ACE and AT1R Gene Polymorphisms and Hypertension in Indian Population

T.F. Ashavaid,^{1*} K.K. Shalia,¹ K.G. Nair,² J.J. Dalal¹

¹Research Laboratories, P.D. Hinduja National Hospital and Medical Research Centre, Mumbai, India

²Breach Candy Hospital and Medical Research Centre, Mumbai, India

The renin angiotensin system (RAS) controls intrarenal blood pressure and sodium balance, and is an important target for anti-hypertensive therapy. Several polymorphisms have been identified within genes encoding RAS that may contribute to the development of elevated blood pressure. The relevance of these polymorphisms in hypertension remains controversial. In this study we have examined 105 hypertensive subjects and 192 controls from the Indian population for I/D polymorphism of angiotensin I converting enzyme (ACE) and A¹¹⁶⁶C polymorphism of angiotensin II type I receptor (AT1R) genes by polymerase chain reaction (PCR) and PCR-based restriction enzyme analysis method, respectively. There was no

significant difference in the distribution of ACE (I/I, I/D, and D/D) and AT1R (A/A and A/C) genotypes between controls and hypertensive subjects. D allele was significantly associated with an early onset of hypertension and although nonsignificant, the frequency was high in subjects with family history of cardiovascular disorders. C¹¹⁶⁶ allele of AT1R did not correlate with the age of onset of hypertension and the frequency was low in subjects with family history. Thus no association was found between ACE and AT1R genotypes and hypertension. However the D allele can be used as a predictor of risk of hypertension in the Indian population. *J. Clin. Lab. Anal.* 14:230–237, 2000. © 2000 Wiley-Liss, Inc.

Key words: renin angiotensin system; angiotensin II; insertion/deletion polymorphism; A¹¹⁶⁶C polymorphism; India

INTRODUCTION

Hypertension is a major risk factor for morbidity and mortality from cardiovascular causes. It is a multifactorial and polygenic disorder where several susceptible genes interact with the environmental factors and play a role in its pathogenesis.

The renin angiotensin system (RAS) is one of the important factors regulating blood pressure (BP) as well as fluid and electrolyte balance and may have an important role in the pathogenesis of hypertension. A genetic variability in the degree of expression of one of the components of its pathway may account for variability in BP or may play a role in mediating high BP.

Angiotensin II, the main effector molecule of this system, has several BP-increasing effects such as vasoconstriction, sodium retention in the kidney and intestine, stimulation of aldosterone secretion, and up-regulation of sympathetic tone (1). To some extent, therapeutic success has been achieved in controlling hypertension using inhibitors of angiotensin-I converting enzyme (ACE)(2) and angiotensin II type I receptor (AT1R)(3). ACE generates angiotensin II and AT1R mediates BP-increasing effects of angiotensin II. It is also observed that polymorphisms identified within these genes may influence therapeutic response to BP-lowering effects (4,5).

The insertion (I)/deletion (D) polymorphism of 287-bp alu repeat sequence in the intron 16 of the ACE gene has been identified (6) which correlates with serum ACE levels with highest values in those with the D allele (7). Studies of the association between ACE genotypes and variation in BP are conflicting. A sib-pair analysis study failed to demonstrate genetic linkage between ACE locus and hypertension (8). Among case-control studies a significant association between hypertension and the I allele has been reported (9,10) while with respect to D allele, positive (11–14) as well as negative (15–21) associations with hypertension have been documented.

In the AT1R gene, a silent polymorphism where cytosine is substituted for adenine (A¹¹⁶⁶→C) at position 1166 in the 3' untranslated region of the gene was identified and was significantly shown to be associated with hypertension (22). These results were confirmed by some (23–27) while denied by oth-

*Correspondence to: Dr. T. 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

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ers (28–33). Furthermore a significant interaction between the ACE and AT1R gene loci in terms of influence on BP variation was reported, the mechanism of which is unclear (10).

In the present study, we have examined the frequencies of I/D polymorphism of ACE gene and A¹¹⁶⁶→C polymorphism of AT1R in hypertensive subjects and in healthy individuals so as to identify their association with hypertension in the Indian population.

MATERIALS AND METHODS

Hypertensive patients were selected from clinical records after attendance at the health check-up clinic of P. D. Hinduja National Hospital and Medical Research Centre. A majority of the subjects were taking antihypertensive medication that involved either β -adrenoceptor blocking agents, calcium channel blockers, calcium antagonists, or sympathetic inhibitors and were on sedatives and tranquilizers. Only seven subjects were on ACE inhibitors and one took an AT1R inhibitor. Out of 105 subjects, 77 showed controlled hypertension (BP < 145/95 mmHg) and 28 subjects had BP > 145/95 mmHg. They had no other major cardiovascular complications, secondary hypertension, or diabetes mellitus. There were 75 males and 30 females within which 27 males and 12 females had a family history of hypertension. Controls were 192 healthy individuals drawn from the same clinic with BP < 140/90 mmHg. They had no history of cardiovascular diseases.

Determination of Biochemical Parameters

Plasma ACE activity was measured by a spectrophotometric method utilizing the synthetic tripeptide substrate N-[3-(2-furyl) acryloyl]-L-phenylalanyl-glycylglycine (Sigma Chemical Company, St. Louis, MO) on a Synchron Cx7 Beckman auto analyzer. Total cholesterol and triglyceride levels also were measured on the Synchron Cx7 Beckman auto analyzer with standard enzymatic methods (Boehringer Mannheim, Germany). High-density lipoprotein (HDL) cholesterol levels were determined in the supernatant after the plasma was subjected to precipitation with MgCl₂ and

phosphotungstic acid (Boehringer Mannheim). Apolipoprotein A1 (apoA1) and apolipoprotein B (apo B) levels were measured on Beckman Array by the rate-nephelometry method. Low-density lipoprotein (LDL) cholesterol was calculated using Friedewald's Formula, (LDL cholesterol (mg/dl) = total cholesterol (mg/dl) – HDL cholesterol (mg/dl) + triglyceride (mg/dl)/5).

Genotype Investigation

DNA extraction

Genomic DNA was extracted from peripheral blood leukocytes using the salting out method as described by Miller et al. (34).

Amplification of the ACE gene

Polymerase chain reaction (PCR) was used for the detection of the presence of the insertion and deletion allele in the intron 16 of the ACE gene that was carried out according to the method of Nakai et al. (35) with slight modification (36). The primer sequence used for the sense oligo was 5'-CTG CAG ACC ACT CCC ATC CTT TCT-3' and for antisense oligo 5'-GAT GTG GCC ATC ACA TTC GTC AGAT-3'. The final volume used for amplification was 50 μ l including 100 ng genomic DNA, 25 pmol of each primer (Geneset, Singapore), 200 μ M of dNTP (MBI Fermentas), 2.5 units of Taq polymerase enzyme (Bangalore-Genei, Bangalore, India), and 10 mM of Tris-HCl buffer (pH 8.3) containing 1.5 mM MgCl₂ and 50 mM KCl. Partial mistyping of I/D heterozygotes was controlled by incorporating 5.0% dimethyl sulfoxide. Amplification was carried out in a Thermal cycler (model 480 Perkin Elmer Cetus, Norwalk, CT) using the following conditions: initial denaturation was carried out at 93°C for 5 minutes, followed by 30 cycles of denaturation at °C for 1.5 minutes, annealing at 58°C for 2 minutes and extension at 72°C for 2.0 minutes. Final extension was performed at 72°C for 10 minutes. The amplified products were resolved on 2.5%-agarose gels and the DNA was visualized by staining with ethidium bromide.

Amplification of the AT1R gene

Mismatch PCR-RFLP strategy was used to detect the A¹¹⁶⁶C polymorphism of the AT1R gene using the method of Hingorani and Morris (37) with slight modification. The sequence of the upstream primer used was 5'-ATA ATG TAA GCT CAT CCA CCA AGA AG-3' and that of downstream primer was 5'-TCT CCT TCA ATT CTG AAA AGT ACT TAA-3'. The downstream primer contains 2 bp mismatch (underlined above) at 3' end such that the 166-bp amplified product incorporates a restriction site for the endonuclease Afl II in the presence of cytosine at nucleotide position 1166 but not in the presence of adenine. Reactions were performed in a volume of 25 μ l containing 100 ng genomic DNA, 25 pmol of each primer (Geneset, Singapore), 200 μ M of dNTP (MBI, Fermentas), 2.5 units of Taq polymerase enzyme (Bangalore-Genei, Bangalore, India), and 10 mM of Tris-HCl buffer (pH 8.3) containing 1.5 mM MgCl₂, 50 mM KCl, and 2.0% dimethyl sulfoxide. Amplification was carried out in a Thermal cycler (model 480 Perkin Elmer Cetus) using following conditions: initial denaturation was carried out at 95°C for 5 minutes followed by amplification for 40 cycles with denaturation at 95°C for 1 minute, annealing at 50°C for 1 minute and extension at 72°C for 2.5 minutes. The final extension was at 72°C for 10 minutes. The amplified product was then incubated with 2.0 units of Afl II restriction endonuclease with manufacturer's buffer and bovine serum albumin (New England Bio-Labs, UK) for 16 hours at 37°C. Digested prod-

ucts were then size separated on 3.0%-agarose gel using ethidium bromide staining.

Statistical Analysis

Values of body mass index (BMI), BP, total cholesterol, HDL cholesterol, ratio of total to HDL cholesterol, LDL cholesterol, triglyceride, and ratio of apo A1 to apo B were expressed as mean $\pm$ SD. Statistical significance of these parameters between two groups was assessed by student's *t*-test. Allele frequencies were deduced from genotype frequencies and the differences between groups were tested by chi-square (χ^2) test. The odds ratio (OR) and confidence interval (CI) were also calculated. A value of $P < 0.05$ was considered to be statistically significant.

RESULTS

PCR amplification of ACE gene revealed a combination of 490-bp product and/or 190-bp product depending on the presence or absence of the insertion fragment. Thus each of the samples revealed one of the three electrophoretic patterns. Subjects with I/I genotype showed a band at 490 bp, subjects with D/D genotype showed a band at 190 bp, and subjects with I/D genotype showed bands at 490 and 190 bp (Fig. 1).

PCR amplification of the AT1R gene revealed 166-bp product that, on digestion with Afl II depending on the absence or presence of C allele, showed a 166-bp band for subjects with A/A genotype and 166- as well as 139-bp bands for the subjects with A/C genotype, respectively (Fig. 2). So far in our study we have not observed a single band of 139 bp which corresponds to the C/C genotype.

The clinical and biochemical parameters of the controls and hypertensive subjects are shown in Table 1. Between the two groups, a statistically significant difference was observed in the BMI, BP level, and lipid profile—except in HDL cholesterol—. Within hypertensive group the same parameters were not different between subjects with and without family history of hypertension (data not shown). The ACE activity in our control group was lowest in subjects carrying I/I (21.07 ± 8.59), genotypes, intermediate in I/D genotypes (36.35 ± 16.62) and highest in D/D (49.26 ± 16.38) genotypes. In the hypertensive group such levels were almost similar to those seen in the control group: 22.50 ± 9.29 , 38.29 ± 10.13 , and 51.28 ± 10.56 in IU/L, respectively. Subjects under treatment with ACE and AT1R inhibitors were not included for calculating serum ACE activity.

The genotype and allele frequencies of ACE and AT1R gene polymorphisms of controls and hypertensive subjects are shown in Table 2. There was no difference observed in the distribution of the ACE genotypes between the two groups. In fact I and D allele frequencies of both the groups were identical: 0.56 and 0.44, respectively. The A/C genotype frequency of hypertensive subjects was 13.33 versus 17.18% in controls. The difference was statistically not significant. The influence of ACE D/D and A/C genotype together on the risk of hypertension also was not seen; this is indicated in Table 3 (Odds Ratio 0.444, $\chi^2 = 0.924$).

Tables 4 and 5 show the distribution of the genotype and allele frequencies of ACE and AT1R gene polymorphisms of control and hypertensive subjects among males and females respectively. It was seen that among the three ACE genotypes

Fig. 1. PCR products from amplification of the ACE gene. Lane M, molecular weight marker Φ X 174 DNA Hae III Digest; lane D/D, 190-bp band; lane I/I, 490-bp band; lane I/D, bands at 490 bp and 190 bp.

Fig. 2. Digested products of the amplified AT1R gene. Lane M, molecular weight marker Φ X 174 DNA Hae III Digest; lane A/A, 166-bp band; lane A/C, bands at 166 bp and 139 bp.

within the control group, I/I genotype was more prevalent in females as compared to I/D and D/D genotypes (OR: 3.31, [CI: 1.78–6.14], $\chi^2 = 14.92$, $P < 0.001$). Correspondingly males were more associated with I/D genotypes (OR: 2.80, [CI: 1.5–5.2], $\chi^2 = 10.92$, $P < 0.001$) as compared to II and DD genotype. In the hypertension group the association of I/I genotype with females was not significant and the frequency of I/D genotype was almost similar to that of male subjects. With respect to AT1R genotypes in the control group, females were significantly associated with the A/C genotype than males. (OR: 2.86 [CI: 1.33–6.15], $\chi^2 = 7.62$, $P < 0.01$). The

A/C genotype frequency was high even in hypertensive females (20.0%) as compared to males (10.66%); however the association was not significant.

In our study there was a significant [$\chi^2 = 8.18$, $P < 0.02$] difference in the ACE genotype frequencies between the group of an early onset of hypertension (age ≤ 40 yrs) [I/I: 4.0 (13.0%), I/D: 17 (57.05%), D/D: 9 (30.0%)] and the group of late onset (age > 40 yrs) [I/I: 32 (43.0%), I/D: 28 (43.0%), D/D: 15 (20.0%)]. D allele was significantly (OR: 2.22, [CI: 1.2–4.05], $\chi^2 = 6.71$, $P < 0.01$) associated with an early rather than late onset of hypertension (0.58 versus 0.39). No such association was seen with respect to AT1R genotypes. The AT1R genotype distribution was A/A 57 (86.36%) and A/C 9 (13.63%) for subjects with an early onset of hypertension against A/A 34 (87.17%) and A/C 5 (12.82%) for those with late onset of hypertension with a C allele frequency of 0.068 and 0.064, respectively.

Within the hypertensive group there was no significant difference between the distribution of ACE and AT1R genotypes when patients were subgrouped according to the presence or absence of cardiovascular disorder (CVD) family history. The distribution of I/I, I/D, and D/D of ACE genotype was 13 (33.33%), 14 (35.90%), and 12 (30.76%) for subjects with such a family history and 23 (35.50%), 31 (46.90%), and 12 (18.18%) for those with none. However the frequency of D allele associated with family history was higher (0.49) than in those subjects without family history (0.42). The distribution of AT1R genotypes A/A and A/C in subjects with family history was 36 (92.31%) and 3 (7.8%) versus 47 (81.03%) and 11 (18.9%) with no family history, respectively. Thus

TABLE 1. Clinical and biochemical characteristics of the controls and hypertensive subjects

	Controls N = 192	Hypertensives N = 105
Age	46.90 $\pm$ 10.77	50.25 $\pm$ 9.78
BMI (Kg/m ²)	23.50 $\pm$ 3.2	27.21 $\pm$ 4.56 ^b
SBP (mmHg) ^a	122.01 $\pm$ 10.1	141.16 $\pm$ 14.43 ^b
DBP (mmHg)	81.56 $\pm$ 7.4	89.0 $\pm$ 7.88 ^b
Total cholesterol (mg/dl)	180.5 $\pm$ 25.6	200.0 $\pm$ 38.42 ^b
HDL cholesterol (mg/dl)	46.12 $\pm$ 8.7	45.5 $\pm$ 9.67 ^c
Ratio	3.91 $\pm$ 1.78	4.64 $\pm$ 1.77 ^d
Triglyceride (mg/dl)	109.0 $\pm$ 29.1	125.0 $\pm$ 44.11 ^b
LDL cholesterol (mg/dl)	109.83 $\pm$ 28.67	120.5 $\pm$ 27.85 ^d
ApoA1/ApoB	1.01 $\pm$ 0.11	0.91 $\pm$ 0.15 ^b

^aSBP, systolic blood pressure; DBP, diastolic blood pressure; NS, nonsignificant.

^b $P < 0.001$.

^cNS.

^d $P < 0.01$.

TABLE 2. Genotype and allele frequencies of ACE and AT1R gene polymorphisms

ACE genotypes	Controls N = 192	Hypertensives N = 105	AT1R genotype	Controls N = 192	Hypertensives N = 105
I/I	67 (34.89%) ^a	36 (34.28%)	A/A	159 (82.81%)	91 (87.61%)
I/D	81 (42.18%)	45 (42.86%)	A/C	33 (17.18%)	14 (13.33%)
D/D	44 (22.91%)	24 (22.85%)			
χ^2	0.014, NS ^b , df ^c = 2			0.756, NS, df = 1	
ACE Allele			AT1R Allele		
I	0.56	0.56	A	0.914	0.933
D	0.44	0.44	C	0.086	0.066
			χ^2	0.691, NS, df = 1	

^aFigures in parentheses indicate percentage of total.^bNS, nonsignificant.^cdf, degree of freedom.

C¹¹⁶⁶ allele was less prevalent in subjects with family history (0.038) than in those without family history (0.094).

In our study all hypertensive subjects were taking antihypertensive medication and therefore association with BP was not analysed. However among those 28 subjects who showed uncontrolled hypertension with BP > 145/95 mmHg, there were 9 subjects with I/I genotype, 11 with I/D genotype, 8 with D/D genotype, only 4 with A/C genotype, and 24 with A/A genotype.

DISCUSSION

This study was designed to determine whether D allele of ACE gene and C¹¹⁶⁶ allele of AT1R gene are common in the hypertensive population in India.

It is quite conceivable that subjects with ACE D/D genotype associated with enhanced expression of ACE, which in turn correlates with high angiotensin II levels, should be more susceptible to hypertension. Various case control studies carried out have shown positive associations in African Americans (11,12), Caucasians (13), and Japanese (14). However in our population, the frequency of D/D genotype did not differ between the controls and the hypertension group: 22.85 versus 22.91% in controls. In the results of a Chinese population study similar to ours, a significant difference was not observed between the two groups (15), and a D/D genotype frequency of a hypertensive group was 23. versus 13.0% in their controls. Negative association also was shown in Italian

(38) and Greek (39) populations; however, in their population overall, D allele (0.56) frequency was reported high as compared to I allele (0.44).

In our test controls population, a remarkable observation was made: among three ACE genotypes, I/I genotype was more significantly associated with females than males—51.31 versus 24.13%. Correspondingly the I/D genotype was more prevalent in males: 51.72 versus 27.63% females. The distribution of the D/D genotype between males and females was almost similar: 24.13 and 21.05%, respectively. I allele was significantly associated with females. A similar association was not seen in the hypertension group as the frequency of the I/I genotype associated with females decreased from 51.31 (of the controls) to 40.0% and a compensatory rise was seen in the I/D genotype, from 27.63 (of the controls) to 43.3%. The frequency of the D/D genotype associated with male hypertensives was high (25.3%) as compared to female hypertensives (16.66%). Overall there was no significant difference, between controls and the hypertensive group, in I- and D-allele frequencies of males and females. There is literature that reports studies carried out by Sugiyama et al. (40), O'Donnell et al. (41), and Fornage et al. (42) in which sex-specific associations were evaluated. The former study showed no correlation while the latter two found evidence of association and genetic linkage of the ACE gene (D/D genotype) with hypertension and BP in men.

In the present study, although association of DD genotype was not seen in hypertensive subjects, the D allele was significantly more associated with an early onset of hypertension (age ≤ 40 years). This is in accordance with the studies by Cheung et al. (43), Nakano et al. (44), and Oshima et al. (45). In fact there were only 4 out of 32 hypertensive subjects with I/I genotype with an early onset of hypertension in contrast to 17 of 45 I/D and 9 of 24 D/D genotype.

ACE D allele has been reported to be significantly prevalent among hypertensive subjects having a parental history of CVD by Tiret et al. (27) (0.68 versus 0.56), Kario et al. (46) (0.46 versus 0.37), and Maeda et al. (47) (0.72 versus 0.52). In our study there was no significant difference in the

TABLE 3. Synergistic effect of D/D and A/C genotype on hypertension

	Frequency of A/C genotypes	
	Hypertensives	Controls
D/D	02	09
I/D + I/I	12	24
	OR -0.444, χ^2 = 0.924, NS ^a , df ^b = 1	

^aNS, nonsignificant.^bdf, degree of freedom.

TABLE 4. Distribution of ACE genotypes according to gender^a

ACE genotype	Controls		Hypertension	
	Male N = 116	Female N = 76	Male N = 75	Female N = 30
I/I	28 (24.13%)	39 (51.31%) ^b	24 (32.07%)	12 (40.0%) ^d
I/D	60 (51.72%) ^c	21 (27.63%)	32 (42.66%) ^c	13 (43.3%)
D/D	28 (24.13%)	16 (21.05%)	19 (25.3%)	05 (16.66%)
χ^2	16.21, $P < 0.001$, df = 2		1.10, NS, df = 1	
I	0.50	0.65#	0.53	0.61##
D	0.50	0.35	0.47	0.39
χ^2	8.53, $P < 0.01$, df = 1		1.2, NS, df = 1	
OR	#1.87		##0.710	
95% CI	1.22–2.84			

^aFigures in parentheses indicate percentage of total; df, degree of freedom; NS, nonsignificant.

^bI/I against I/D + D/D $\chi^2 = 14.92$, $P < 0.001$, OR: 3.31 (1.78–6.14).

^cI/D against I/I + D/D $\chi^2 = 10.92$, $P < 0.001$, OR: 2.8 (1.5–5.2).

^dI/I against I/D + D/D $\chi^2 = 0.608$, NS, OR: 1.41 (0.59–3.4).

^eI/D against I/I + D/D $\chi^2 = 0.0038$, NS, OR: 0.973.

genotype distribution between hypertensives with and without family history. However, subjects having a family history had a higher frequency of D/D genotype (30.76 vs. 18.18%) and D allele (0.49 vs. 0.42%) than did subjects having no family history.

In contrast to the deletion polymorphism of the ACE gene that is associated with the serum ACE activity, no clear phenotype has been determined in conjunction with A/C genotype of AT1R. Moreover the AT1R A¹¹⁶⁶C polymorphism is located in the 5'-end of the 3' untranslated region of the gene. Therefore it was proposed that its association with CVD was due to its linkage with another unidentified functional variant that might alter the responsiveness of this receptor (22).

In our study the distribution of AT1R genotypes between hypertensive and control subjects was not statistically different. The frequency of A/C genotype was in fact lower in the hypertensive group as compared to the controls. We have not observed C/C genotype so far. The C¹¹⁶⁶ allele frequency was 0.066 in the hypertensive group as compared to 0.086 in the controls. Homozygous C/C genotype also was not reported

by Fan et al. (26) in the Chinese population. However they have shown a significantly higher prevalence of A/C genotype in hypertensive subjects than in controls with C¹¹⁶⁶ allele frequency: 0.698 vs. 0.041.

In our control population C¹¹⁶⁶ allele was more significantly associated with females than males (0.138 versus 0.059). A nonsignificant, similar trend was seen in the hypertension group as well (0.1 versus 0.053). However our results do not agree with those of Tired et al. (27) who have reported increased frequency of C¹¹⁶⁶ allele with hypertensive females compared to controls (0.3 versus 0.23).

There also was no correlation between an early onset of hypertension with the presence of a strong family history of CVD and A¹¹⁶⁶C polymorphism of AT1R in our study. In fact the frequency of the A/C genotype was lower in subjects with family history (0.038) than in those without family history (0.094). This is similar to Tired et al.'s (27) results, which also reported a significantly lower C¹¹⁶⁶ allele frequency in those having family history (0.19) than in those without (0.31).

TABLE 5. Distribution of AT1R genotypes according to gender^a

ACE genotype	Controls		Hypertensives	
	Male N = 116	Female N = 76	Male N = 75	Female N = 30
A/A	112 (88.88%)	47 (72.30%)	67 (89.30%)	24 (80.0%)
A/C	15 (11.81%)	18 (27.69%)*	8 (10.66%)	6 (20.00%)*
χ^2	7.62, $P < 0.01$, df = 1		1.61, NS, df = 1	
OR	*2.86		***2.09	
95% CI	1.33–6.15		0.657–6.64	
A	0.942	0.862	0.947	0.900
C	0.059	0.138**	0.053	0.100****
χ^2	6.90, $P < 0.01$, df = 1		1.5, NS, df = 1	
OR	**2.56		****1.97	
95% CI	1.24–5.26		0.653–5.94	

^aFigures in parentheses indicate percentage of total; df, degree of freedom; NS, nonsignificant.

Thus our results do not agree with those of Bonnardeaux et al. (22) who have reported a significant association of C¹¹⁶⁶ allele with hypertension (0.36 against 0.28, $\chi^2 = 6.8$, $P < 0.01$). Their subjects had a strong family history as well as an early onset of hypertension and have not shown any significant preferential gender-specific association in the study.

A significant synergistic interaction between the ACE and AT1R gene on hypertension was reported by Hingorani et al. (10). In our test population we examined the interaction of ACE D/D and AT1R A/C genotype on the risk of hypertension and statistically found no association. The ACE D/D genotype and hypertension were not restricted to subjects carrying C allele.

The serum ACE levels in controls were according to ACE genotypes, and were highest in D/D genotypes, lowest in I/I genotypes, and intermediate in I/D genotypes, all in accordance with our previous published data (36). In the hypertension group, seven subjects treated with ACE inhibitors and one with AT1R inhibitor were excluded in calculating serum ACE levels. In the remaining similar, genotypic influence was seen as in controls which is also reported by Moris et al (48). No difference in ACE levels was observed among corresponding genotypes between hypertensives and controls.

In our study the prevalence of the D/D genotype was higher in hypertensive males than in females, but the figures did not reach statistical significance. The D/D genotype and D-allele frequency were high in subjects with family history of hypertension and were significant in those with the early onset of hypertension (age ≤ 40 years). A/C genotype frequency associated with females was higher than males in both groups and in subjects without family history as compared with those with family history of CVD.

From our study it can be seen that in this highly heterogeneous multigenetic and multifactorial disease, strong genetic effects are present only in a small subgroup of hypertensives and may not show any overall detectable contribution. Our findings show a lack of association between the I/D- and A/C-allele marker system and clinical diagnosis of hypertension, and suggest that they are not useful risk markers of susceptibility to hypertension but do make a minor contribution in the polygenic context.

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