 Int J Hum Genet, 5(4): 247-252 (2005)

The Insertion I/ Deletion D polymorphism of Angiotensin-Converting Enzyme (ACE) Gene Increase the Susceptibility to Hypertension and / or Diabetes

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KEYWORDS Hypertension; Type II diabetes; ACE polymorphism

ABSTRACT The causes of hypertension and type II diabetes (NIDDM) are mainly unknown, but they arise from interplay between several genetic and environmental factors. Hence the present study was aimed to investigate whether the Insertion I/ Deletion D polymorphism of angiotensin-converting enzyme (ACE) gene increase the susceptibility to hypertension and / or diabetes. ACE gene was genotyped in 200 hypertension patients, 100 type II diabetic patients and 200 age and sex matched controls. From the present data it was observed that in hypertension patients genotypic and allelic frequencies were significantly deviated from Hardy-Weinberg equilibrium ($p<0.05$). The DD genotype was strongly associated with hypertension [odds ratio (OR) = 2.02, confidence interval (CI) = 1.14-3.58, $p<0.05$] and remained so when patients with type II diabetes were excluded from the analysis (OR = 2.07, CI = 1.10-3.93, $p<0.05$) and significant association was not obtained in diabetic patients without hypertension. From the present results, it was concluded that *D* allele of ACE gene protects against diabetes, however it increases susceptibility to hypertension particularly when associated with type II diabetes.

INTRODUCTION

Patients with hypertension have a high risk of developing severe complications, such as diabetic nephropathy, retinopathy and cardiovascular disease. Type 2 diabetes is an important complication of hypertension and is observed in more than 30% of patients with hypertension (Dodson.1990). Studies demonstrating familial clustering of diabetic nephropathy, cardiovascular disease and hypertension (Seaquist et al. 1989; Earle et al. 1992; Freire et al. 1994) suggest that, in addition to poor blood pressure and glycemic control, genetic factors may affect susceptibility to the development of hypertensive micro-and macroangiopathy. In this context, genetic polymorphisms of the renin-angiotensin system (*RAS)* are attractive candidates to be studied, since inhibition of the activity of this system has shown to retard the development of diabetic complications, such as nephropathy and retinopathy (Lewis et al. 1993; Chaturvedi et al. 1998).

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Renin angiotensin system may play an important role in blood pressure regulation and acts as a key regulator of Sodium homeostasis. The gene coding for Angiotensin converting Enzyme *(ACE)* regulates vascular tone through the activation of angiotensin II, a potent vasoconstrictor (Timmermans et al. 1993), and inactivation of bradykinin (Atlas. 1998), a non-apeptide belonging to a class of active peptides (kinins) that are released from tissue to produce a variety of effects, including arterial vasodilatation and venoconstriction. The insertion/ deletion (I/D) polymorphism of the ACE gene is characterized by the presence (I) or absence (D) of a 287-bp alu repeat sequence within intron 16 of the *ACE* gene. *ACE* polymorphism appears to have a significant impact on narrowing of blood vessels that offer protection against type II diabetes but if these carriers do eventually develop the disease, they would face more serious complications.

The *ACE* I/D polymorphism is also associated with overall plasma ACE levels (Rigat et al. 1990). Patients homozygous for the D allele are characterized by elevated plasma levels of ACE compared with patients homozygous for the I allele, which might explain a diversity in the response to *ACE* inhibition (Marre et al. 1997).

Furthermore, the ACE I/D polymorphism has been suggested to play an important role in the individual antiproteinuric response to ACE inhibition (Jacobsen et al. 1998). Therefore, because the I/D polymorphism affects ACE and angiotensin II levels, it may also affect an individual's sensitivity to insulin and hence ACE might be a good candidate gene for determining insulin resistance.

The ACE insertion (I)/ deletion (D) polymorphism is not only effective in playing a role in hypertension and diabetes (Ruiz et al. 1994; Vijay et al. 2001) but also participates in cardiac complications (Cambien et al. 1992; Marian et al.1993; Schunkert et al. 1994, Lindpaintner et al. 1995). However, these studies yielded conflicting results: some claiming positive associations with *ACE* genotype, and others contradicting. Several case-control studies of hypertensive diabetes have demonstrated a positive association of the ACE I/D polymorphism (Bengtsson et al. 1999; Staessen et al. 1997). However the influence of ethnic background on the link between hypertension and/or diabetes and ACE polymorphism still remains unresolved. It is important to look for the gene association in the Asian Indian population in view of high prevalence of hypertension and diabetes.

Hence the present study was carried out to investigate the association of I/D polymorphism with hypertension and / or diabetes and its role in increasing the susceptibility to hypertension and / or diabetes.

MATERIAL AND METHODS

The study was carried out in 200 hypertensive patients (HTN), 100 normotensive type II diabetic patients (NDM), reported at CARE hospital aged 30-65 years and 200 age and sex matched random controls. Further, hypertensive group was sub-divided into 2 groups: Non-diabetic individuals with hypertension (NHTN, n=125), and type II diabetic individuals with hypertension (HDM, n=75).

Data were collected from each patient on clinical variables including age, height, weight, body mass index, cigarette smoking, alcohol consumption and family history etc. Diagnosis of hypertension and diabetes was based on the physical and clinical examination of patients by the doctors followed by appropriate laboratory and other investigations.

Hypertension and diabetes were defined according to WHO criteria (EC report 1998, VIIth JNC report 2003). Patients with secondary hypertension, nephropathies and Cardiac abnormalities were excluded from the present study.

Determination of ACE Genotype: DNA was isolated from whole blood using standard protocols (Miller et al. 1988) and ACE gene sequence was amplified by polymerase chain reaction (PCR-Thermo cycler, Biometra, U.S.A) initially using a flanking primer pair (Rigat et al. 1992) and subsequently when necessary with a primer pair that recognizes the insertion specific sequences for confirmation of specificity of the amplification reactions (Shanmugan et al. 1993). The amplicons were separated on 2% agarose gel electrophoresis (Bangalore Genei Ltd, Bangalore) and the bands were visualized under U.V light.

Statistical Analysis: Statistical comparisons between group means were done by ANOVA, while proportions were compared by means of χ^2 test. Deviations from the Hardy-Weinberg equilibrium were tested with χ^2 analysis. Multiple logistic regressions were used to test the effect of ACE genotypes on the likelihood of hypertension and / or diabetes while controlling for other confounding factors. The odd's ratios (OR) together with the 95% confidence interval (CI), comparing the allelic distribution in the four study groups were also calculated. Two-tailed p values less than 0.05 were considered significant. The SPSS package (10.0 version) was used to perform statistical analysis.

RESULTS

Table 1 describes baseline characteristics of patients with hypertension and /or diabetes and control subjects. It was observed that patient groups significantly differed from control subjects with respect to age, BMI, SBP ($p<0.05$). Significant difference for DBP was observed only in HTN patients as compared to controls ($p<0.05$). When comparisons were made with in the patient groups, it was observed that HTN patients were slightly older, with overweights than NDM group. HDM patients were observed to have elevated systolic and diastolic blood pressure (Table 1) as compared to NDM patients and controls. A greater proportion of males were observed to have hypertension in complication with diabetes. Significantly greater proportions of smokers and alcoholics were observed among NHTN patients as compared to other patient groups and controls

Table 1: Base line characteristics observed in patients with hypertension and/or diabetics and control subjects

Patients	*Variable*			*Controls (n=200)*
	NHTN (n=125)	*HDM (n=75)*	*NDM (n=100)*	
Mean age (Yrs)	50.9±0.94	[1]51.3±1.16	49.14±1.03	45.06±0.53
Mean BMI (Kg/m^2)	[1]26.7±0.43	26.1±0.41	26.5±0.48	25.4±0.29
Mean SBP (mm/Hg)	151.5±2.28	[1]152.8±2.81	125.6±0.70	119.9±0.50
Mean DBP (mm/Hg)	92.3±0.90	93.1±1.01	81.4±0.31	80.0±0.24
Male Sex (%)	67.9	75.4	66.8	73.5
Smokers (%)	26.9	24.6	26	9
Alcoholics (%)	[2]43.6	36.1	40	16.5
SLS (%)	29.5	[2]32.8	20	16
Familial (%)	[2]42.3	37.7	36	18.5

1. ANOVA, P<0.05
2. χ^2 test, P<0.05
NHTN- non-diabetic hypertension, HDM- hypertension with diabetes, NDM- normotensive diabetes, BMI=Body mass Index, SLS = Sedentary life style.

(p<0.05). Significantly greater proportion of physically inactive individuals was observed with hypertension in combination with diabetes. A strong genetic component was observed in NHTN patients as compared to other groups.

Table 2 describes the distribution of *ACE* genotypes in the four observational groups. It was observed that the prevalence of DD homozygotes was high (47.8%) in NHTN patients as compared to *NDM* patients (24.0%) and controls (22.5%). ID heterozygote frequency was high in HDM patients (37.4%) as compared to NDM patients (24%). In contrast prevalence of II homozygotes was high in NDM patients (52.0%) as compared to controls (40.0%).

Table 2: Distribution of ACE genotypes in the four observed groups

ACE genotype	*Patients*						*Controls*	
	NHTN		*HDM*		*NDM*			
	n	*%*	*n*	*%*	*n*	*%*	*n*	*%*
DD	59*	47.2	25	33.3	24	24.0	45	22.5
ID	35	28.0	28	37.3*	24	24.0	75	37.5
II	31	24.8	22	29.4	52	52.0	80	40.0

* p <0.05

The genotypic and allele frequencies differed significantly in HTN patients from the control group (p<0.05). No significant differences were observed in the distribution of the ACE I/D genotypes between *NDM* patients and control subjects (Table 2). The distributions of *ACE* genotypes observed were in agreement with the Hardy-Weinberg proportion except for NHTN group (p<0.05).

The adjusted odds of prevalence of hypertension and/or diabetes (Table 3a) revealed a strong association of DD genotype with hypertension taking II genotype as reference (odds ratio OR-2.02, 95% confidence interval CI- 1.14-3.58, p<0.05). And remained so in NHTN patients (OR-2.08, 95% CI- 1.10-3.93, p<0.05) and HDM patients (OR-1.98, 95% CI- 0.95-4.12, p<0.05). However, when ID genotype was taken as reference (Table 3b), HDM patients had 2.5 (95% CI- 1.73- 3.47, P<0.05) times higher odds of hypertension as compared to NDM patients and nearly 1.2 times risk (95% CI-1.09-2.18) as compared to NHTN patients. There was no association with NDM patients.

Table 3a: Odd's of prevalence of hypertension and/ or diabetes

Genotype	*Odd's ratio*[a]	*95% CI*	
		Lower limit	*Upper limit*
HTN			
DD vs. II	2.02*	1.14	3.58
ID vs. II	1.73	1.03	2.90
NHTN			
DD vs. II	2.08*	1.10	3.93
ID vs. II	2.06	1.01	4.21
HDM			
DD vs II	1.98*	0.95	4.12
ID vs. II	1.36	0.68	2.72
NDM			
DD vs II	0.82	0.38	1.78
ID vs. II	0.49	0.23	1.05

[a] Multivariate adjusted odd's ratio
* p<0.05

DISCUSSION

We examined ACE gene polymorphism, one of the important genes in rennin-angiotensin system in hypertension, type 2 diabetes (NIDDM) and healthy control groups. Genetic factors, life style modification, obesity are potential risk factors to improve the complications in hypertension (Reaven.1988; VIIth JNC report. 2003). Renin angiotensin system should modulate a risk for hypertension and its complications.

Table 3b: Odd's of prevalence of hypertension and/ or diabetes

Genotype	*Odd's ratio*[a]	*95% CI*	
		Lower limit	*Upper limit*
NHTN			
DD vs. ID	2.15*	1.09	3.18
HDM			
DD vs ID	3.35*	1.73	3.47
NDM			
DD vs ID	0.85	0.49	1.46

[a] Multivariate adjusted odd's ratio
* $p<0.05$

Previous studies revealed a strong association between ACE I/D polymorphism and hypertension (Katsuya etal.1995; Mastana and Nunn. 1997). There are also previous reports showing negative association between ACE genotypes and hypertension (Sagnella et al. 1999). Vijay et al (2001) did not find any association between ACE genotypes type 2 diabetes. We also did not find any association between ACE genotypes and type 2 diabetes. The findings of our study are in agreement with some previous studies (Mastana and Nunn. 1997; Vijay et al. 2001; and Pasha et al. 2002).

Our data also showed an association between ACE DD genotype and hypertensive type 2 diabetes. We found that carrying DD genotype in these patients 2.5 times increased than normotensive diabetic patients, and 1.2 times increased than non-diabetic hypertensive patients, suggesting that the DD genotype is associated with an increased susceptibility to diabetic complications in patients with hypertension. Our study appears to be similar to previous reports (Staessen et al. 1997; Bengtsson et al.1999). Bengtsson et al. (1999) reported a significant association of D allele with hypertension but not in diabetes and also suggested that D allele might increase the susceptibility to hypertension, particularly in hypertensive type 2 diabetic patients. Results obtained from the present study could be of prognostic value in identifying individuals at risk for diabetic nephropathy in HDM patients as suggested by earlier studies (Doi et al. 1996; Ohno et al. 1996; Fujisawa et al. 1998; Yoshida et al. 1999;Vijay et al. 2001).

The observation made in the present study revealed the protective role of *D allele* in NDM patients offering insulin sensitivity as suggested by Katsuya et al. (1995). In a different context Lee et al. (2002) have reported a strong association of II genotype with insulin resistance in NIDDM patients providing genetic evidence for the clustering of the metabolic syndrome or insulin resistance syndrome.

In conclusion, observations from the present study clearly indicate the strong association of *D* allele with hypertension and its protective role in diabetes. The *D* allele increases the susceptibility to hypertension particularly when associated with type II diabetes leading to the progression of complications like diabetic nephropathy. At present the indications are that ACE may have a central position in energy metabolism and primarily acts as an enzyme of importance to the vascular and inflammatory systems.

Future studies will show whether all these associations and pathophysiological aspects of *ACE* and its basic geno- and pheno-types will lead not only to a better understanding of hypertension and/ or diabetes, as well as its many complications, but may also lead to identification of at-risk patients and/or improved pharmacological or non-pharmacological interventions.

ACKNOWLEDGEMENTS

We extend our gratitude to Dr. K. Vishweshwar Rao, (former statistician, National Institute of Nutrition) for his valuable guidance in the statistical analysis.

REFERENCES

Atlas SA 1998. The renin-angiotensin system revisited: classical and nonclassical pathways of angiotensin formation. *Mt Sinai J Med*, **65:** 87-96.

Bengtsson K, Orho-Melander M, Lindblad U, Melander O, Bog-Hansen E, Ranstam J, Rastam L et al, 1999. Polymorphism in the angiotensin converting enzyme but not in the angiotensinogen gene is associated with hypertension and type 2 diabetes: the Skaraborg Hypertension and diabetes project. *J Hypertens*, **17(11):** 1569-75.

Cambien R, Poirier O, Lecerf L, Evans A, Cambou JP,

Arveiller D et al 1992. Deletion polymorphism in the gene for angiotensin-converting enzyme is a potent risk factor for myocardial infarction. *Nature*, **359:** 641-644.

Chaturvedi N, Sjolie AK, Stephenson JM, Abrahamian H, Keipes M, Castellarin A et al 1998. Effect of lisinopril on progression of retinopathy in normotensive people with type 1 diabetes. *Lancet*, **351:** 28–31.

Dodson PM 1990. Epidemiology and pathogenesis of hypertension in diabetes. In: AH Barnett, PM Dodson (Eds.) *Hypertension and Diabetes*. London: Science Press.

Doi Y, Yoshzimi H, Yoshinari M, Lion K, Yamamoto M, Ichikawa K, et al 1996. Association between a polymorphism in the angiotensin-converting enzyme gene and microvascular complication in Japanese patients with NIDDM. *Diabetologia*, **39:** 97-102.

Earle K, Walker J, Hill C, Viberti G 1992. Familial clustering of cardiovascular disease in patients with insulin dependent diabetes and nephropathy. *N Eng J Med*, **326:** 673–677.

Freire MBS, Ferreira SRG, Vivolo MA, Oliveira JM, Zanella MT 1994. Familial hypertension and albuminuria in normotensive type I diabetic patients. *Hypertension*, **23(1)**: 1256–1258.

Fujisawa T, Ikegami H, Kawaguchi Y, Hamada Y, Ueda H, Shintani M, et al 1998. Meta-analysis of association of insertion/deletion polymorphism of angiotensin I converting enzyme gene with diabetic nephropathy and retinopathy. *Diabetologia*, **41:** 47-53.

Jacobsen P, Rossing K, Rossing P, Tarnow L, Mallet C, Poirier O et al 1998. Angiotensin converting enzyme gene polymorphism and ACE inhibition in diabetic nephropathy. *Kidney Int*, **53:** 1002-1006.

Katsuya T, Horiuchi M, Chen YDI, Koike G, Pratt RE, Dzau Vj et al 1995. Relations between deletion polymorphism of the angiotensin-converting enzyme gene and insulin resistance, glucose intolerance, hyperinsulinemia, and dyslipidemia. *Arterioscler Thromb Vasc Biol*, **15:** 779-782.

Lee Y-J, Tsai JCR 2002. ACE gene insertion/deletion polymorphism associated with 1998. World Health Organization definition of metabolic syndrome in Chinese type 2 diabetic patients. *Diabetes Care*, **25(6):** 1002–8.

Lewis EJ, Hunsicker LG, Bain RP, Rohde RD 1993. The effect of angiotensin-converting-enzyme inhibition on diabetic nephropathy. *N Eng J Med*, **329:** 1456–1462.

Lindpaintner K, Pfeffer MA, Kreutz R, Stampfer MJ, Grodstein F, Lamotte F et al 1995. A prospective evaluation of angiotensin-converting gene polymorphism and the risk factor of ischemic heart disease. *N Engl J Med*, **332:** 706-711.

Marian AJ, Yu Q, Workman R, Greve G, Roberts R 1993. Angiotensin-converting enzyme polymorphism in hypertrophic cardiomyopathy and sudden cardiac death. *Lancet*, **342:** 1085-1086.

Marre M, Jeunemaitre X, Gallois Y 1997. Contribution of genetic polymorphism in the renin angiotensin system to the development of renal complications in insulin dependent diabetes. Genetique de la nephropathie diabetique (GENEDÌAB) study group. *J Clin Ìnvest*, **9:** 1585-95.

Mastana S, Nunn J 1997. Angiotensin-converting enzyme deletion polymorphism is associated with hypertension in a Sikh population; *Hum, Hered*, **47:** 250– 253.

Miller SA, Dykes DD, Polesky HF 1988. A simple salting out procedure for extracting DNA from human nucleated cells. *Nucleic Acids Res.*, **16:** 1215.

Ohno T, Kawazu S, Tomono S 1996. Association analyses of the polymorphisms of angiotensin-converting enzyme and angiotensinogen genes with diabetic nephropathy in Japanese non-insulin-dependent diabetics. *Metabolism*, **45:** 218-22.

Pasha MAQ, Khan AP, Kumar R, Ram RB, Grover SK, Srivastava KK et al 2002. Variation in angiotension-converting enzyme gene insertion/deletion polymorphism in Indian population of different ethnic origins. *J Bio Sci*, **27(1):** 67-70.

Reaven GM 1988. Role of insulin resistance in human disease. Banting lecture 1988. *Diabetes*, **37:** 1595-1607.

Rigat B, Hubert C, Alhenc-Gelas F, Cambien F, Corvol P, Soubrier F 1990. An insertion/deletion polymorphism in the angiotensin 1-converting enzyme gene accounting for half the variance of serum enzyme levels. *J Clin Invest*, **86:** 1343-1346.

Rigat B, Hubert C, Corval P, Soubrier F 1992. PCR detection of the insertion/deletion polymorphism of he human angiotensin converting enzyme gene (DCP[1]) dipetidyl carboxy peptidase I). *Nucleic Acids Res*, **20:** 1433.

Ruiz J, Blanche H, Cohen N, Velho G, Cambien F, Cohen D, et al 1994. Insertion/deletion polymorphism of the angiotensin-converting-enzyme gene is strongly associated with coronary artery disease in non-insulin dependent diabetes mellitus. *Proct Natl Acad Sci USA*, **91:** 3662-665.

Schunkert H, Hense HW, Holmer SR, Stender M, Perz S, Keil U et al 1994. Association between a deletion polymorphism of the angiotensin-converting-enzyme gene and left ventricular hypertrophy. *N Engl J Med*, **330:** 1634-1638.

Seaquist ER, Goetz FC, Rich S, Barbosa J 1989. Familial clustering of diabetic kidney disease. Evidence for genetic susceptibility to diabetic nephropathy. *N Eng J Med*, **320:** 1161-1165.

Shanmugan V, Sell KW, Saha BK 1993. Mistyping ACE heterozygotes. *PCR Meth Appls*, **3:** 120-1.

Staessen JA, Wang JG, Ginocchio G, Petrov V, Saavedra AP, Soubrier F et al 1997. The deletion/insertion polymorphism of the angiotensin converting enzyme gene and cardiovascular renal risk. *J Hypertens*, **15:** 1579-1592.

The Expert Committee on the Diagnosis and Classification of Diabetes Mellitus 1998. Report of the Expert Committee on the Diagnosis and Classification of Diabetes Mellitus. *Diabetes Care*, **21(1):** S5-S22.

The Seventh Report of the Joint National Committee on Prevention, Detection, Evaluation and Treatment of High Blood Pressure 2003. The National High Blood Pressure Education Program Committee. *JAMA*, **289:** 2560-2572.

Timmermans PBMWM, Wong PC, Chiu AT, Herblin

WF, Benfield P, Carini DJ et al 1993. Angiotensin II receptor and angiotensin II receptor antagonists. Pharmacol Rev, **45:** 205-251.

Vijay V, Yanqing Z, Karthik B,Stephen D, Snehalatha C, Ramadchandran A et al 2001. Association between ACE gene polymorphism and Diabetic Nephropathy in south Indian patients. *J Pancreas*, **2(2):** 83-87.

Yoshida H, Kuriyama S, Atsumi Y, Tomonari H, Mitarai T, Hamaguchi A, et al 1996. Angiotensin I converting enzyme gene polymorphism in non-insulin dependent diabetes mellitus. *Kidney Int*, **50:** 657-64.