Coronary Heart Disease, Hypertension, and Angiotensinogen Gene Variants in Indian Population

K.G. Nair, K.K. Shalia, T.F. Ashavaid,* and J.J. Dalal

Research Laboratories, P.D. Hinduja National Hospital and Medical Research Centre, Mumbai, India

Gene encoding components of the renin angiotensin system (RAS) have been implicated with the increased risk of cardiovascular disease (CVD). Two variants of the angiotensinogen (AGT) gene, M235T and T174M, have been shown to be associated with increased risk of hypertension. In the present study, we examined the association of these two polymorphisms and their synergistic interaction with the angiotensin I-converting enzyme (ACE) deletion homozygote genotype (D/D) on subjects with coronary heart disease (CHD) and hypertension. We studied 131 healthy individuals, 141 angiographically verified CHD patients, and 159 hypertensive subjects. The identification of the ACE and

AGT gene polymorphisms was carried out using a PCR-based restriction endonuclease digestion method. There was no significant difference in the distribution of the M235T and T174M variants between the two test groups and the control group. Association was also not seen when analysis was carried out in patients when subgrouped according to the extent of the severity of the disease. In addition, the risk was not restricted to subjects carrying the D allele of the ACE gene and T235T of AGT. M235T and T174M variants do not contribute to the increased risk of CHD or hypertension in the Indian population. J. Clin. Lab. Anal. 17:141–146, 2003.

© 2003 Wiley-Liss, Inc.

Key words: renin angiotensin system; coronary heart disease; hypertension

INTRODUCTION

In recent years, the evidence of the pathologic role of angiotensin II in cardiovascular disease (CVD) and of the proved efficacy of angiotensin I-converting enzyme (ACE) inhibitors and angiotensin II type I receptor (AT1R) inhibitors in preventing major cardiovascular events has focused interest on the genetics of the renin angiotensin system (RAS). Extensive studies on the insertion (I)/ deletion (D) polymorphism of the ACE gene, A/C 1166 of the AT1R gene, and the M235T and T174M variants of the AGT gene have been documented (1). The importance of these variations as risk factors for CVD, however, remains uncertain. In the Indian population, polymorphisms of the ACE (2) and AT1R (3) gene were not found to be associated with coronary artery disease (CAD). In another study carried out on hypertensive subjects, association of the D allele was not observed. However, the D allele was significantly associated with an early onset (≤ 40 years) of hypertension (4). In the present paper, we discuss the role of two molecular variants of the AGT gene; M235T and T174M, with hypertension and coronary heart disease (CHD) and their significant interaction with the

D allele of the ACE gene in increasing the risk of CHD and hypertension.

MATERIALS AND METHODS

The study group was comprised of 141 angiographically verified CAD patients. Each had at least one coronary artery with 50% luminal diameter obstruction. Another test group was comprised of 159 hypertensive patients who were selected from clinical records after attendance at the health clinic of the P.D. Hinduja National Hospital and Medical Research Centre. They had no other major cardiovascular complications,

Grant sponsor: National Health and Education Society (NHES) of P.D. Hinduja National Hospital and Medical Research Centre, Mumbai, India.

*Correspondence to: Tester F. Ashavaid, Consultant Biochemist, Head, Department of Laboratory Medicine, P.D. Hinduja National Hospital and Medical Research Centre, Veer Savarkar Marg, Mahim, Mumbai 400 016, India. E-mail: dr_tashavaid@hindujahospital.com

Received 8 April 2002; Accepted 12 December 2002
DOI 10.1002/jcla.10084

Published online in Wiley InterScience (www.interscience.wiley.com).

secondary hypertension, or diabetes mellitus. A total of 131 healthy individuals with BP <140/90 mmHg were taken as controls from the same clinic and were age and sex matched with patients. They had no history of cardiovascular diseases or any other organic disease.

Analysis of biochemical parameters was carried out on a Synchro Cx 7 analyzer (Synchro, Inc., Lafayette, IN) using commercially available kits as described previously (4). Plasma AGT was measured with a two-step procedure, including the conversion of AGT to angiotensin I by renin (0.005 units) (5) from Sigma (Sigma Chemical Company, St. Louis, MO), followed by the measurement of angiotensin I by radioimmunoassay (RIANEN Angiotensin I assay system, DuPont, Boston, MA).

Genomic DNA was extracted from peripheral blood leukocytes using a salting out procedure as described by Miller et al. (6). I/D polymorphism of the ACE gene was carried out as described previously (4). M235T and T174M variants of the AGT gene were genotyped using a PCR-based restriction endonuclease digestion method (7). (See Figs 1 and 2)

Statistical Analysis

Quantitative variables were expressed as mean $\pm$ standard deviation. Statistical significance of these

parameters between the two groups was assessed by Student's *t*-test. Genotype distribution and allele frequencies were compared between groups using the chi-square (χ^2) test. The odds ratio (OR) and 95% confidence interval (CI) were calculated to evaluate the risk. A value of $P < 0.05$ was considered to be statistically significant.

RESULTS

Table 1 shows the clinical and biochemical profiles of the study participants in the two test groups and control group. In both the test groups, there was not much difference in the ACE level as compared to the control group but they were highest in subjects with D/D genotype, lowest in subjects with I/I genotype and intermediate in subjects with I/D genotype as previously described (2,4). In both the test groups, subjects taking ACE and AT1R inhibitors were not included for calculating ACE levels. AGT levels were significantly high in both the CHD and hypertensive group as compared to the control group. High levels of AGT in the hypertension group were observed with the M235T genotype, while in the control group and the CHD group it was associated with the M235M genotype.

The genotype analysis of M235T and T174M variants is depicted in Tables 2 and 3 for the CHD and the

Fig. 1. M235T variants of the angiotensinogen gene. Lane MW contains molecular weight marker ϕ X 174 DNA Hae III Digest, lane MM contains band at 266 bp, lane TT contains band at 303 bp, and lane MT contains band at 266 bp as well as at 303 bp.

Fig. 2. T174M variants of the angiotensinogen gene. **Lane MW** contains molecular weight marker ϕ X 174 DNA Hae III Digest, **lane TT** contains band at 303 bp, **lane MM** contains band at 210 bp and at 93 bp, and **lane TM** contains band at 303 bp, at 210 bp, as well as at 93 bp.

TABLE 1. Clinical and biochemical profile of the healthy individuals, CHD patients and hypertensives

	Controls	CHD patients	Hypertensive patients
Age (years)	48.05 $\pm$ 5.78	55.61 $\pm$ 4.75	50.25 $\pm$ 9.78
Sex (male/female)	78/53	116/25	109/50
Hypertension (with/without)	—	64/77	
Cholesterol (mg/dl)	181.9 $\pm$ 29.85	196.13 $\pm$ 24.07 ^a	165 $\pm$ 20.65 ^a
Triglycerides (mg/dl)	103.8 $\pm$ 33.31	149.16 $\pm$ 76.56 ^a	131 $\pm$ 15.65 ^a
HDL C (mg/dl)	47.78 $\pm$ 4.43	30.66 $\pm$ 8.61 ^a	39.45 $\pm$ 6.47 ^b
Ratio	3.5 $\pm$ 1.5	6.8 $\pm$ 4.25 ^a	4.1 $\pm$ 2.5 ^b
LDL C (mg/dl)	109.2 $\pm$ 28.7	145 $\pm$ 28.0 ^a	110 $\pm$ 25.3 ^b
ApoA1/ApoB	1.01 $\pm$ 0.095	0.91 $\pm$ 0.096 ^a	1.01 $\pm$ 0.096 ^b
Plasma ACE levels (IU/L)	33.61 $\pm$ 13.62 n = 130	37.23 $\pm$ 12.68 ^b n = 130	35.13 $\pm$ 11.65 ^b n = 150
Plasma angiotensinogen levels (pmol/ml)	145.10 $\pm$ 65.10 n = 87	245.71 $\pm$ 106.9 ^a n = 85	164.85 $\pm$ 65.0 ^a n = 120

^a $P < 0.001$.

^bNS, not significant.

hypertension group, respectively. Within the control group, the frequencies of the M235M, M235T, and T235T variants were 8.4%, 30.53%, and 61.06%, respectively, while that of T174T, T174M, and M174M genotypes were 77.86%, 20.61%, and 1.53%, respectively. The distribution of the genotypes was according to the Hardy Weinberg equilibrium. There was only a marginal difference in the distribution of these genotypes in the CHD as well as in the hypertensive group from that of the control group.

Within the CHD group, the data of M235T and T174M variants were further analyzed according to the presence or absence of diabetes mellitus or hypertension and between subjects having single vessel disease and those having double and triple vessel disease. There was

no significant difference in the distribution observed. CHD patients with low risk factors (without diabetes mellitus or hypertension or with single vessel disease) were compared with controls as well; however, no significant association was observed even then (data not shown).

Within the hypertension group, the distribution of these genotypes was analyzed according to the age of onset of hypertension (≤ 40 years and > 40 years) and presence or absence of family history. The statistical analysis did not show any significant change in the distribution between the two criteria. Significant difference in the distribution was also not seen when subjects with low risk factors (late onset of hypertension (> 40 years) and no family history) were compared against our

Table 2. Distribution of genotype and allele frequencies of M235 T and T174 M genotypes of controls and CHD patient groups

M235 T	MM	MT	TT	T174M	TT	TM	MM
Controls (N = 131)	11 (8.4%)	40 (30.53%)	80 (61.06%)	Controls (N = 131)	102 (77.86%)	27 (20.61%)	02 (1.53%)
M/F	7/4	24/16	47/33	M/F	60/42	17/10	01/01
CHD Patients (N = 141)	09 (6.38%)	36 (25.53%)	96 (68.09%)	CHD patients (N = 136)	111 (81.62%)	25 (18.38%)	00
M/F	9/0	26/10	81/15	M/F	90/21	23/02	
	$\chi^2 = 1.49$, df = 2, NS			$\chi^2 = 0.583^a$, df = 1, NS			
	M	T			T	M	
Controls	62 (0.24)	200 (0.76)		Controls	231 (0.88)	31 (0.12)	
CHD patients	54 (0.19)	228 (0.81)		CHD patients	247 (0.91)	25 (0.09)	
	$\chi^2 = 1.65$, df = 1, NS			$\chi^2 = 0.99$, df = 1, NS			

Figures in the parentheses indicate percentage to the respective total in case of genotype analysis and allele frequency in case of allele analysis.

^aData for M174 M genotype is pooled with T174 M genotype for calculating chi square.

df, degree of freedom; NS, not significant.

Table 3. Distribution of genotype and allele frequencies of M235 T and T174 M genotypes of controls and hypertensive groups

M235T	MM	MT	TT	T174M	TT	TM	MM
Controls (N = 131)	11 (8.4%)	40 (30.53%)	80 (61.06%)	Controls (N = 131)	102 (77.86%)	27 (20.61%)	02 (1.53%)
M/F	7/4	24/16	47/33	M/F	60/42	17/10	01/01
Hypertensive patients (N = 159)	15 (9.43%)	60 (37.74%)	84 (52.83%)	Hypertensive patients (N = 134)	104 (77.61%)	29 (21.64%)	01 (0.746%)
M/F	10/05	40/20	59/25	M/F	67/37	17/12	00/01
	$\chi^2 = 2.003$, df = 2, NS			$\chi^2 = 0.002^a$, df = 1, NS			
	M	T			T	M	
Controls	62 (0.24)	200 (0.76)		Controls	231 (0.88)	31 (0.12)	
Hypertensive patients	90 (0.28)	228 (0.72)		Hypertensive patients	237 (0.88)	31 (0.12)	
	$\chi^2 = 1.60$, df = 1, NS			$\chi^2 = 0.008$, df = 1, NS			

Figures in the parentheses indicate percentage to the respective total in case of genotype analysis and allele frequency in case of allele analysis.

^aData for M174 M genotype is pooled with T174 M genotype for calculating chi square.

df, degree of freedom; NS, not significant.

Table 4. Synergistic effect of DD of ACE and TT of M235 T variants in CHD patients and hypertensive patients

	T235 T genotypes		T235 T genotypes	
	CHD patients	Controls	Hypertensive patients	Controls
D/D	25	23	14	23
I/D + I/I	68	55	69	55
	$\chi^2 = 0.143$ df = 1, NS OR = 0.879 (95% CI 0.45–1.71)		$\chi^2 = 3.62$ df = 1, NS OR = 0.485 (95% CI 0.228–1.029)	

df, degree of freedom; NS, not significant.

control group (data not shown). Within the hypertension group, data analysis according to blood pressure was not carried out because all the hypertensive patients included were on antihypertensive medication.

We also analyzed the significant interaction between the ACE D/D and AGT T235T genotypes in increasing the risk of CHD. As stated in our previous studies (2,4), the ACE I/D polymorphism was not associated with

CHD. In the present study in both the groups, the risk was not found to be associated with subjects carrying the DD genotype and T235T as well (Table 4). With respect to the T174M genotype, the CHD group had none and the hypertension group had one subject with a mutant homozygous M174M genotype. The ACE gene polymorphism of which was I/D was heterozygous for a M235T AGT gene polymorphism. The two subjects in

the control group with the M174M genotype were also of the I/D genotype and were homozygous mutant for the M235T variant.

DISCUSSION

The present study investigated the relationship between genetic polymorphisms, M235T and T174M, in the gene encoding angiotensinogen (AGT), and the risk of hypertension and CHD in the Indian population. In our study comprising of 159 hypertensive subjects, 141 angiographically verified CHD patients, and 131 healthy subjects, we did not find significant association of mutant variant of both the genotypes with these patient groups. Mutant variant was also not associated with the extent of severity of the disease or patients having low risk. Significant interaction with the ACE gene in increasing the risk of CHD or hypertension was also not observed.

The AGT gene has been mapped on chromosome 1q4 (8) and implicated in essential hypertension through both genetic linkage and allelic association by Jeunemaitre et al. (9), initially in both Utah and French Caucasians. They studied M235T ($T^{704} \rightarrow C$) and T174M ($C^{521} \rightarrow T$) variants in exon 2 and reported that subjects carrying M235T genotypes with or without the T174M variant (0.14 and 0.33) were more associated with hypertensives than with controls (0.09 and 0.28). Since then, several studies have been carried out to show the association of M235T and T174M genotypes of AGT with CHD (10–20) and hypertension (21–28), wherein positive as well as negative reports are documented.

Staessen et al. (29) in a meta-analysis of 69 studies of the AGT gene (until April, 1998) with CVD have shown that the presence of the T 235 allele was associated with increased risk of hypertension in Whites, but not in Blacks or Asians. Further atherosclerotic complications were not correlated with M235T polymorphism in any population. In their study, an important observation was that prevalence of the T allele was 0.77 in Blacks and 0.78 in Asians, but reached only 0.42 in Caucasians. Later studies have also reported similar findings (10,25). Bloem et al. (30) have studied M235T variants in Black and White subjects from Indianapolis and have reported the T235 allele frequency as 0.41 for Whites and 0.81 for Blacks. In our study on the Indian population, T235 allele frequency (0.76) was similar to Blacks and Asians and noticeably different from Whites. Staessen et al. (29) further state that the higher prevalence of the T allele in the former two races may have provided an insufficient power to demonstrate an association using the uncommon M235M genotype as the reference group.

The distribution of the T174M genotype does not show a marked difference between various ethnic groups, and the M174 allele frequency (0.12) in our

population was similar to that reported in Caucasians (9,21), Chinese (25), and Japanese (12,17,27).

The T235 allele has been associated with a 10%–20% increase in plasma AGT level (9). Winkelmann et al. (10) reported a stepwise increase in the AGT levels with the T allele. Bloem et al. (30) reported the same but only in Whites while in Blacks the influence of M235T genotypes was not seen. In our study, highest mean levels of AGT in controls and CHD patients were associated with the M235M genotype and in the hypertensive group, with M235T genotype.

In conclusion, we fail to detect an association of M235T or T174M variants of the AGT gene with CHD or hypertension, indicating that these polymorphisms may not be a risk factor for CHD or hypertension in the Indian population.

REFERENCES

1. Ashavaid TF, Shalia KK, Altaf AK, Todur SP, Nair KG, Nair SR. Gene polymorphism and coronary risk factors in Indian population. *Clin Chem Lab Med* 2002;40:975–985.
2. Joseph A, Nair KG, Ashavaid TF. Angiotensin converting enzyme gene polymorphism in coronary artery disease: the Indian scenario. *Clin Chem Lab Med* 1998;36:621–624.
3. Nair KG, Ashavaid TF, Shalia KK, Dalal JJ. Angiotensin II type I receptor A/C1166 gene polymorphism and coronary artery disease [Abstract]. *Indian Heart J* 1999;51:601.
4. Ashavaid TF, Shalia KK, Nair KG, Dalal JJ. ACE and AT1R gene polymorphisms and hypertension in Indian population. *J Clin Lab Anal* 2000;14:230–237.
5. Yang G, Merrill DC, Thompson MW, et al. Functional expression of the human angiotensinogen gene in transgenic mice. *J Biol Chem* 1994;269:3247–3250.
6. Miller SA, Dykes DD, Polesky HF. A simple salting out procedure for extraction of DNA from human nucleated cells. *Nucleic Acids Res* 1988;16:1215.
7. Fernandez-Llama P, Poch E, Oriola J, Boley A, Rivera F, Revert L. Angiotensinogen gene M235T and T174M polymorphisms in essential hypertension: relation with target organ damage. *Am J Hypertens* 1998;11:439–444.
8. Gaillard-Sauech I, Mattei MG, Claude E, Coronel P. Assignment by in situ hybridization of the angiotensinogen gene to chromosome band 1q4, the same region as the human renin gene. *Hum Genet* 1990;84:341–343.
9. Jeunemaitre X, Soubrier F, Kotelevtsev YV, et al. Molecular basis of human hypertension: role of angiotensinogen. *Cell* 1992;71:169–180.
10. Winkelmann BR, Russ AP, Nauck M, et al. Angiotensinogen M235T polymorphism is associated with plasma angiotensinogen and cardiovascular disease. *Am Heart J* 1999;137:698–705.
11. Katsuya T, Koike G, Yee TW, et al. Association of angiotensinogen gene T235 variant with increased risk of coronary heart disease. *Lancet* 1995;345:1600–1603.
12. Cong ND, Hamaguchi K, Saikawa J, Hara M, Sakata T. A polymorphism of angiotensinogen gene at codon 174 and coronary artery disease in Japanese subjects. *Am J Med Sci* 1998;316:339–344.
13. Tiret L, Blanc H, Ruidavets J, et al. Gene polymorphism of the renin-angiotensin system in relation to hypertension and parental history of myocardial infarction and stroke: the PEGASE Study.

- Projet d'Etude des Genes de l'Hypertension Arterielle Severe a moderee Essentielle. *J Hypertens* 1998;16:37-44.
14. Reinhardt D, Sigusch HH, Vogt SF, et al. A common variant of the angiotensinogen gene and the risk of coronary artery disease in German population. *Pharmazie* 2000;55:69-71.
 15. Ortega HE, Fernandez-Aceituno MA, Esparragon RFJ, et al. The involvement of the renin-angiotensin system gene polymorphisms in coronary heart disease. *Rev Esp Cardiol* 2002;55:92-99.
 16. Ishigami T, Umemura S, Iwamoto T, et al. Molecular variant of angiotensinogen gene is associated with coronary atherosclerosis. *Circulation* 1995;91:951-954.
 17. Ichihara S, Yokota M, Fujimura T, et al. Lack of association between variants of the angiotensinogen gene and the risk of coronary artery disease in middle aged Japanese men. *Am Heart J* 1997;134:260-265.
 18. Ludwig EH, Boerckl IB, Ellison RC, et al. Association between candidate loci in angiotensin converting enzyme and angiotensinogen with coronary heart disease and myocardial infarction; the NHLBI Family Heart Study. *Ann Epidemiol* 1997;7:3-12.
 19. Petrovic D, Zorc M, Kanic V, Peterlin B. Interaction between gene polymorphisms of renin-angiotensin system and metabolic risk factors in premature myocardial infarction. *Angiology* 2001;52:247-252.
 20. Jeunemaitre X, Ledru F, Battaglia S, et al. Genetic polymorphisms of the renin-angiotensin system and angiographic extent and severity of coronary artery disease: the CORGENE Study. *Hum Genet* 1997;99:66-73.
 21. Caulfield M, Lavender P, Farrall M, et al. Linkage of the angiotensinogen gene to essential hypertension. *N Engl J Med* 1994;330:1629-1633.
 22. Hata A, Namikawa C, Sasaki M, et al. Angiotensinogen as a risk factor for essential hypertension in Japan. *J Clin Invest* 1994;93:1285-1287.
 23. Suwazono Y, Kobayashi E, Sakurada I, Ohkubo Y, Nogawa K, Kido T. Association of the angiotensinogen gene (M235T, T174M) and the angiotensin I-converting enzyme (I/D) with blood pressure in Japanese workers. *Blood Press* 1999;8:23-28.
 24. Rotimi C, Morrison L, Cooper R, et al. Angiotensinogen gene in human hypertension. Lack of an association of the 235T allele among African Americans. *Hypertension* 1994;24:591-594.
 25. Niu T, Chen C, Yang J, et al. Blood pressure and the T174M and M235T polymorphisms of the angiotensinogen gene. *Ann Epidemiol* 1999;9:245-253.
 26. Fornage M, Turner ST, Sing CF, Boerwinkle E. Variation at the M235T locus of the angiotensinogen gene and essential hypertension: a population-based case-control study from Rochester, Minnesota. *Hum Genet* 1995;96:295-300.
 27. Morise T, Takeuchi Y, Takeda R. Rapid detection and prevalence of the variants of the angiotensinogen gene in patients with essential hypertension. *J Intern Med* 1995;237:175-180.
 28. Rutledge DR, Browe CS, Kubilis PS, Ross EA. Analysis of two variants of the angiotensinogen gene in essential hypertensive African-Americans. *Am J Hypertens* 1994;7:651-654.
 29. Staessen JA, Kuznetsova T, Wang JG, Emelianov D, Vlietinck R, Fagard R. M235T angiotensinogen gene polymorphism and cardiovascular renal risk. *J Hypertens* 1999;17:9-17.
 30. Bloem LJ, Manatunga AK, Tewksbury DA, Pratt JH. The serum angiotensinogen concentration and variants of the angiotensinogen gene in White and Black children. *J Clin Invest* 1995;95:948-953.