 1997; Russ *et al.*, 1993). For ACE I/D polymorphism 100 ng of DNA was amplified using Gene Amp PCR system 9700 from applied biosystem. The cycling conditions were initial denaturation for 3 min at 94°C followed by 30 cycles of: denaturation for 30 sec at 94°C, annealing at 58°C for 45 sec, extension at 68 °C for 2 min, then final extension was done at 68°C for 7 min. Reaction mixtures consisted of 1.25 units of thermostable Taq polymerase, 0.2 mM of each dNTP, 1.5 mM $MgCl_2$, 2 mM dimethylsulphoxide (DMSO) and 0.5 μmol of each primer, made up to a final volume of 50 μL The primers used were: sense-5'CTGGAGACCACTCCCATCCTTTCT3' and antisense- 5'GATGTGGCCATCACATTCGTC AGAT3'. The reaction products were electrophoresed on 2% agarose gel and visualized under ultraviolet (UV) light after ethidium bromide staining. Subjects were classified according to the presence or absence of a 287 base pairs insertion at intron 16 of ACE gene as II, ID, DD. Preferential amplification of the smaller 190 bp deletion allele (D) in ID heterozygote has led to their mistyping as DD homozygote so they were retyped using an insertion (I) specific sense primer 5'-TGGGACCACAGCGC CCGCCACTAC-3'; antisense: 5'-GCCAGCCCTCC.

CATGCCCATAA-3' (primers were supplied by Alpha DNA, 4401 Notre-Dame St.w.). and were then subjected to denaturation at 94°C for 3 min followed by 32 cycles of 94°C for 30 sec, 67°C for 30 sec and 72°C for 30 sec, followed by 67°C for 30 sec and 72°C for 10 min; (Mayer *et al.*, 2002). The products were separated on 1.5% agarose gel and visualized under UV light after ethidium bromide staining.

Regarding AGT M235T polymorphism, amplification was done using the following primer sequences: sense- 5'CCGTTTGTGCAGGGCCTGGCTCTCT3' and antisense: 5'CAGGGTGCTGTCCA CACTGGACCCC3'. Reactions were carried out in 25 μL volumes under standard conditions (1.5 mmol L^{-1} MgCl2, 50 μmol L^{-1} for each dNTP, 10 mmol L^{-1} tris/HCl, 50 mmol L^{-1} KCl, 1 μmol L^{-1} primers, 1 U Ta q DNA polymerase per sample The PCR conditions were initial denaturation at 94°C for 3 min, followed by 30 cycles of 1 min denaturation at 94°C, 1 min annealing at 68°C and 1 min extension at 72°C, then final extension at 72 for 10 min was done. The product of PCR was subjected to digestion by restriction enzyme Tth 111I (New England BioLabs, Missisauga, ON, Canada) for 3 h at 65°C. The M to T point mutation creates a detection site at position 235 and the digested fragments were separated by electrophoresis in 2.5% agarose gel (Caulfield *et al.*, 1995).

Statistical analysis: Statistical analysis was carried out using SPSS statistical package for social sciences (version 11.5, SSPS Inc, Chicago, IL). Pearson's Chi Square test was used for testing the categorical data of genotype association with hypertension and estimation of the expected genotype proportions were calculated and compared to the observed proportion according to Hardy Weinberg

law. Allele frequencies were calculated by gene counting method. The relation of I/D alleles with the presence of hypertension was also tested considering both recessive effect of deletion allele (DD vs. DI+II) and a dominant effect of same allele (DD+DI vs. II) (Siani *et al.*, 2004), for AGT (TT vs. TM+MM), then (TT+TM vs. MM). The presence of possible synergistic effect of AGT and ACE on hypertension was assessed. Odds Ratios (OR) for these comparisons were calculated with 95% Confidence Interval (CI), the significance level was set at p-value<0.05. Student *t* test was used to compare means of metabolic risk factors of hypertension as BMI, fasting blood glucose, serum lipids, urea and creatinine in both normotensive and hypertensive persons. One way ANOVA and linear regression analysis were used to test the influence of genotypes and metabolic risk factors on systolic and diastolic blood pressure.

RESULTS

Means of Age and metabolic risk factors are shown in (Table 1). Distributions of genotypes and allele frequency in control and hypertensive patients are shown in Table 2, Fig. 1-2.

ACE polymorphism: ACE I/D polymorphism was tested in all studied 203 samples (93 normotensive with mean age 42+7.3 and 110 hypertensive with mean age 45+8.2. No deviation from Hardy Weinberg equilibrium was observed ($\chi^2 = 1.2$, p>0.05) D allele frequency was 0.56 and I allele was 0.44 in all studied individuals. In agarose gel two bands were visualized, that of

Table 1: Clinical criteria of hypertensive and normotensive control group

Parameter	Normotensive	Hypertensive subjects (110)	p value	t value
Ages (years)	42±7.3	45±8.2	>0.05	-1.7
BMI (kg m^{-2})	28.02±11	28.8±4.5	>0.05	0.67
SBP (mmHg)	131.4±13.4	147.8±13.5*	<0.01	8.6
DBP (mmHg)	82±7.2	95.6±6.3*	<0.01	14.35
Serum total cholesterol (mg dL^{-1})	179.6±42.2	201±52.5*	<0.01	3.2
LDL (mg dL^{-1})	130.4±51.1	131.1±40.67	>0.05	0.108
HDL (mg dL^{-1})	40.1±6.5	42.4±11.8	>0.05	1.67
Triacylglycerols (mg dL^{-1})	127.9±31	139.5±53.02	>0.05	1.85
Serum glucose (mg $dL^{-1)}$	112.9±35.9	124.2±26.2	>0.05	2.5
Serum creatinine (mg dL^{-1})	0.88±0.16	0.91±0.18	>0.05	2.07
Serum urea (mg dL^{-1})	21.7±8.6	23.3±8.6	>0.05	1.3

Values in mean±(SD), *Statistically significance

Table 2: The risk of hypertension in relation to different genotypes and alleles of ACE and AGT genes

Genotype	Normal	Hypertension	OR	95% CI	χ^2	P
I/D ACE gene (n = 203)	n = 93	n = 110				
DD	26.9% (25/93)	39.9% (34/110)	1.2	0.66-2.24	0.4	0.32
ID+II	73.1% (68/93)	69.1% (76/110)				
D allele	55% (102/186)	58% (127/220)	1.12	0.76-1.7	0.31	0.3
I allele	45% (84/186)	42% (93/220)				
AGT M235T (n = 123)	n = 61	n = 62				
TT	19.7% (12/61)	35.5% (22/62)	2.25*	1-5.1*	3.8*	0.038
MT+MM	80.3% (49/61)	64.5% (40/62)				
T allele	54% (66/122)	61% (76/124)	1.3	0.81-2.23	1.3	0.15
M allele	46% (56/122)	39% (48/124)				

Data presented percentage (No.) *Statistically significant difference, OR: Odd ratio, CI:Cconfidence interval, χ^2: Pearson chi-square

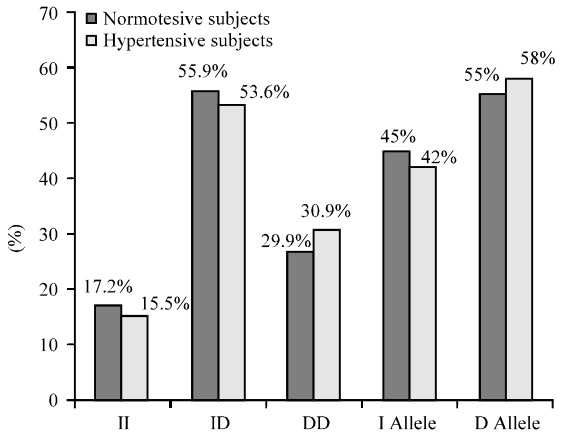

Fig. 1: Distribution of ACE I/D genotypes and allele frequency in hypertensive and control group

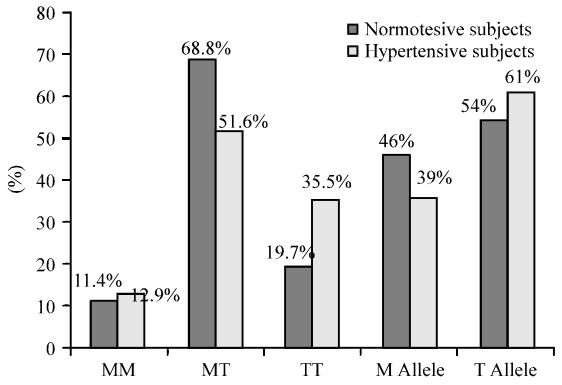

Fig. 2: Distribution of AGT T235M genotypes and allele frequency in hypertensive and control group

insertion was 490 bp and that of deletion was 190 bp (Fig. 4). When DD samples were amplified using the I specific primer a 335 bp fragment was recorded, this was considered as an ID variant (Fig. 5).

As shown in Fig. 1, 2; ACE genotypes DD, ID and II frequencies in control group were 26.9% (25), 55.9% (52) and 17.2% (16), respectively. In hypertensive group it was 30.9% (34), 53.6% (59) and 15.5% (17), respectively ($p>0.05$, χ^2 was 0.42). as shown in Table 2, the frequency of DD genotype was 30.9% (34/110) in hypertensive group and (ID+II) genotypes were 69.1% (76/110), as compared to control group at which DD genotype was 26.9% (25/93) and (ID+II) genotypes were 73.1% (68/93), the risk assessment showed mild increase in risk for hypertension (OR = 1.2, 95% CI = 0.66-2.24, $p>0.05$). The lower risk association was observed at allele level as D allele was found in 0.58 hypertensive patients and I was found in 0.42 (OR = 1.12, 95% CI = 0.76-1.66) as compared to 0.55 D allele and 0.45 I allele in normal control.

AGT polymorphism: AGT M235T genotype was analyzed in 123 subjects, 61 normotensive with mean age 43.1+6.9 and 62 hypertensive with mean age 46.1+7.6 years. The

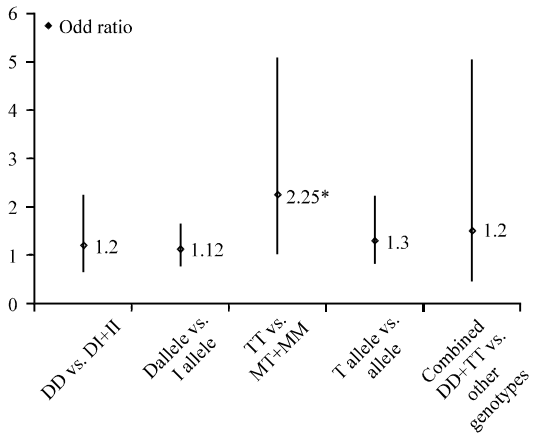

Fig. 3: The risk of hypertension in relation to different genotypes and allele of ACE and AGT genes, *Significant association at $p<0.05$

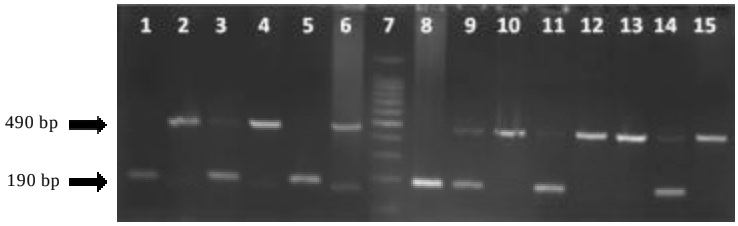

Fig. 4: Gel electrophoresis of ACE I/D: Lanes 1, 3, 5, 8, 11 are DD homozygote with single band at 190, lanes 2, 4, 10, 12, 13 are II homozygote with one band 490, lanes 6, 9, 14 are ID heterozygote with two bands and lane 7 is 100 bp DNA marker

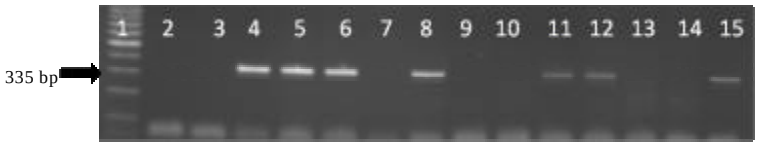

Fig. 5: Gel electrophoresis using insert specific primers: Lanes 4, 5, 6, 8, 11, 12, 15 show single bands of the insertion fragment of 335 bp so considered ID heterozygote, while lanes 2, 3, 7, 9, 10, 13, 14 No. bands indicating DD homozygote, Lane 1 shows 100 bp DNA marker

products of restriction were detected by agarose gel 2.5% as one fragment 141 bp for TT and 165 for MM and two bands 141, 165 bp for MT (Fig. 6).

Allele frequency of AGT M235T was in Hardy Weinberg Equilibrium in the cases but not in the control group. This may be explained by the small size of tested samples and there was genetic drift toward higher frequency of MT individuals (66.8%) instead of TT individuals (11.4%) in this sample. As shown in Fig. 2, AGT M235T genotypes TT, MT and MM in the normotensive control group were 19.7% (12), 68.8% (42) and 11.4% (7), respectively, whereas among hypertensive group

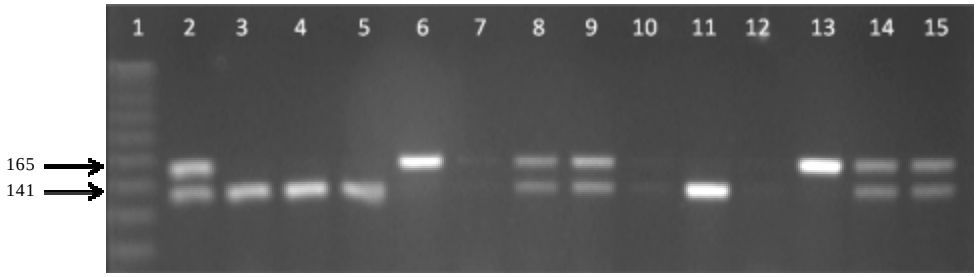

Fig. 6: Gel electrophoresis of AGT T235M: Samples after digestion with Tth 111 I, lanes 3, 4, 5, 11 homozygote TT with one band 141 bp, lanes 6, 13 are homozygote, MM with one band 165 bp, lanes 2, 8, 9, 14, 15 heterozygote MT with two bands at 141 and 165 lane 1 is 50 bp DNA marker

Table 3: Regression analysis results of correlation of metabolic risk factors and genotypes with systolic and diastolic blood pressure

	SBP		DBP	
Parameter	R^2	p value	R^2	p value
Serum total cholesterol (mg dL^{-1})	0.076	0.012	0.2	<0.001*
LDL (mg dL^{-1})	0.091	0.007*	0.279	<0.001*
HDL (mg dL^{-1})	0.044	0.06	0.011	0.356
Triacylglycerols (mg dL^{-1})	0.014	0.297	0.013	0.306
Serum glucose (mg dL^{-1})	0.049	0.123	0.004	0.659
Serum creatinine (mg dL^{-1})	0.003	0.511	0.02	0.085
Serum urea (mg dL^{-1})	0.015	0.139	0.034	0.028*
DD genotype	0.002	0.584	0.007	0.314
TT genotype	<0.001	0.925	<0.001	0.889

*Statistically significant difference

it was 35.5% (22), 51.6% (32) and 12.9% (8), respectively (p>0.05, $\chi 2$ was 4.3). The frequency of T allele was 0.61 and the frequency of M allele was 0.39 in the hypertensive group as compared to 0.54 and 0.46 in control group with OR 1.3, 95%CI 0.8-2.2.

As shown in Table 2, in hypertensive group TT was 35.5% (22/62) while MT+MM genotype was 64.5% (40/62), in comparison to control group at which TT genotype was 19.7% (12/61) and MT+MM genotype was 80.3% (49/61). Upon calculating the risk for hypertension in TT genotype as shown in Fig. 3 OR = 2.25, 95%CI 1-5.1, $\chi^2 = 3.84$, p = 0.039 indicating significant difference.

Combined DD and TT when compared with other genotypes gave lower risk with OR = 1.2, 95%CI 0.36-3.77 declaring absence of synergistic effect between AGT and ACE polymorphism on the incidence of hypertension.

Metabolic risk factors: In order to assess the link of ACE and AGT gene polymorphisms to other risk factors of hypertension, we compared clinical parameters among different genotypes; but no significant association was noticed. Linear regression analysis showed that only total cholesterol (TC) and LDL were predictors for both systolic and diastolic BP, values for TC were (r = 0.28, R^2 = 0.076, p = 0.012 for SBP, r = 0.53, R^2 = 0.21, p<0.01 for DBP), values for LDL were (r = 0.3, R^2 = 0.091, p = 0.007 for SBP, r = R^2= 0.279, p<0.01 for DBP) (Fig. 7 and Table 3).

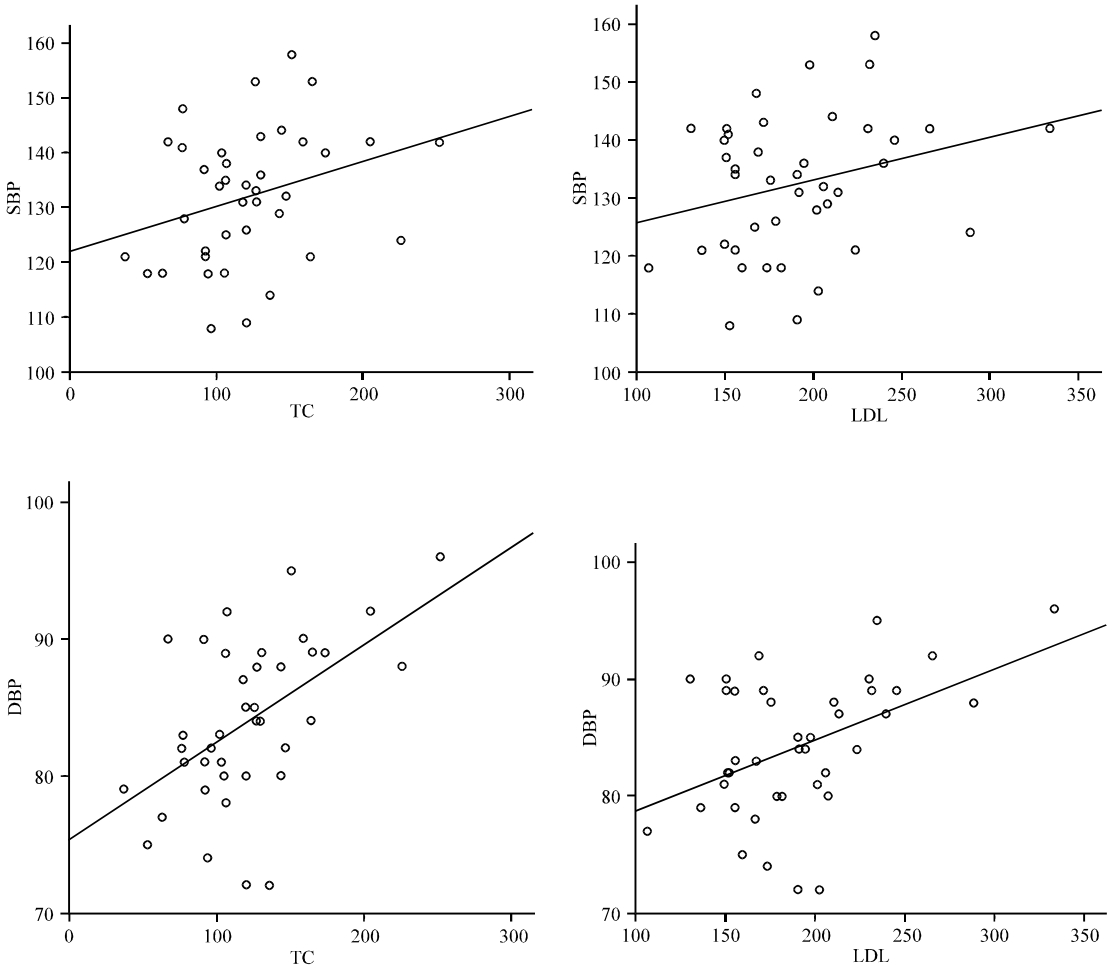

Fig. 7: Linear regression curve to correlate lipid profile with diastolic and systolic blood pressure, TC: Total cholesterol, LDL: Predictors of systolic, SBP: Diastolic and DBP: Blood pressure

DISCUSSION

In spite of the great advance in understanding the pathogenesis and proper management of hypertension still there is progressive increase in the rate of its incidence and prevalence. In addition, hypertension is still one of the most common problems in medicine and is responsible for high cardiovascular morbidity and mortality. Overall, 26.4% of the adult population worldwide in 2000 had hypertension (Kearney *et al.*, 2005). At the present time about one billion people worldwide have high blood pressure and that number is expected to increase to 1.56 billion by 2025. The prevalence of high blood pressure is predicted to increase by 24% in developed countries and by 80% in developing regions such as Africa and Latin America (Bakris and Ritz, 2009).

RAS is involved in homeostasis of blood pressure (Bonnardeaux *et al.*, 1994); overstimulation of RAS causes an increase in blood pressure and ACE inhibitors have been acknowledged as the first line agents for the treatment of hypertension and a variety of cardiovascular disorders (Paliwal *et al.*, 2011). Consequently mutation in the genes of RAS components may be involved in pathophysiology of hypertension.

The results of the present study identified higher frequency of D allele which was 0.58 in hypertensive subjects as compared to 0.55 in control group, with odd ratio (OR)1.12, (95%CI 0.76-1.7); risk was slightly increased in homozygous DD genotype (30.9%) in distinction to combined DI+II genotypes (69.1%) with (OR 1.2, CI 0.66-2.24).

In this aspect we agreed with several previous studies who recorded higher frequency of D allele and DD genotype in hypertensive patients, as Tripathi *et al.* (2006) who reported high incidence of hypertension in DD genotype (87%) as compared to II+ID (65%). Similarly, a study of Ramachandran *et al.* (2008) showed that D allele was found in 60.92% in hypertensive as compared to 22.86% in control and Rallidis *et al.* (2009) also found higher prevalence of DD genotype (62.5%) among hypertensives as compared to 35.6% in normotensives with p = 0.01. Statistically significant associations with essential hypertension were also identified for DD genotype of ACE I/D polymorphism (OR 1.61, 95% CI 1.32-1.98, p<0.0001) in a study by Ji *et al.* (2010). Another study on Hellenic population recorded that ID and DD genotypes of ACE were associated with increase risk as compared to II genotype (OR 1.78, CI 1.03-3.07) (Tsezou *et al.*, 2008). Lack of statistical significance in our results, could be explained by the limited number of investigated subjects.

Various reports are available explaining the mechanism of elevated blood pressure in association with DD genotype at cellular level (Lewis *et al.*, 1993). Individuals with DD genotype have serum ACE levels and intracellular ACE activity twice than those of II genotype (Rigat *et al.*, 1990; Costerousse *et al.*, 1993). High ACE activity leads to increase angiotensin II level promoting expression of gross factor and proliferation of Mesangial cells and matrix leading to glomerulonephrosis and hypertension (Mitch, 1995). Possible mechanism is stimulation of oxidative stress which promotes atherosclerosis and hypertension as reported by Ghazi *et al.* (2009). A study by Uddin *et al.* (2007) proved association of D allele with diabetic nephropathy, suggesting that D allele may affect blood pressure through renal homeostatic disorder.

The current study revealed obvious association between AGT M235T polymorphism and incidence of hypertension as T allele was more frequent in hypertensive subject (0.61) than in normal subjects (0.54) with mild risk (OR = 1.3, 95% CI 0.8-2.23); the association became statistically significant when we compared homozygous TT genotype to MT+MM genotypes (OR = 2.25, 95% CI = 1-5.1). These results were in accordance with previous meta-analysis studies which reported significant increase in risk of hypertension in Caucasians with T allele and TT genotype. OR of T vs. M was 1.2 (95% CI = 1.11 -1.29) (Kunz *et al.*, 1997), OR TT vs. MM was 1.31 (Staessen *et al.*, 1999), OR TT vs. MM was 1.19 (95% CI= 1.1-1.3) (Sethi *et al.*, 2003) and OR 1.36 for TT genotype and 1.98 for T allele (Say *et al.*, 2005), OR 1.54, 95% CI 1.16-2.03, p = 0.002 (Ji *et al.*, 2010). Positive association of T allele was found on several studies done on Japanese population (Hata *et al.*, 1994; Nishiuma *et al.*, 1995; Yugar-Toledo *et al.*, 2011), Chinese and Taiwanese population (Ji *et al.*, 2010; Niu *et al.*, 1999; Chiang *et al.*, 1997). The Mechanism by which M235T variant contributes to pathogenesis of hypertension is poorly understood, AGT M235T variant has been found to be in complete linkage disequilibrium with guanine to adenosine transition at 6 bp upstream of the initiation site of transcription (Inoue *et al.*, 1997). In vitro test of promoter activity and DNA binding studies with nuclear protein showed that this nucleotide substitute affects the basal transcription rate of this gene in various cell lines thereby the AGT T235 variant and increase plasma AGT levels (Jeunemaitre *et al.*, 1992) and hence might contribute to the elevation of blood pressure. Some investigators recorded combined effect of D allele of ACE and T allele of AGT on hypertensive patients with increase liability to cardiovascular

complications as left ventricular hypertrophy (Kurbanova and Eliseyeva, 2010), but this was not supported by our results as there was decrease risk of incidence of hypertension in patients with combined alleles especially homozygous genotypes TT and DD.

Opposing studies by Bloem *et al.* (1997), Mondry and Loh (2006), Saab *et al.* (2011) found TT homozygous more in normotensive than in hypertensive subjects. In addition many literatures reported lack of association between ACE I/D polymorphism and hypertension (Tascilar *et al.*, 2009; Arfa *et al.*, 2010). Other studies on Caucasians found no difference for risk in any genotype (Glavnik and Petrovic, 2007), similar results were found in Korean women (Kim, 2009), Chinese (Jiang *et al.*, 2009) and Iranian people (Nakhjavani *et al.*, 2007). This inconsistency was explained by large difference in frequency of ACE alleles in different populations and ethnic groups and difference in the bases of choice and numbers of studied sample population, this may be added to the polygenic nature of hypertension.

In the present study we analyzed association of metabolic risk factors with incidence of hypertension and their correlation to ACE and AGT gene polymorphisms to investigate if there is possible interaction between them and RAS system polymorphism, we found positive correlation between hypertension and total cholesterol and LDL cholesterol levels which was in accordance with several earlier studies (Latiffah and Hanachi, 2008; Tassaduqe *et al.*, 2003), but no significant association was detected, so mostly these genetic variants doesn't have significant enhancement of risk factors for hypertension but their mechanism of action is restricted to RAS system vascular and salt regulatory effect, same finding was mentioned by Tascilar.

The purpose of investigating the strength of association between effect of ACE and AGT gene polymorphism on blood pressure is to predict response to certain anti hypertensive treatment which will be reflected on the choice of a suitable regimen.

CONCLUSION

The current study confirmed the potential association of AGT gene polymorphism and hypertension in Egyptian patients which may explain different responses to antihypertensive agents especially those acting on RAS, while ACE I/D polymorphism needs more analysis on wider scale of population genotypes.

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