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Candidate Gene Polymorphisms of Renin Angiotensin System and Essential Hypertension in a South Indian Tamilian Population

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ABSTRACT Genetic variants of renin angiotensin system (RAS) gene play a significant role in the pathogenesis of essential hypertension and cardiovascular diseases. In the present study, we investigated the association of RAS gene polymorphisms with hypertension by analyzing the polymorphisms *ACE* ID, *AGT* T207M, M268T and *AGT1R* A1166C in 462 hypertensive patients and 444 healthy subjects. Genotyping was determined by allele specific PCR, PCR-RFLP and RT-PCR Taqman assay. The *ACE* ID heterozygous (OR=1.5: 95% CI: 1.0-2.3, p<0.05) and ACE DD homozygous genotype (OR=1.7: 95% CI: 1.2-2.8, p<0.01) was found to be significantly associated with hypertension. There was no significant association between *AGT* T207M, M268T and *AGT1R* A1166C gene polymorphisms and hypertension. Gender-specific analysis showed *ACE* ID heterozygous genotypes were positively associated with hypertension among male hypertensives (OR=1.9: 95% CI: 1.1-2.6, p<0.01). Significant gene-gene interaction was observed between *ACE* ID and *AGT* M268T polymorphisms (OR=2.0; 95% CI: 1.2-3.5, p<0.01). Our results suggest that *ACE* ID polymorphism is associated with hypertension. Further, gene-gene interaction between *ACE* ID and *AGT* M268T gene polymorphisms further modified the risk of essential hypertension.

INTRODUCTION

Essential hypertension (EH) is a polygenic disorder resulting from the interaction of several genetic and environmental factors. EH has been recognized as a major contributing risk factor in several cardiovascular ailments. Renin angiotensin system (RAS) plays an important role in the regulation of blood pressure. Association between candidate gene polymorphisms of RAS and EH has been reported in different populations (Danser and Schunkert 2000).

Genes encoding RAS include angiotensin converting enzyme gene (*ACE* ID), angiotensinogen gene (*AGT* T207M and M268T) [previously recognized as *AGT* T174M and M235T] and angiotensin II type 1 receptor gene (*AGT1R* A1166C) polymorphisms. About 78 molecular variants of human *ACE* gene have been reported (Rieder et al. 1999), since the identification of its gene sequence (Soubrier et al. 1988). Among them, *ACE* ID polymorphism has been extensively studied in all cardiovascular disorders. The presence or absence of 287 bp in intron 16 of *ACE* gene leads to insertion/deletion polymorphism (Rigat et al. 1990; Rigat et al. 1992). The presence of deletion allele with elevated serum ACE levels (Rigat et al. 1990; Danser et al. 1995) eventually leads to hypertension (Higaki et al. 2000), myocardial infarction (Cambien et al. 1992), and cardiomyopathy (Rai et al. 2008). Similarly, *AGT* (Gaillard et al. 1989; Jeunemaitre et al. 1992b) and *AGT1R* genes were cloned and several molecular variants were identified (Furta et al. 1992; Takayanagi et al. 1992; Bonnardeaux et al. 1994; Ishanov et al. 1997).

Numerous studies have been carried out on the association between *AGT* T207M and M268T gene polymorphisms and cardiovascular disorders (Ishanov et al. 1997; Pilbrow et al. 2007). Increase in levels of angiotensinogen in T268 homozygous variant leads to increase in blood pressure (Bloem et al. 1997). The *AGT* M268T polymorphism was found to be in linkage disequilibrium with T207M and promoter region A -6 G polymorphisms. Haplotype analysis of *AGT* T207M and M268T revealed a significant association with hypertension among the Caucasian and Taiwan Chinese populations

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(Jeunemaitre et al. 1992a; 1992b; Wang et al. 2002; Wu et al. 1997). Association between C/C homozygous variant genotype of *AGT1R* A1166C and increased vasoconstriction in human arteries has been reported by Amant et al.(1997). Various studies on RAS gene polymorphisms in relation to hypertension showed contradictory results across different ethnic populations with both positive and negative results (Higaki et al. 2000; Jeunemaitre et al. 1992; Bonnardeaux et al. 1994; Ashavaid et al. 2000; Bhavani et al. 2004; Randhawa et al. 2006; Nair et al. 2003; Nejatizadeh et al. 2008).

Very few studies have described the association of *ACE* ID (22-24) and *AGT* gene (Nair et al. 2003; Nejatizadeh et al. 2008) polymorphisms in relation to EH among Indian population. Hence, we sought to investigate the association between RAS gene polymorphisms and susceptibility to EH in south Indian Tamilian population**.** Further, we also investigated the gender specificity and gene-gene interactions of RAS gene polymorphisms in relation to EH. Tamilians are Dravidian people from the Indian sub-continent, living in southern parts of India and north-eastern Sri Lanka. They are ethnically, linguistically and culturally related to other Dravidian people of the sub-continent. An estimated 77 million Tamilians reside in India and other parts of the world (Tamil people 2009).

MATERIALS AND METHODS

Study Participants

The study was carried out in 906 ethnically matched unrelated subjects including 462 essential hypertensives (228 men and 234 women) and 444 healthy controls (201 men and 243 women) aged 30-60 years. The hypertensive cases were diagnosed and selected from the outpatient clinics of hypertension and internal medicine (JIPMER hospital, Pondicherry, India). All of them were residents of Tamilnadu and Pondicherry for atleast three generations. Patients receiving antihypertensive medications for more than 3 months (or) newly diagnosed hypertensive patients with systolic blood pressure more than 140 mmHg and/or diastolic blood pressure more than 90 mmHg on 2 or more consecutive visits were considered to be hypertensives (European Society of Hypertension-European society of Cardiology Guidelines, 2003). The age of hypertension is defined as the time when BP recordings fulfilled the inclusion criteria of hypertension on 2 consecutive visits before starting the medication or when antihypertensive medication is initiated. Patients with other significant illnesses that might affect the outcome of investigation e.g., diabetes mellitus, hyperlipidemia, liver or renal disease, congestive cardiac failure and recent episode of myocardial infarction were excluded. Pregnant and lactating women and those receiving medications for other indications that could affect BP were also excluded.

The control group had no personal or family history of hypertension in the first degree relatives with systolic blood pressure less than 130 mmHg and diastolic blood pressure less than 85 mmHg. Patients who visited the outpatient clinics with minor illness without hypertension, diabetes mellitus, hyperlipidemia and family history of hypertension in previous records were recruited as controls. None of the control groups were receiving antihypertensive therapy, treatment for heart disease or hormone replacement therapy during the time of investigation. Plasma lipid profile and blood glucose level were measured after overnight fasting for both hypertensive and normotensive subjects to rule out diabetes and hyperlipidemia. A detailed family history relating to their pedigree was taken to identify if any of the close relatives of the volunteers or patients were hypertensives. Details such as identification characteristics, body weight and height, drug history were recorded. All the participants were interviewed using a standardized questionnaire with regard to their lifestyle, smoking, alcohol consumption and drug intake. In all subjects, height was measured to the nearest centimeter and weight to the nearest 0.1 kg, which was used for calculation of BMI (kg/m^2). Blood pressure was measured in the right arm by resting the subjects for 10 minutes and by using standard sphygmomanometer and the average of three readings taken 2 minutes apart was recorded. The study was approved by the institutional ethics committee and written informed consent was obtained from all the participants who opted to participate in this study.

Genotyping Methods

Five milliliter of venous blood was collected with ethylene diamine tetra acetic acid (EDTA)

as anticoagulant. The genomic DNA was extracted from the peripheral leucocytes using standard phenol-chloroform extraction method. *ACE* ID polymorphism was determined by allele specific Polymerase Chain Reaction (PCR) (Tiret et al. 1992). The primers for PCR amplification of *ACE* ID were 5'-CTGGAGAG CCACTCCCATCCTTTCT-3' (Forward) and 5'-GACGTGGCCATCACATTCGTCAGAT-3' (Reverse). The PCR products were run on 2% agarose gel electrophoresis. The different fragments obtained were 490bp II, 190bp DD, and 490bp and 190bp ID. The samples with homozygous deletion DD by this assay were retyped using a third insertion specific primer 5'-TTTGAGACGGAGTCTCGCTC-3'. This was done to rule out the possibility of mistyping individuals due to preferential amplification of the deletion fragment over the longer insertion fragment (Shanmugam et al. 1993).

AGT T207M and *AGT1R* A1166C polymorphisms were identified with PCR-RFLP method. The primers used for *AGT* T207M were 5'-GATGCGCACAAGGTCCTG -3' (Forward) and 5'-CAGGGTGCTGTCCACACT GGCTCGC-3' (Reverse). The amplified 303 bp products were digested with the restriction enzyme *NcoI* and incubated overnight at 37°C (Caufield et al. 1994). The digested products were separated based on their size using 8% polyacrylamide gel electrophoresis. The different fragments obtained were 303 bp in homozygous wild type TT, 211 and 92 bp in homozygous variant MM and 303, 211 and 92 bp in case of heterozygous variant TM. The primers for *AGT1R* A1166C polymorphism were 5'-GAAGCCTGCACCATGTTTTGA-3' (Forward) and 5'-GGCTTTGCTTTGTCTT GTTG-3' (Reverse). The amplified 229 bp products were digested with restriction enzyme *Dde1* as described earlier (Bonnardeaux et al. 1994) and the digested PCR products were separated by 8% polyacrylamide gel electrophoresis. The different fragments obtained were 229 bp in homozygous wild type AA, 117 and 112 bp in homozygous variant CC and 229, 117 and 112 bp in case of heterozygous variant genotype AC. All the heterozygous and homozygous variant genotype samples were genotyped again for confirmatory purpose.

Genotyping for *AGT* M268T polymorphism was carried out by Real Time Thermocycler (7300 Applied Biosystems) using Taqman SNP genotyping assay with fluorogenic 5' nuclease chemistry to enable the detection of the specific PCR product. The PCR reaction was carried out in duplicate in a 20 µL final volume which contained 10 µL of Taqman Universal PCR master mix (2X), 0.5µL of 20X working stock of SNP genotyping assay, 4.5µL of genomic DNA diluted in DNase free water and 5 µL of Milli Q water. The allelic discrimination analysis was finally performed with 7300 SDS software.

Statistical Analysis

Statistical analysis was done using the Statistical Packages for Social Sciences software. (SPSS, Windows version release 13, SPSS Inc., Chicago, Illinois, USA). Demographic details of hypertensive cases and controls that were continuous variables were compared using Student's unpaired *t*-test, whereas dichotomous variables were compared by Chi square and/or Fisher's exact test. Differences in allele frequencies and genotype distribution between hypertensive cases and controls were compared by Chi square and/or Fisher's exact test. The association between genotypes and hypertension was analysed by calculating the crude odds ratio (OR) and 95% confidence interval (95% CI) using Chi square and/or Fisher's exact test. The adjusted OR was calculated using unconditional logistic regression and the low risk genotype was designated as reference category. For analyzing gene-gene interactions, stratified variables were generated and included in the logistic model, simultaneously with appropriate indicator variables. Linkage disequilibrium values for the pair of dimorphisms were measured by using Helix Tree software (Golden helix™ CA, USA). Haplotypes of *AGT* T207M and M268T were constructed using the EM algorithm. P value < 0.05 was used as the level of significance.

RESULTS

Demographic Details of Study Subjects

No significant differences was observed in sex distribution, BMI, heart rate, alcohol consumption, dietary habits, total cholesterol, triglycerides, high density and very low density lipoprotein cholesterol levels (Table 1). The

Table 1: Demographic details of study subjects

Parameter	*Hypertensive cases (n = 462)*	*Controls (n = 444)*	*p value*
Sex M/F	228/234	201/243	0.2
Age (Years)	45.1 ± 0.4	47.4 ± 0.4	0.01
BMI (kg/m^2)	23.0 ± 0.3	22.9 ± 0.2	0.6
Systolic blood pressure (mmHg)	153.4 ± 0.8	117.5 ± 0.4	0.0001
Diastolic blood pressure (mmHg)	97.3 ± 0.5	78.2 ± 0.3	0.0001
Alcohol users	124 (27)	100 (22.5)	0.2
Smokers	99 (21.4)	40 (9)	0.001
Diet			
Vegetarian	423 (91.6)	414 (93.2)	0.4
Non-vegetarian	39 (8.4)	30 (6.8)	
Total cholesterol (mg / dL)	176.0 ± 1.5	173.7 ± 1.7	0.08
Triglycerides (mg / dL)	120.8 ± 2.9	117.9 ± 2.3	0.2
HDL cholesterol (mg / dL)	41.0 ± 0.4	41.0 ± 0.4	0.6
LDL cholesterol (mg /dL)	111.5 ± 1.3	105.4 ± 1.3	0.01
VLDL (mg / dL)	24.9 ± 0.5	23.4 ± 0.5	0.09

Values are mean ± SEM and numbers and percentage

mean age (45.1 ± 0.4 vs. 47.4 ± 0.4, $p<0.01$) and smokers (21.4% vs. 9%, $p<0.001$) were higher in cases when compared to controls. However, there were significant differences in age, SBP, DBP, smoking patterns and low-density lipoprotein-cholesterol level between the hypertensive cases and controls. Potential confounders such as age and smoking were taken for multiple logistic regression analysis using suitable indicator variables.

Genotype Distribution of RAS Gene Polymorphisms among Cases and Controls

The distribution of RAS gene polymorphisms is summarized in Table 2. There was a signifi-

Table 2: RAS gene polymorphisms and essential hypertension among hypertensive cases and controls

Polymorphism	*Cases (n=462)*	*Controls (n=444)*	*OR (95% CI)*	*p value*
ACE I/D				
II	124 (26.8)	153 (34.5)	1.0	0.05
ID	211 (45.7)	190 (42.8)	1.4 (1.0-1.9)	0.02
DD	127 (27.5)	101 (22.7)	1.5 (1.1-2.2)	0.009
Allele				
D	0.50	0.44	1.3 (1.1-1.5)	
I	0.50	0.56		
AGT T207M				
TT	376 (81.4)	354 (79.7)	1.0	
*TM+MM	86 (18.6)	90 (20.3)	0.9 (0.6-1.2)	0.6
Allele				
T	0.91	0.90	1.0 (0.7-1.3)	0.6
M	0.09	0.10		
AGT M268T				
MM	70 (15.2)	75 (16.9)	1.0	
MT	164 (35.5)	170 (38.3)	0.9 (0.6-1.4)	0.9
TT	228 (49.3)	199 (44.8)	1.2 (0.8-1.8)	0.3
Allele				
M	0.33	0.36	1.3 (0.9-1.4)	0.2
T	0.67	0.64		
AT1R A1166C				
AA	420 (90.9)	399 (89.9)	1.0	
*AC+CC	42 (9.1)	45 (10.1)	0.9 (0.6-1.4)	0.7
Allele				
A	0.95	0.95	0.9 (0.6-1.5)	0.9
C	0.05	0.05		

Values in parenthesis indicate percentage
*One homozygous variant genotype of *AGT* T207M and *AGT1R* A1166C polymorphism in hypertensive cases were combined with heterozygous genotype for analysis.

Table 3: Gender specific distribution of RAS gene polymorphisms among the study subjects

Genes and Genotypes	*Male*			*Female*		
	Cases (n=228)	*Controls (n=201)*	*p value*	*Cases (n=234)*	*Controls (n=243)*	*p value*
ACE ID						
II	51 (22.4)	66 (32.8)		73 (31.2)	87 (35.8)	
ID	111 (48.7)	83 (41.3)	0.03	100 (42.7)	107 (44.0)	0.7
DD	66 (28.9)	52 (25.9)	0.08	61 (26.1)	49 (20.2)	0.1
AGT T207M						
TT	184 (80.7)	157 (78.1)		192 (82.1)	197 (81.1)	
*TM+MM	44 (19.3)	44 (21.9)	0.5	42 (17.9)	46 (18.9)	0.8
AGT M268T						
MM	30 (13.2)	31 (15.4)		36 (15.4)	35 (14.4)	
MT	86 (37.7)	83 (41.3)	0.4	76 (32.5)	96 (39.5)	0.3
TT	112 (49.1)	87 (43.3)	0.1	122 (52.1)	112 (46.1)	1.0
AT1R A1166C						
AA	210 (92.1)	182 (90.5)		210 (89.7)	217 (89.3)	
*AC+CC	18 (7.9)	19 (9.5)	0.6	24 (10.3)	26 (10.7)	0.8

Values in parenthesis indicate percentage
*One homozygous variant genotype of *AGT* T207M and *AGT1R* A1166C polymorphism in hypertensive cases were combined with heterozygous genotype for analysis.

cant association between the *ACE* ID [II vs. ID, OR=1.4 (95% CI: 1.0-1.9), $p < 0.05$] and DD genotypes and hypertension [II vs. DD, OR= 1.5 (95% CI: 1.1-2.2), $p < 0.02$]. When the association between *ACE* ID genotype and hypertension was analyzed after adjusting the confounding factors, the risk for hypertension was further increased in ID heterozygous and DD homozygous genotype carriers (Table 4). There were no significant differences in the genotypes and alleles for *AGT* M268T, T207M and *AGT1R* A1166C gene polymorphisms between hypertensive patients and controls before and after adjusting the confounding factors.

Gender Specific Distribution of RAS Gene Polymorphisms among Cases and Controls

The frequency of *ACE* ID heterozygous genotype was higher among male hypertensive patients compared with the control group (48.7% vs. 41.3%, $p< 0.03$). The prevalence of the *ACE* D allele was higher in male cases when compared to male controls (53.3 % vs. 46.5 %, $p<$

Table 4: Odds ratio for RAS gene polymorphisms among the study subjects

Genotypes	*All subjects*		*Males*		*Females*	
	OR (95% CI)	*p value*	*OR (95% CI)*	*p value*	*OR (95% CI)*	*p value*
ACE ID						
II	1.0		1.0		1.0	
ID	1.5 (1.0-2.3)	0.05	1.9 (1.1-2.6)	0.01	1.1 (0.7-1.7)	0.8
DD	1.7 (1.2-2.8)	0.01	1.6 (1.0-2.8)	0.06	1.6 (0.9-2.5)	0.1
AGT T207M						
TT	1.0		1.0		1.0	
*TM+MM	1.0 (0.7-1.3)	0.6	0.9 (0.5-1.4)	0.4	0.9 (0.5-1.5)	0.6
AGT M268T						
MM	1.0		1.0		1.0	
MT	1.1 (0.8-1.7)	0.8	1.1 (0.6-1.9)	0.1	0.8 (0.5-1.4)	0.3
TT	1.3 (0.9-2.1)	0.2	1.4 (0.7-2.4)	0.2	1.0 (0.7-1.7)	0.8
AT1R A1166C						
AA	1.0		1.0		1.0	
*AC+CC	1.0 (0.5-1.9)	0.9	0.7 (0.4-1.6)	0.3	1.0 (0.5-1.9)	1.0

Values in parenthesis indicate percentage (Table 3)
Odds ratio and p value adjusted for confounding factors such as smoking in males and age in females.
*One homozygous variant genotype in *AGT* T207M and *AGT1R* A1166C were combined with heterozygous genotype for analysis.

Table 5: Gene-gene interaction of RAS gene polymorphisms among cases and controls

Genotypes	*Cases (n=462)*	*Controls (n=444)*	*OR (95% CI)*	*p value*
ACE ID & *AGT* T207M				
ACE II & *AGT* TT	108 (23.4)	128 (28.8)	1.0	
ACE II & *AGT* TM or MM	23 (5.0)	34 (7.7)	0.7 (0.5-134)	0.6
ACE ID or DD & *AGT* TT	265 (57.3)	226 (50.9)	1.4 (0.9- 2.1)	0.08
ACE ID or DD & *AGT* TM or MM	66 (14.3)	56 (12.6)	1.4 (0.9- 2.2)	0.2
ACE ID & *AGT* M268T				
ACE II & *AGT* MM	19 (4.1)	27 (6.1)	1.0	
ACE II & *AGT* MT/TT	104 (22.5)	135 (30.4)	1.1 (0.6- 2.1)	0.9
ACE ID or DD & *AGT* MM	49 (10.6)	48 (10.8)	1.4 (0.6- 2.8)	0.3
ACE ID or DD & *AGT* MT or TT	290 (62.8)	234 (52.7)	2.0 (1.2- 3.5)	0.01
ACE ID & *AT1R* A1166C				
ACE II & *AT1R* CC	113 (24.5)	136 (30.6)	1.0	
ACE II & *AT1R* AC or CC	13 (2.8)	16 (3.6)	1.0 (0.5- 2.1)	0.7
ACE ID or DD & *AT1R* AA	300 (64.9)	263 (59.2)	1.4 (0.98-1.9)	0.08
ACE ID or DD & *AT1R* AC or CC	36 (7.8)	29 (6.5)	1.5 (0.9- 2.6)	0.2

Values in parenthesis indicate percentage.
Odds ratio and p value adjusted for confounding factors age, smoking and genotypes.

0.06) whereas there was no significant association between *AGT* T207M, M268T and *AGT1R* A1166C gene polymorphisms and hypertension among the male and female cases and controls (Table 3).

Values of some confounding factors were higher in male hypertensive patients compared with male controls, including smoking (43.4 % vs. 20%, $p < 0.001$) and LDL cholesterol (112.5 ± 1.4 vs.107.4 ± 1.3, $p< 0.01$). Age was higher in female controls than in female cases (46.8 ± 0. vs. 43.4 ± 0.5, $p < 0.01$). These confounding factors were adjusted by multiple logistic regression analysis to calculate the adjusted odds ratio as shown in Table 4. When the association between the hypertensive risk and RAS genotypes was analysed, ID genotype of *ACE* was significantly associated with hypertension among male subjects [II vs. ID; OR=1.9; 95% CI: 1.1-2.6, $p<0.01$)]. There was no significant difference in the genotype and allelic distribution of *AGT* M268T, T207M and *AGT1R* A1166C gene polymorphisms amongst the male and female cases and controls even after adjusting the confounding variables.

Gene-gene Interaction of RAS Gene Polymorphisms

The high risk genotypes (heterozygous and homozygous variants) of RAS gene polymorphisms were investigated by comparing them with no risk genotypes (wild-type reference genotype) for gene-gene interaction. Individuals carrying the combination of both heterozygous and homozygous variant genotypes of *ACE* ID and *AGT* M268T were found to have a twofold higher risk for hypertension (Table 5). The risk was not significant between the combinations of other gene polymorphisms and hypertension among the cases and controls (Table 6).

Haplotypes of AGT (M268T and T207M) Gene Polymorphisms

The two polymorphisms, *AGT* T207M and M268T were found to be in weak linkage disequilibrium (LD correlation, r value = 0.9, $p < 0.07$). Four different haplotypes were constructed as shown in Table 7. The haplotype frequencies were almost equally distributed and did not differ significantly among cases and controls.

DISCUSSION

Cardiovascular disorders resulted in 2.3 million deaths in the year 1990 and are expected to be doubled by the year 2020. Hypertension accounts for 1.2 million deaths due to coronary heart disease and 0.5 million deaths due to stroke in India (Gupta et al. 2004). The present study is the largest case-control study to describe the role of RAS gene polymorphisms and susceptibility to EH in any of the Indian population.

When the influence of all the three genes (4 SNPs) were analyzed separately, the *ACE* ID heterozygous and homozygous DD genotype of *ACE* gene were found to be associated with

Table 6: Gene-gene interactions (three way) of *ACE* ID, *AGT* M268T, T207M and *AT1R* A1166C genotypes on hypertension risk

Genotypes	*Cases (n=462)*	*Controls (n=444)*	*OR [a] (95%CI)*	*p value*
ACE ID, *AGT* T207M & M268T				
II, TT & MM	20	24	1.0	
II, TT & MT or TT	101	122	1.3 (0.6-2.8)	0.6
II, TM or MM & MM	3	3	1.1 (0.2-7.8)	0.8
II, TM or MM & MT or TT	12	13	1.0 (0.3-3.2)	0.9
ID or DD ,TT & MM	38	41	1.5 (0.6-3.3)	0.5
ID or DD, TT & MT or TT	255	212	2.0 (1.0-4.1)	0.09
ID or DD, TM or MM & MM	7	7	1.3 (0.3-5.1)	0.7
ID or DD, TM or MM & MT or TT	26	22	2.1 (0.8-5.1)	0.1
ACE ID, *AT1R* A1166C & *AGT* T207M				
II, AA & TT	103	116	1.0	
II, AA & TM or MM	22	30	0.7 (0.4-1.5)	0.8
II, AC or CC & TT	10	12	0.8 (0.3-2.2)	0.8
II, AC or CC & TM or MM	2	4	0.7 (0.2-4.0)	0.9
ID or DD, AA & TT	237	205	1.5 (1.0-2.1)	0.06
ID or DD, AA & TM or MM	54	48	1.5 (0.9-2.4)	0.1
ID or DD, AC or CC & TT	27	22	1.6 (0.8-3.1)	0.3
ID or DD, AC or CC & TM or MM	7	7	1.0 (0.3-3.2)	0.6
ACE ID, *AT1R* A1166C & *AGT* M268T				
II, AA & MM	19	24	1.0	
II, AA & MT or TT	108	122	1.3 (0.6-2.8)	0.7
II, AC or CC & MM	2	3	1.1 (0.2-7.8)	0.9
II, AC or CC & MT or TT	10	13	1.0 (0.3-3.2)	0.7
ID or DD, AA & MM	38	41	1.5 (0.6-3.3)	0.5
ID or DD, AA & MT/TT	255	212	2.0 (1.0-4.1)	0.04
ID or DD, AC or CC & MM	7	7	1.3 (0.3-5.1)	0.8
ID or DD, AC or CC & MT or TT	23	22	2.1 (0.8-5.1)	0.1

[a]Odds ratio adjusted for age, smoking and other genotypes

hypertension whereas there was no association between *AGT* T207M, M268T and *AGT1R* A1166C gene polymorphisms and hypertension. Interestingly, I/D heterozygous genotype was found to be significantly associated with hypertension in men, but not in women. Previous studies on *ACE* ID gene polymorphism in the Indian population showed a significant association with DD genotype and diastolic blood pressure in men (Bhavani et al. 2004), whereas another study did not show such association with DD genotype in hypertensive men (Ashavaid et al. 2000). In the present study, mean difference in blood pressure and resting heart rate could not be evaluated among the different genotype groups since many of our hypertensive subjects were on antihypertensive medication. When the effect of RAS gene polymorphisms on blood pressure was analyzed in newly diagnosed hypertensive subjects alone, significant differences were not observed among the different genotype groups. The observed differences in the results of our study and previous Indian study may be due to difference in the selection of study subjects (Bhavani et al. 2004). We have excluded the subjects with diabetes mellitus, family history of hypertension, and patients with other cardiovascular disorders whereas their study included the confounding risk factors of hypertension (Bhavani et al. 2004). However, our results are similar with the Japanese (Higaki et al. 2000), Indians (Bhavani et al. 2005), Turkish study (Agachan et al. 2003), African-Americans (Duru et al. 1994) and Framingham heart study (O'Donnell et al. 1998). Earlier, it was proposed that subjects homozygous for deletion DD

Table 7: Haplotypes of *AGT* T207M and M268T gene polymorphism

Haplotypes	*Cases (n = 924)*	*Controls (n = 888)*	*OR (95% CI)*	*p value*
T, M	560 (60.6)	512 (57.6)	1.0	
M, M	272 (29.4)	286 (32.2)	0.9 (0.7-1.1)	0.4
T, T	76 (8.2)	64 (7.2)	1.1 (0.8-1.5)	0.7
M, T	16 (1.7)	26 (3.0)	0.6 (0.3-1.2)	0.1

Values in parenthesis indicate percentage.

genotypes had higher ACE levels and heterozygous ID subjects had intermediate ACE levels (Rigat et al. 1990; Martinez et al. 2000). But studies also showed negative association between ACE genotypes and ACE levels (Jeunemaitre et al. 1992a). Association between *AGT* T207M and M268T polymorphisms with hypertension was first explained by Jeunemaitre et al. (1992b) and later on, studies among different ethnic groups showed association between M268T polymorphism and increased plasma angiotensinogen levels in T allele carriers with hypertension (Bloem et al. 1997; Caulfield et al. 1994; Caulfield et al. 1995; Paillard et al. 1999). A meta analysis by Staessan et al. (1997) comprising 69 studies have shown that the presence of T268 allele was associated with increased risk of hypertension in Caucasians but not in Africans-Africans and Asians. Nejatizadeh et al. (2008) reported a positive association between *AGT* T268 allele and hypertension in a North Indian population. Contrary to the previous report, a study conducted by Nair et al. in western Indians failed to find association between M268T polymorphism and hypertension and the results are similar with our study (Nair et al. 2003). The genotype and allelic distribution of *AGT* T207M did not show significant difference with hypertension and the results of our study are consistent with Indians (Nair et al. 2003; Nejatizadeh et al. 2008), Caucasians (Caulfield et al. 1994) and Russians (Mustafina et al. 2002). In the present study, we could not observe any significant association between *AGT1R* A1166C gene polymorphism and hypertension. A case-control study by Bonnardeaux et al. showed significant association between A1166C polymorphism and hypertension in Caucasians (Bonnardeaux et al. 1994). Later, similar results were reported (Dzida et al. 2001). However, our results are in agreement with the other studies reported in Indians (Ashavaid et al. 2000), and Chinese population (Liu et al. 2002) that did not show significant association between A1166C polymorphism and hypertension.

Hypertension is a polygenic disease and its genetic susceptibility is influenced by multiple gene polymorphisms. Polymorphisms of individual genes may impart susceptibility to a small extent and it is likely that multiple genes are involved in its pathogenesis (Williams et al. 2000; Porto et al. 2003). Many studies have described the association of these polymorphisms with hypertension on the basis of single locus. However, these studies have not explained the association with multiple gene-gene interaction. Therefore, we analyzed the RAS genes to determine whether genotypes in combination alter the hypertension susceptibility. The present study observed a significant association between the combinations of *ACE* ID and *AGT* M268T gene polymorphisms whereas a study by Nair et al. (2003) did not find significant interaction between *ACE* ID and *AGT* M268T polymorphism in Indian subjects. It was earlier explained that both the polymorphisms *AGT* T207M and M268T were in complete linkage disequilibrium and the haplotype combinations of variant alleles were higher in hypertensive cases when compared to controls (Jeunemaitre et al. 1992a). In the present study, both the polymorphisms were found to be in weak linkage disequilibrium. Haplotype combinations of *AGT* 207T and 268T were found to be higher in hypertensive cases as compared to controls in North Indians (Nejatizadeh et al. 2008). However, there were no differences in the haplotypes constructed in our study. The strength of our study includes strict selection of unrelated cases and controls in a homogenous population. The study would have been strengthened with the measurements of circulating ACE and AGT level for genotype-phenotype correlation.

To conclude, the present study showed a significant association between *ACE* ID polymorphism and hypertension. However, such association was not observed for *AGT* T207M, M268T and *AGT1R* A1166C gene polymorphisms. The findings of our study deviated from other Indian studies and other populations. This may be due to the presence of unidentified or other important polymorphisms in the RAS gene, difference in selection of study subjects and influence of environmental factors. The present study results imply that *ACE* ID polymorphism is a predisposing factor for hypertension. Further, the risk was modified with the combined effects of *ACE* ID and *AGT* M268T variant genotypes (gene-gene interaction) which resulted in significantly increased risk to hypertension.

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