Angiotensin Converting Enzyme Gene Polymorphism (Insertion/Deletion) and Hypertension in Adult Asian Indians: A Population-Based Study from Calcutta, India

Mithun Das, Susil Pal, Arnab Ghosh

Human Biology, Volume 80, Number 3, June 2008, pp. 303-312 (Article)

Published by Wayne State University Press

Angiotensin Converting Enzyme Gene Polymorphism (Insertion/Deletion) and Hypertension in Adult Asian Indians: A Population-Based Study from Calcutta, India

MITHUN DAS,[1] SUSIL PAL,[2] AND ARNAB GHOSH[3]

Abstract The angiotensin converting enzyme (*ACE*) gene insertion/deletion polymorphism has been identified as a potential genetic risk factor for essential hypertension. The purpose of the present study is to investigate the association of the insertion/deletion polymorphism of the *ACE* gene with essential hypertension in adult Asian Indians. Three hundred fifty (184 males and 166 females) adult (30 years and older) Asian Indians of Calcutta and its suburbs participated in this population-based cross-sectional study. Anthropometric measures, lipids profiles, blood glucose, and blood pressure measures were collected from participants. *ACE* insertion/deletion polymorphism was determined by agarose gel electrophoresis and D/D typing was further reconfirmed using insertion-allele-specific amplification. Essential hypertension was defined as a systolic blood pressure (SBP) greater than 160 mm Hg and/or a diastolic blood pressure (DBP) greater than 90 mm Hg or use of any antihypertensive treatment by participants. Significantly higher SBP, DBP, and mean arterial pressure were recorded in *D*/*D* subjects compared to *I*/*I* and *I*/*D* subjects. We also observed that the odds of being hypertensive were 7.483 (95% CI = 1.746, 30.192) in *D*/*D* individuals compared to those carrying one or no *D* alleles. This finding suggests that *ACE* insertion/deletion polymorphism is associated with essential hypertension in Asian Indians. Moreover, individuals who are homozygous for the *D* allele of the *ACE* gene are more likely to have essential hypertension.

Cardiovascular disease (CVD) will be the largest cause of death and disability in India by 2020, with 2.6 million Indians predicted to die from coronary heart disease (CHD), which constitutes 54.1% of all CVD deaths (Ajay et al. 2002). Nearly half of these deaths are likely to occur in young to middle-aged individuals (about 30 to 60 years). In people of Indian origin, death from CVD occurs at least a decade earlier than in their counterparts in countries with established market economies. The Global Burden of Disease Study estimated that about 52% of

[1]Department of Anthropology, Sree Chaitanya College, Habra, West Bengal, India.
[2]Human Genetic Engineering Research Centre, Calcutta, India.
[3]Department of Anthropology, Visva Bharati University, Sriniketan 731 236, West Bengal, India.

Human Biology, June 2008, v. 80, no. 3, pp. 303–312.

KEY WORDS: *ACE* GENE POLYMORPHISM, HYPERTENSION, OBESITY, ATHEROSCLEROSIS, ASIAN INDIANS.

CVD deaths occur before the age of 60 years in India compared to 23% in countries with an established market economy. The growing burden of CVD in India could be attributed to the increasing prevalence of CVD risk factors, especially hypertension, dyslipidemia, diabetes, visceral obesity, physical inactivity, and tobacco use (McKeigue et al. 1991; Ajay et al. 2002; Ghosh 2007). Essential hypertension is considered one of the prime contributing factors to explain this exaggerated rate. In India, there has been a steady increase in the prevalence of hypertension over the last few decades. However, few studies on the prevalence of essential hypertension have been undertaken in the culturally heterogeneous Indian population. The prevalence of hypertension in India was merely 4% in 1951 and went up to 29% during the early 1990s (Dubey 1954; Gopinath et al. 1994). Epidemiological studies carried out during the late 1990s demonstrated that the rising prevalence of hypertension in India was as high as 50% in both sexes (Gupta et al. 1995, 2002, 2004; Hazarika et al. 2002).

A number of known genetic factors affect blood pressure regulation; angiotensin converting enzyme (ACE) is one of them. ACE is a key component of the renin-angiotensin system (RAS) and is considered important in the etiopathogenesis of hypertension and CVD (Crews and Williams 1999). ACE is a well-known zinc-metallopeptidase that converts angiotensin I into the potent vasoconstrictor angiotensin II and that ultimately degrades bradykinin (a powerful vasodilator) for regulation of vascular tone cardiac functions (Baudin 2002). Out of several allelic polymorphisms, an insertion/deletion polymorphism, a short intronic sequence, could account for half the variance in plasma ACE level, resulting in large interindividual variability (Rigat et al. 1990; Barley et al. 1994). Several studies have shown a positive association of *ACE* insertion/deletion polymorphisms and hypertension and other cardiovascular risk factors (Mastana and Nunn 1997; Zee et al. 1999; Zhu et al. 2003; Schut et al. 2004; Jimenez et al. 2007; Higaki et al. 2000; Tai and Tan 2004; Slowik et al. 2007; Mattace-Raso et al. 2007; Barbalic et al. 2006; Xu et al. 2004; Thomas et al. 2000), and other studies have shown a negative association (Vassilikioti et al. 1996; Todoroki et al. 2003; Renner et al. 2002; Chowdhury et al. 1998) as a result of considerable interindividual and ethnic variation. In a six-year follow-up study, Di Pasquale et al. (2004) observed and suggested that the *ACE* DD polymorphism seems to be associated with a higher incidence of hypertension in baseline healthy subjects, irrespective of other conventional risk factors.

In India, few studies have been undertaken so far to examine *ACE* insertion/deletion polymorphism and hypertension (Nair et al. 2003; Ashavaid et al. 2000, 2002; Mastana and Nunn 1997; Singh et al. 2006; Tripathi et al. 2006). However, most of these studies are case-control in nature. To the best of our knowledge, only one population-based study has been undertaken so far; in that study a significant prevalence (association) of the *ACE D* allele in hypertensive subjects compared to normotensive subjects was observed (Mastana and Nunn 1997). A significant positive association between the *D* allele and hypertension has also been observed in studies pertaining to people of Indian origin, for example,

Bangladeshis (Morshed et al. 2002) and Pakistanis (Ismail et al. 2004). This in turn raises the possibility that in people of Indian origin, variations in or near the *ACE* gene locus may contribute to the development and severity of hypertension (Pasha et al. 2002).

Keeping this view in mind, we aimed in the present population-based cross-sectional study to look at whether there is any significant association between *ACE* insertion/deletion polymorphisms and essential hypertension in adult Asian Indians living in eastern India.

Materials and Methods

Study Population. The data were collected on adult men and women, age 30 years and older, from Calcutta and its suburbs. Three hundred fifty individuals (184 males and 166 females) participated in the study. Before the study, a public advertisement was circulated regarding the study with the help of local council officials. Individuals were selected randomly after they responded to the local advertisement. The response rate was as high as 85%. The institutional ethical committee of Visva Bharati University (Sriniketan, West Bengal, India) approved the study. Written consent was obtained from all participants before actual commencement of the study.

Anthropometric Measurements. Anthropometric measures (height, weight, waist circumference, and hip circumference) were taken using standard anthropometric techniques (Lohman et al. 1988). Height and weight were measured to the nearest 0.1 cm and 0.5 kg, respectively. Waist and hip circumferences were obtained to the nearest 0.1 cm using a nonelastic tape. Body mass index (BMI, in kg/m^2) was calculated accordingly.

Blood Pressure Measurements. Two morning systolic (SBP) and diastolic (DBP) blood pressure measurements were taken using a sphygmomanometer and stethoscope and were averaged for analysis. A third measurement was taken only when the difference between the two measurements was greater than 5 mm Hg, and the readings were averaged for analysis. A 5-min relaxation period between measurements was maintained for all subjects. Prior medical records (e.g., use of antihypertensive drugs) regarding blood pressure was also considered. Mean arterial pressure (MAP) was calculated accordingly. Subjects were considered hypertensive if they had an SBP greater than 160 mm Hg and/or a DBP greater than 90 mm Hg or were currently using any antihypertensive medication. The rest of the subjects were considered normotensive.

Genotyping. DNA was extracted from isolated serum using a QIAamp Kit (Qiagen, Hilden, Germany). Genomic DNA was amplified using the polymerase chain reaction (PCR) with a thermal cycler (Bio-Rad Corporation, Hercules, California).

Table 1. Characteristics of the Participants ($n = 350$)

	Males (n = 184)		*Females (n = 166)*		
Variable	*Mean*	*SD*	*Mean*	*SD*	*P Value*
Age (yrs)	54.04	12.40	48.48	11.57	<0.001
BMI (kg/m^2)	22.37	4.09	23.20	4.37	0.066
Systolic blood pressure (mm Hg)	132.97	24.02	137.21	24.52	0.104
Diastolic blood pressure (mm Hg)	82.22	11.41	83.48	10.55	0.286
Mean arterial pressure (mm Hg)	107.57	16.53	110.35	16.63	0.119

Insertion/deletion polymorphism was determined using agarose gel electrophoresis with ethidium bromide. *D*/*D* polymorphism was further reconfirmed using an insertion-allele-specific amplification according to the standard protocol (Lindpaintner et al. 1995). The following primers were used:

Forward: 5′-GCCCTGCAGGTGTCTGCAGCATGT-3′
Reverse: 5′-GGATGGCTCTCCCCGCCTTGTCTC-3′
Insertion-specific, forward: 5′-TGGGACCACAGCGCCCGCCACTAC-3′
Insertion-specific, reverse: 5′-TCGCCAGCCCTCCCATGCCCATAA-3′

To study the *ACE* insertion/deletion polymorphism and its effect on blood pressure measures, we considered DNA samples of individuals belonging to the highest (90th) and the lowest (10th) percentiles of blood pressure centiles (percentiles).

Statistical Analyses. Descriptive statistics such as mean and standard deviation (SD) of anthropometric and blood pressure measures were calculated by sex. The sex differences in anthropometric and blood pressure variables were calculated using an unpaired *t* test. The proportion of *I*/*I*, *D*/*D*, and *I*/*D* individuals by blood pressure status was also undertaken using a chi-square test. The distribution and difference in genotype frequency was examined using binomial test analysis. To find out the effect of genotypes on blood pressure status, we calculated the odds ratio (adjusted for age) using a binary logistic regression (dependent variable was blood pressure status, that is, hypertensive or normotensive).

All statistical analyses were computed using SPSS (PC+ version 10.0). A p value less than 0.05 (two-tailed) was considered significant.

Results

The characteristics of the participants by sex are presented in Table 1. No significant sex differences for BMI, SBP, DBP, and MAP were observed; age did show a significant sex difference ($p < 0.001$). The prevalence of essential hypertension

Table 2 Distribution of Hypertensive and Normotensive Individuals by Age Category

	Hypertensive		*Normotensive*		
Age Group (Years)	*Males*	*Females*	*Males*	*Females*	*Total*
30–39	4	8	16	31	59
40–49	9	22	35	26	92
50+	66	56	54	23	199
Total	105	80	79	86	350

by age category and sex is presented in Table 2. The prevalence of hypertension was considerably higher in females (51.8%) than in males (42.9%).

Because there was no significant effect of sex on blood pressure measures (results not shown), male and female samples were pooled to differentiate the highest and lowest groups (90th vs. 10th percentile). The proportions of *D*/*D*, *I*/*I*, and *I*/*D* individuals according to blood pressure status are presented in Table 3. We observed significant differences ($\chi^2 = 20.7$; $p < 0.001$) in the distribution of insertion/deletion genotypes by blood pressure status (hypertensive vs. normotensive). Furthermore, we noticed significant differences in genotype frequencies between the highest and lowest percentiles, with the frequency of the *D*/*D* genotype being relatively high in the 90th percentile. The frequencies of the *I* and *D* alleles were 0.259 and 0.471, respectively. The chi-square test revealed a significant difference for the frequency of the *D* allele between the percentile groups (results not shown). In the 90th percentile, the frequency of the *D* allele was 0.600 compared to a *D* allele frequency of 0.471 in the 10th percentile. The binomial test (results not shown) revealed a significant difference (z value of 3.046, $p < 0.05$) in allele frequencies between hypertensive and normotensive individuals.

Blood pressure measures according to the *ACE* insertion/deletion polymorphisms are presented in Table 4. Significantly higher SBP, DBP, and MAP were recorded in *D*/*D* subjects compared to *I*/*I* and *I*/*D* subjects (Table 4). A two-way comparison (Table 5) of blood pressure measures and genotypes revealed significant differences between *I*/*D* and *D*/*D* individuals for SBP and DBP.

Table 3. Proportion of *D*/*D*, *I*/*I*, and *I*/*D* Individuals According to Blood Pressure Status

Genotype	*Normotensive*	*Hypertensive*	*Total*
I/*I*	14 (40.0)	12 (34.2)	26
I/*D*	18 (51.4)	4 (11.4)	22
D/*D*	3 (8.5)	19 (54.2)	22
Total	35 (100)	35 (100)	70

$\chi^2 = 20.7$, df 2; $p < 0.001$.
Percentages are in parentheses.

Table 4. Difference in Mean Blood Pressure According to Genotype

	Systolic Blood Pressure		*Diastolic Blood Pressure*	
Genotype	*Mean*	*SD*	*Mean*	*SD*
I/I	137.35	41.71	79.58	18.76
I/D	116.82	30.47	74.50	12.94
D/D	165.55	31.28	93.82	13.48

The odds ratio (adjusted for age) of *ACE* insertion/deletion polymorphisms are presented in Table 6. We observed that the odds of being hypertensive for the *D/D* genotype was 7.483 (95% CI = 1.746, 30.192) compared to the *I/I* genotype (odds ratio = 1).

Discussion

In the present investigation we examined whether a significant association exists between *ACE* insertion/deletion polymorphisms and hypertension in adult Asian Indians living in the eastern part of Calcutta. The prevalence of hypertension was comparatively higher in females (51.8%) than in males (42.9%). This fact reiterates that Indian woman may be comparatively worse off than men in many aspects of risk for CVD (Ghosh 2007). Importantly, the *D/D* genotype was significantly associated with essential hypertension. Furthermore, a significant difference in average blood pressure was observed among individuals with the *D/D* genotype compared to the *I/I* or *I/D* genotype. However, no significant difference was observed for SBP and DBP between *I/I* and *I/D* individuals. Moreover, a significant difference in both allele ($p < 0.05$) and genotype (< 0.001) frequencies exists between the highest (90th) and the lowest (10th) percentile groups with respect to blood pressure measures. It seems reasonable to argue that *ACE* insertion/deletion polymorphism is playing an important role in regulation of blood pressure in this population. Apparently, the *D/D* genotype predisposes Asian Indians to hypertension and presumably to metabolic syndrome. Further studies incorporating a representative sample size of Asian Indians across the Indian diaspora are required to further our understanding of the genetic etiology of essential hypertension in this population.

Table 5. Two-Way Comparison of Systolic and Diastolic Blood Pressure by Genotype

	Systolic Blood Pressure		*Diastolic Blood Pressure*	
Two-Way Comparison	*t*	*p*	*t*	*p*
I/I vs. *I/D*	1.915	0.0617	1.072	0.2894
I/I vs. *D/D*	2.609	0.0122	2.968	< 0.0047
I/D vs. *D/D*	5.234	< 0.0001	4.850	< 0.0001

Table 6. Odds Ratio (Adjusted for Age) of *I*/*D* and *D*/*D* Genotypes vs. Hypertension (Dependent Variable Is Hypertensive vs. Normotensive)

			95% Confidence Interval	
Genotype	*Odds Ratio*[a]	*p Value*	*Lower Limit*	*Upper Limit*
I/*D*	0.258	0.047	0.160	0.980
D/*D*	**7.483**	0.0007	1.746	30.192

a. Odds ratio was computed considering the odds of *I*/*I* genotype = 1.

Investigations across populations provide a substantial database on *ACE* insertion/deletion polymorphism and associated hypertension (Table 7). Ethnic background appears to influence *ACE* gene insertion/deletion polymorphism globally. The analysis of distribution of the *ACE* polymorphism and activity within and across the major human groups appears to be useful in identifying the mechanisms contributing to the emergence of common chronic diseases, such as hypertension, CHD, type 2 diabetes mellitus, and diabetic nephropathy (as part of RAS). Such comparative studies could be of significant clinical relevance and utility in the upcoming field of pharmacogenomics (Pasha et al. 2002).

This study's major limitation was that it was performed on a relatively small sample size and therefore is not representative of Asian Indians. Because of considerable ethnic and cultural heterogeneity in Asian Indians, it is necessary to study other ethnic groups to see whether the observed trends exist among them also. The lack of assessment of plasma angiotensin and angiotensin-converting enzyme further limits the study results.

Acknowledgments The DNA typing of the present study was carried out at the Human Genetic Engineering Research Centre (HGERC), Calcutta, India. We are thankful to all the participants for their sincere cooperation during data collection.

Received 17 September 2007; revision received 5 May 2008.

Literature Cited

Ajay, V. S., R. Gupta, J. Panniyammakkal et al. 2002. *National Cardiovascular Disease Database*. Delhi: Ministry of Health and Family Welfare, Government of India, and Geneva: World Health Organization.

Ashavaid, T. F., K. K. Shalia, A. A. Kondkar et al. 2002. Gene polymorphism and coronary risk factors in Indian population. *Clin. Chem. Lab. Med.* 40:975–985.

Ashavaid, T. F., K. K. Shalia, K. G. Nair et al. 2000. *ACE* and *AT1R* gene polymorphisms and hypertension in Indian population. *J. Clin. Lab. Anal.* 14:230–237.

Barbalic, M., T. Skaric-Juric, F. Cambien et al. 2006. Gene polymorphisms of the renin-angiotensin system and early development of hypertension. *Am. J. Hypertens.* 19:837–842.

Barley, J., A. Blackwood, N. Carter et al. 1994. Angiotensin converting enzyme insertion/deletion polymorphism: Association with ethnic origin. *J. Hypertens.* 12:955–957.

Table 7. Association Studies Between *ACE* Insertion/Deletion (I/D) Polymorphism and Essential Hypertension

Study	*Phenotype Studied*	*Genes Screened*	*Population(s) Studied*	*Nature of Study*	*Major Findings*
Barnas et al. (1997)	Type 2 diabetes mellitus, hypertension, and metabolic control	*ACE* (I/D)	Austrians	Case-control	*ACE* I/D genotype was an independent risk factor for the prevalence of diabetic nephropathy. *D/D* genotype was more frequent in patients with diabetic nephropathy along with high blood pressure and renal disease.
Cooper et al. (1997)	Obesity and blood pressure	*ACE*, *AGT*	Jamaicans	Population	Obesity may alter the levels of *ACE* and *AGT* and may provide a pathway through which obesity promotes elevated blood pressure.
Mastana and Nunn (1997)	Essential hypertension	*ACE* (I/D)	Indians (Sikhs)	Population	Positive association between *ACE* I/D polymorphism and essential hypertension.
Chowdhury et al. (1998)	Essential hypertension	*ACE* (I/D)	Bangladeshis	Case-control	No significant association of *ACE* I/D polymorphism and hypertension.
Morshed et al. (2002)	Essential hypertension	*ACE* (I/D)	Bangladeshis	Population	Positive association between *ACE* I/D polymorphism and essential hypertension.
Strazzullo et al. (2003)	Abdominal adiposity	*ACE* (I/D), *AGT* (M235T), *AGTR1* (A1166C)	Italians	Population (males only)	*ACE* I/D polymorphism was a significant predictor of overweight and abdominal adiposity in men. *D/D* homozygosity was associated with larger increase in body weight, blood pressure in aging individuals, and higher incidence of overweight.
Zhu et al. (2003)	Essential hypertension	RAS	European Americans and African Americans	Case-control	Association was confirmed in haplotype analysis for *REN*, *AGTR1*, and *ACE* in African Americans but was less significant in European Americans. Individual variation in the RAS genes may contribute to hypertension risk.
Ismail et al. (2004)	Essential hypertension	*ACE* (I/D)	Pakistanis	Case-control	Positive association between *ACE* I/D polymorphism and essential hypertension.
Present study (2008)	Essential hypertension	*ACE* (I/D)	Asian Indians (Bengalees)	Population	*Odds* of being hypertensive were 7.48 in *D/D* individuals.

Barnas, U., A. Schmidt, A. Illievich et al. 1997. Evaluation of risk factors for the development of nephropathy in patients with IDDM: Insertion/deletion angiotensin converting enzyme gene polymorphism, hypertension, and metabolic control. *Diabetologia* 40:327–331.

Baudin, B. 2002. New aspects on angiotensin-converting enzyme: From gene to disease. *Clin. Chem. Lab. Med.* 40:256–265.

Chowdhury, A. H., M. M. Zaman, K. M. Haque et al. 1998. Association of angiotensin converting enzyme (*ACE*) gene polymorphism with hypertension in a Bangladeshi population, Bangladesh. *Med. Res. Counc. Bull.* 24:55–59.

Cooper, R., N. McFarlane-Anderson, F. I. Berinett et al. 1997. *ACE*, angiotensinogen, and obesity: A potential pathway leading to hypertension. *J. Hum. Hypertens.* 11:107–111.

Crews, D. E., and S. L. Williams. 1999. Molecular aspects of blood pressure regulation. *Hum. Biol.* 71:475–503.

Di Pasquale, P., S. Cannizzaro, and S. Paterna. 2004. Does angiotensin-converting enzyme gene polymorphism affect blood pressure? Findings after 6 years of follow-up in healthy subjects. *Eur. J. Heart Fail.* 6:11–16.

Dubey, V. D. 1954. A study on blood pressure amongst industrial workers of Kanpur. *J. Indian State Med. Assoc.* 23:495–498.

Ghosh, A. 2007. Comparison of anthropometric, metabolic, and dietary fatty acids profiles in lean and obese dyslipidemic Asian Indian subjects. *Eur. J. Clin. Nutr.* 61:412–419.

Gopinath, N., S. L. Chadha, P. Jain et al. 1994. An epidemiological study of obesity in adults in the urban population of Delhi. *J. Assoc. Physicians India* 42:212–215.

Gupta, R., V. P. Gupta, M. Sharna et al. 2002. Prevalence of coronary heart disease and risk factors in an urban Indian population. *Indian Heart J.* 54:59–66.

Gupta, R., H. Prakash, S. Majumdar et al. 1995. Prevalence of coronary heart disease and coronary risk factors in an urban population of Rajasthan. *Indian Heart J.* 47:331–338.

Gupta, R., M. Sarna, J. Thanvi et al. 2004. High prevalence of multiple coronary risk factors in Punjabi Bhatia community. *Indian Heart J.* 56:646–652.

Hazarika, N. C., D. Biswas, K. Narain et al. 2002. Hypertension and its risk factors in tea garden workers of Assam. *Natl. Med. J. India* 15(2):63–68.

Higaki, J., S. Baba, T. Katsuya et al. 2000. Deletion allele of angiotensin converting enzyme gene increases risk of essential hypertension in Japanese men: The Suita Study. *Circulation* 101:2060–2065.

Ismail, M., N. Akhtar, M. Nasir et al. 2004. Association between the angiotensin-converting enzyme gene insertion/deletion polymorphism and essential hypertension in young Pakistani patients. *J. Biochem. Mol. Biol.* 37:552–555.

Jimenez, P. M., C. Conde, A. Casanegra et al. 2007. Association of *ACE* genotype and predominantly diastolic hypertension: A preliminary study. *J. Renin-Angiotensin-Aldosterone Syst.* 8:42–44.

Lindpaintner, K., M. A. Pfeffer, and R. Kreutz. 1995. A prospective evaluation of an angiotensin converting enzyme gene polymorphism and the risk of ischemic heart disease. *New Engl. J. Med.* 332:706–711.

Lohman, T. G., A. F. Roche, and R. Martorell. 1988. *Anthropometric Standardization Reference Manual.* Champaign, IL: Human Kinetics.

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

Mattace-Raso, F. U., M. P. Sie, T. J. van der Cammen et al. 2007. Insertion/deletion gene polymorphism of the angiotensin-converting enzyme and blood pressure changes in older adults: The Rotterdam Study. *J. Hum. Hypertens.* 21:736–740.

McKeigue, P. M., B. Shah, and M. G. Marmot. 1991. Relation of central obesity and insulin resistance with high diabetes prevalence and cardiovascular risk in South Asians. *Lancet* 337:382–386.

Morshed, M., H. Khan, and S. Akhteruzzaman. 2002. Association between angiotensin-I converting enzyme polymorphism and hypertension in selected individuals of the Bangladeshi population. *J. Biochem. Mol. Biol.* 35:251–254.

Nair, K. G., K. K. Shalia, T. F. Ashavaid et al. 2003. Coronary heart disease, hypertension, and angiotensinogen gene variants in Indian population. *J. Clin. Lab. Anal.* 12:411–418.

Pasha, M. A., A. P. Khan, R. Kumar et al. 2002. Variations in angiotensin-converting enzyme gene insertion/deletion polymorphisms in Indian populations of different ethnic origins. *J. Biosoc. Sci.* 27:67–70.

Renner, W., E. Pabst, B. Paulweber et al. 2002. The angiotensin-converting-enzyme insertion/deletion polymorphism is not a risk factor for peripheral arterial disease. *Atherosclerosis* 165:175–178.

Rigat, B., C. Hubet, F. Alhenc-Gelas et al. 1990. An insertion-deletion polymorphism in the angiotensin-I converting enzyme gene accounting for half the variance of serum enzyme levels. *J. Clin. Invest.* 86:1343–1346.

Schut, A. F. C., G. S. Bleumink, B. H. C. Stricker et al. 2004. Angiotensin converting enzyme insertion/deletion polymorphisms and the risk of heart failure in hypertensive subjects. *Eur. Heart J.* 25:2143–2148.

Singh, P. P., I. Naz, A. Gilmour et al. 2006. Association of *APOE* (*Hha*1) and *ACE* (I/D) gene polymorphisms with type 2 diabetes mellitus in northwest India. *Diabetes Res. Clin. Pract.* 74:95–102.

Slowik, A., T. Dziedic, J. Pera et al. 2007. *ACE* genotype, risk, and causal relationship to stroke: Implications for treatment. *Curr. Treat. Options Cardiovasc. Med.* 9:198–204.

Strazzullo, P., R. Iacone, L. Iacoviello et al. 2003. Genetic variation in the renin-angiotensin system and abdominal adiposity in men: The Olivetti Prospective Heart Study. *Ann. Intern. Med.* 138:17–23.

Tai, E. S., and C. E. Tan. 2004. Genes, diet, and serum lipid concentrations: Lessons from ethnically diverse populations and their relevance to coronary heart disease in Asia. *Curr. Opin. Lipidol.* 15(1):5–12.

Thomas, G. N., R. P. Young, B. Tomlinson et al. 2000. Renin-angiotensin-aldosterone system gene polymorphisms and hypertension in Hong Kong Chinese. *Clin. Exp. Hypertens.* 22:87–97.

Todoroki, M., J. Minami, T. Ishimitsu et al. 2003. Relation between the angiotensin-converting enzyme insertion/deletion polymorphism and blood pressure in Japanese male subjects. *J. Hum. Hypertens.* 17:713–718.

Tripathi, G., P. Dharmani, F. Khan et al. 2006. High prevalence of *ACE DD* genotype among North Indian end-stage renal disease patients. *BMC Nephrol.* 17:7–15.

Vassilikioti, S., M. Doumas, S. Douma et al. 1996. Angiotensin converting enzyme gene polymorphism is not related to essential hypertension in a Greek population. *Am. J. Hypertens.* 9:700–702.

Xu, Q., Y. H. Wang, W. J. Tong et al. 2004. Interaction and relationship between angiotensin converting enzyme gene and environmental factors predisposing to essential hypertension in Mongolian population of China. *Biomed. Environ. Sci.* 17:177–186.

Zee, R. Y., P. M. Ridker, M. J. Stampfer et al. 1999. Prospective evaluation of the angiotensin converting enzyme insertion/deletion polymorphism and the risk of stroke. *Circulation* 99:331–333.

Zhu, X., Y. P. Chang, D. Yan et al. 2003. Associations between hypertension and genes in the renin-angiotensin system. *Hypertension* 41:1027–1034.