A Study of Angiotensin Converting Enzyme (ACE) Gene Polymorphism in Essential Hypertension among a Business Community in Punjab


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ABSTRACT The present study was carried out to investigate the association of the angiotensin-converting enzyme deletion/insertion polymorphism with hypertension and its role in increasing the susceptibility to hypertension among Bania population in Punjab. The study blood samples consisted of 50 normal (28 males and 22 females) healthy individuals and 50 hypertensive, age and sex matched (28 males and 22 females) individuals. The genotype frequencies were found to be 0.22, 0.32 and 0.46 for II, DD, ID genotype in hypertensives. The same has been found for normotensives to be 0.26, 0.18 and 0.56 for II, DD and ID respectively. The observed overall genotype distributions were consistent with Hardy-Weinberg equilibrium. The present analysis suggested that the genetic variation at the ACE gene may be associated with some determinants for blood pressure.

INTRODUCTION

Many studies have demonstrated the genetic linkage between ACE gene and blood pressure variations (Jeunemaitre et al. 1992; Chiang et al. 1996; Nakano et al. 1998; Ashavaid et al. 2000; Cox et al. 2002; Morshed et al. 2002). Despite of the fact that significant positive association of ACE gene with hypertension has been reported by many authors. However, many other studies (Dura et al. 1994; Barley et al. 1996; O'Donnell 1998) have failed to identify such associations. It has been suggested (Barley et al. 1994; Stassen et al. 1997) that this inconsistent association may be due to the fact of different ethnicity of the population groups and environmental heterogeneity. Therefore, it is of interest to study regarding the deletion/insertion (D/I) polymorphism of ACE gene and blood pressure regulation. Hence, the present study was carried out to investigate the association of I/D polymorphism of ACE gene with hypertension and its role of increasing the susceptibility of hypertension among Bania population in Punjab.

MATERIALS AND METHODS

The study included a total of 100 individuals of both sexes, consisting 50 each age and sex matched controls and patients with hypertension. These individuals were selected from the Bania community in Punjab. This community is mostly engaged in business and trade. They are primarily vegetarians and non-alcoholic. However, many male members of the family prefer alcoholic drink time to time. They are living in patrilocal society with by and large nuclear family system in single household sharing both genes and environment. The age ranges of selected subjects are between 40 to 70 years.

Blood pressure was measured using a mercury sphygmomanometer in a sitting position on with the right forearm placed horizontal on the table, as recommended by the American Heart Association (1981). All efforts were made to minimize the factors which may influence blood pressure. Hypertension was defined as systolic blood pressure >140 mm Hg accompanied by diastolic blood pressure >90 mm Hg. None of the subjects had evidence of cardiac or renal diseases. About 5ml of peripheral blood samples were collected in a pre-labeled screw cap vial containing 20% EDTA. The specimens were transported from the place of collection to the laboratory on dry ice and were then stored at -20°C till further analysis. DNA was isolated from whole blood using standard protocol of Gill et al. (1987). To determine the ACE genotype, genomic DNA samples were amplified by a polymerase chain reaction (PCR) using primer pair such as 5' - CTG GAG AGC CAC TCC CAT CCT TTC T-3'
(sense) and 5' GAC GTC GCC ATC ACA TTC GTC AGA T 3' (antisense). The PCR reaction mixture contained 100 ng genomic DNA, 0.2 µM of each primer, 200 µM dNTP, 10 mM of Tris-HCL buffer (PH 8.3), 1.5 mM MgCl₂ (PH 8.3) and 0.024 units of Taq polymerase enzyme in a final reaction volume of 15.0 µl. After initial denaturation at 95°C for 5 minutes, the DNA was amplified by 30 cycles of denaturation at 94°C for 45 seconds, annealing at 56°C for 45 seconds and primer extension at 72°C for 45 seconds. Final extension was performed at 72°C for 10 minutes. The amplified PCR products were separated by electrophoresis on 2% agarose gel and the DNA was visualized under UV transilluminator after staining with ethidium bromide. The insertion (I) allele was detected as band of 490 bp fragment and deletion (D) allele was identified as a band of 190 bp fragment. All the data were analyzed by SPSS 10.0. Two-tailed probability levels for statistical significance are reported. The relevant null hypothesis on odds ratio (Matched Pairs) in the present case is tested $H_0: \text{OR}_M = 1$.

RESULTS

Anthropometric and physiometric characteristics of the control and hypertensive subjects are presented in table I. The two study groups were well matched for sex, age and sample size. The mean systolic (SBP) and diastolic (DBP) blood pressures, BMI and WHR were significantly higher ($P<0.001$) among hypertensive subjects than in control subjects. However, there is no significant mean age difference between both groups. The ACE genotype and allele frequencies distribution of control and hypertensive subjects are shown in table 2. The frequencies of II, ID and DD genotype among the control group were 26% (n=13), 54% (n=27) and 20% (n=10) respectively, whereas, in hypertensive group the same were found to be 22% (n=11), 32% (n=16) and 46% (n=23) respectively. There is significant difference ($P<0.05$) observed in the distribution of ACE genotype polymorphism between the two groups. It has been observed that the ACE DD genotype was significantly ($P<0.05$) higher in hypertensive subjects, whereas, ID genotype was significantly ($P<0.05$) higher in control subjects. The frequency of the D allele is also more frequent but not significant in hypertensive subjects than in control. The distribution of genotype frequencies associations of ACE gene polymorphisms between male and female among control and hypertensive subjects are given in table 3. The results showed that among three genotypes within control group, ID genotype was significantly more prevalent in male as compared to other two genotypes (odds ratio:3.0; CI: 0.152-5.84; $\chi^2=14.0$, $P<0.001$). Whereas, among female, II genotype is comparatively more prevalent but not significantly differ (odds ratio: 0.692; $\chi^2=1.45$, ns). In the hypertensive group, both male and female are more associated with DD genotype as compared to other two genotypes. However, these

<table>
<thead>
<tr>
<th>Variables</th>
<th>Controls (n=50)</th>
<th>Hypertensive (n=50)</th>
<th>p</th>
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<tr>
<td>Age (years)</td>
<td>Mean ± SD</td>
<td>Mean ± SD</td>
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<tr>
<td>BMI (Kg/m²)</td>
<td>26.56 ± 3.19</td>
<td>29.32 ± 3.25</td>
<td>&lt;0.001</td>
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<tr>
<td>WHR</td>
<td>0.851 ± 0.005</td>
<td>0.971 ± 0.006</td>
<td>&lt;0.001</td>
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<tr>
<td>SBP (mm Hg)</td>
<td>129.31 ± 10.13</td>
<td>148.23 ± 9.11</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>DBP (mm Hg)</td>
<td>85.19 ± 8.79</td>
<td>92.14 ± 7.37</td>
<td>&lt;0.001</td>
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<table>
<thead>
<tr>
<th>Genotypes</th>
<th>Controls (n=50)</th>
<th>Hypertensive (n=50)</th>
<th>p</th>
</tr>
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<tbody>
<tr>
<td>II</td>
<td>13 (26%)</td>
<td>11 (22%)</td>
<td>$\chi^2=8.11$, &lt;0.001</td>
</tr>
<tr>
<td>ID</td>
<td>27 (54%)</td>
<td>16 (32%)</td>
<td></td>
</tr>
<tr>
<td>DD</td>
<td>10 (20%)</td>
<td>23 (46%)</td>
<td></td>
</tr>
<tr>
<td>Alleles</td>
<td>I</td>
<td>0.51</td>
<td>0.47</td>
</tr>
<tr>
<td></td>
<td>D</td>
<td>0.29</td>
<td>0.53</td>
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associations are not statistically significant (odds ratio (male)=0.867; \( \chi^2 =0.286, \text{ns} \); (female)=0.833, \( \chi^2 =0.364, \text{ns} \)).

**DISCUSSION**

In the present study, it has been investigated the association of ACE insertion/deletion (I/D) polymorphism with hypertension among selected individuals from Bania (a business community) population in Punjab. The study was carried out between two groups (control and hypertensive) which were perfectly matched for age and sex. However, they were significantly different with respect to BMI, WHR, SBP and DBP. The study suggested a possible positive association of DD polymorphism with hypertension in Bania Population in Punjab. Many studies (Cambien et al. 1992; Tiret et al. 1993; Duru et al. 1994; Morise et al. 1994; Barley et al. 1996; Samani et al. 1996; Zaid et al. 1998; Turgay et al. 1999; Higaki et al. 2000) have reported positive ACE DD genotype association with hypertension. The frequency of D allele (53%) in hypertensive group is more prevalent as compared to other allele between both the groups. Although, the difference is not significant (\( \chi^2=0.0032, \text{ns} \)). In the present study, sex specific association of DD genotype with hypertension has not been seen. The possible reason for negative association of DD genotype with hypertension between both sexes may be relatively smaller sample size. However, it is interesting to note that in control group ID genotype was more significantly (P<0.001) associated with male, whereas, II genotype is more prevalent (41%) in female though it is not statistically significant. Therefore, the present data suggest DD genotype have some association with hypertensive male and female individuals, although, the data did not show any statistical significance.

**ACKNOWLEDGEMENT**

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**REFERENCES**


Acute Hypertension and Hypertensive Crisis in Children

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Introduction:
Pediatric Hypertension is defined as systolic or diastolic blood pressure (BP) exceeding the 95th percentile for gender, age, and height. The risk of hypertension increases with the Body Mass Index (BMI). Approximately 30% of children with BMI greater than 95th percentile have hypertension. The spectrum of hypertension that presents to the Emergency Department ranges from mild and asymptomatic to a true hypertensive emergency.

A definition of hypertension ideally is based on a threshold level of blood pressure that divides those at risk for adverse outcomes from those who have no increased risk. The important conclusions of the fourth report on the diagnosis, evaluation, and treatment of high blood pressure in children and adolescents of The National High Blood Pressure Education Program Working Group on High Blood Pressure in Children and Adolescents. (Pediatrics 2004; 114: 555-576) are as follows:

• Hypertension is defined as average systolic and/or diastolic blood pressure >95th percentile for gender, age, and height on >3 occasions.
• Pre hypertension is defined as average systolic or diastolic pressures between 90-95th percentile. These children should be observed carefully and evaluated if risk factors like obesity are present; tracking data suggest that this subgroup is more likely to develop overt hypertension over time than normotensive children.
• Adolescents with blood pressure levels more than 120/80 mm Hg should be considered pre hypertensive.
• A patient with blood pressure levels >95th percentile in a physician’s office or clinic, who is normotensive outside a clinical setting, has white-coat hypertension. Ambulatory blood pressure monitoring is helpful for confirmation.
• If the blood pressure is >95th percentile, it should be staged. If stage 1 (95th percentile to the 99th percentile plus 5 mm Hg), measurements should be repeated on 2 more occasions. If hypertension is confirmed, evaluation should proceed. If blood pressure is stage 2 (>99th percentile plus 5 mm Hg), prompt referral should be made for evaluation and therapy. If the patient is symptomatic, immediate referral and treatment are indicated.
• All children should have yearly blood pressure evaluation beyond 3 years of age. There is an increased risk of hypertension in children with history of hypertension in family members, those who are obese, had
IUGR or have urinary infections and renal scars.

**Evaluation:**
When confronted with newly diagnosed hypertension in the child, the physician should consider three important issues: 1) Is the hypertension primary or secondary? 2) Is there evidence of target organ damage? and 3) Are there associated risk factors that would worsen the prognosis if the hypertension were not treated immediately?

A brief, but careful history and physical examination should be performed. Some key features in the history would be the duration and onset of hypertension, degree of compliance with any drug therapy, and possibility of renal disease (any history of urinary tract infections, hematuria, edema, or umbilical artery catheterization). One should also enquire for any history of joint pain, palpitations, weight loss, flushing, weakness, drug ingestion, headaches, nausea, vomiting and a family history of renal disease or hypertension.

After several determinations of the blood pressure, a focused physical examination should be performed immediately. One should check for any evidence of neurologic dysfunction and left ventricular dysfunction / cardiac failure. Fundoscopy should be performed looking for hemorrhage, infarcts or papilledema. The peripheral pulses should be palpated carefully. Weak and delayed femorals suggest coarctation of aorta. Any discrepancy in the upper and lower extremity BP measurements should be noted. The presence of an abdominal bruit suggests renovascular hypertension. An improper cuff size can significantly record a wrong blood pressure. By convention, an appropriate cuff size is a cuff with an inflatable bladder width that is at least 40% of the arm circumference at a point midway between the olecranon and the acromion. For such a cuff to be optimal for an arm, the cuff bladder length should cover 80% to 100% of the circumference of the arm. Blood pressure measurements are overestimated to a greater degree with a cuff that is too small than they are underestimated by a cuff that is too large. If a cuff is too small, the next largest cuff should be used, even if it appears large.

**Etiology**

Hypertension is usually described as primary (essential) or secondary due to a definable cause. The secondary cause will be found more likely when the patient is younger and hypertension is more severe. Most acute hypertension in childhood is due to glomerulonephritis. Chronic hypertension is commonly associated with renal parenchymal disease and only a small proportion have renovascular hypertension, pheochromocytoma or coarctation of the aorta. Late in the first decade and into the second decade of life, primary hypertension begins to predominate. Coarctation of the aorta accounts for one third cases of hypertension in neonatal period and infancy. Renovascular causes are amongst the curable forms of
Pregledni članak


Pleotropni učinci polimorfizma ACE

Pleiotropic effects of ACE polymorphism

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Sažetak

Angiotenzin-konvertirajući enzim (engl. angiotensin converting enzyme, ACE) ima vitalnu ulogu u normalnoj ljudskoj fiziologiji zbog svojeg izravnog djelovanja u sustavu renin-angiotensin-aldosteron (RAAS), sustavu kinin-kallikrein, in vitro razgradnji amiloid-beta-peptida, aktivnosti GPI-aze (glikozilfosfatidylinozitol, GPI) te u signalnoj transdukciji. Budući da na aktivnost ACE snažno utječe insercijsko-delecijski (I/D) polimorfizam gena ACE, prikupljeno je mnoštvo podataka, kako bi se razjasnila udrženost I/D polimorfizma s kardiovaskularnim i ostalim bolestima poput šećerne bolesti, dijabetes, korone bolesti srca, moždanog udara, hipertenzije, Alzheimerove bolesti, karcinoma i Parkinsonove bolesti. Ovaj će se pregled ograničiti na učinak I/D polimorfizma gena ACE na dugovječnost s obzirom na patofiziologiju nekih bolesti.

Ključne riječi: polimorfizam ACE; dugovječnost; prerana smrtnost; kardiovaskularne bolesti

Abstract

Angiotensin converting enzyme (ACE) has vital role in normal functioning of the human body due to its direct involvement in the renin-angiotensin-aldosterone system (RAAS), kinin-kallikrein system, in vitro degradation of amyloid beta-peptide, GPIase (glycosphosphatidylinositol, GPI) activity and in signal transduction. As ACE activity level is strongly influenced by ACE insertion/deletion (I/D) polymorphism, a huge body of data has been generated to elucidate the association of I/D polymorphism with cardiovascular and non cardiovascular diseases like diabetes, diabetic nephropathy, diabetic retinopathy, atherosclerosis, coronary heart diseases and stroke, hypertension, Alzheimer’s disease, cancer and Parkinson’s disease. This review will be limited to the effect of ACE I/D polymorphism on longevity considering the pathophysiology of several diseases.

Key words: ACE polymorphism; longevity; early mortality; cardiovascular diseases

Angiotensin konvertirajući enzim (ACE)

Angiotensin konvertirajući enzim (engl. angiotensin converting enzyme, ACE) je karkošpektidazna ovisna o kloridima i cinku, prisutna na površini epitelnih i endotelnih stanica. Kod ljudi se mogu naći njegove dvije izoforme. Jedna je veći protein sastavljen od 1300 aminokiselina (150–180 kDa) i zove se somatski ACE (sACE), zbog prisutnosti u somatskim tkivima. sACE se može usidriti u plazmatskoj membrani preko transmembranske domene ili može biti prisutan u krvi u topljivom obliku (1). Druga je izoforma manji protein sastavljen od 730 aminokiselina (100–110 kDa), prisutan samo u testisima te se stoga i zove

Angiotensin converting enzyme

Angiotensin converting enzyme (ACE) is a chloride and zinc dependent carboxypeptidase enzyme present on the surface of epithelial and endothelial cells. In humans, two isoforms exist. One is larger protein, composed of 1300 amino acids (150–180 kDa) and is called somatic ACE (sACE), due to its presence in somatic tissues. sACE can be anchored in plasma membrane through transmembrane domain, or be present in plasma in the soluble form (1). The other isoform is a smaller protein composed of 730 amino acids (100–110 kDa), present only in testicles and called germinal form or testicular form (tACE). Its function

Višestruka PCR metoda (engl. multiplex PCR) i PCR u stvar- nom vremenu (engl. real-time PCR) također su se upot- rebljavale u otkrivanju I/D polimorfizma, no niti jedna od njih nije uspjela zamijeniti Shangumanovu metodu, zbog problema povezanih s obradom nakon same meto- de PCR, kao što su elektroforeza na agaroznom gelu kod višestruke PCR i visoki troškovi PCR u stvarnom vremenu (19,20). Nedavno su Koyama i sur. (2008.) opisali brzu i jed- nostavnu tehniku koja obuhvaća denaturirajuću tekućin- sku kromatografiju visoke djelotvornosti (engl. denatu- ring high performance liquid chromatography, DHPLC). U uvjetima koji nisu denaturirajući, ona analizira PCR pro- dukt za probiranje I/D polimorfizma u epidemiološkim genetskim istraživanjima (21). Ta metoda otkljanja mogućnost pogrešnog određivanja i nudi 100% točnost kod I/D heterozigota, no zbog visokih troškova kromatografije nije isplativa.

**Učinci polimorfizma I/D na ljudsko zdravlje**

Utjecaj I/D polimorfizma na patofiziološke uvjete kroz aktivnost ACE rezultira je mnoštvo podataka koji svje- doče o njegovoj povezanosti s nekoliko bolesti (Tablica 1.). U ovom se pregledu o njegovoj ulozi u ljuskonom zdrav- lju raspravlja u svjetlu prethodnih istraživanja o nekoliko pokazatelja zdravlja populacije.

**Rast i polimorfizam ACE**

Unutarnji okolina ima očigledan učinak na ekspres-iju gena ACE koji napoljetku utječe na trudnoću i po- četnu težinu novorođenčadi. Kajanti et al. (2004.) su pri- mjetili povezanost I/I genotipa s kraćim trajanjem trud- noće i većom težinom kod poroda, što kod odraslih neiz- ravno znači da postoje manji izgledi za razvoj koronarne bolesti srca, šećerne bolesti tipa 2, inzulinske rezistencije i metaboličkog sindroma (22-24). Tako je, dakle, razvijen koncept da je alel I odgovoran za veću tjelesnu težinu pri porodu. Taj je koncept bio prihvatljiv sve dok Hindmar- sh i sur. (2007.) nisu ustvrdili kako ne postoji povezanost genotipa ACE novorođenčeta ili njegovih roditelja sa po- rođajnom težinom (25). Međutim, I/I genotip pokazao je svoju pozitivnu ulogu u ranom rastu djece koja su unutar were adopted by different scientists. Shanguman et al. (1993) used 5% dimethyl sulfoxide (DMSO) and sense primer from the 5’ end of the insertion sequence, along with the standard antisense primer (17). Later on, step down PCR modification in this method improved the accuracy of the method (18).

**Effects of I/D polymorphism on human health**

The influence of I/D polymorphism on pathophysiological conditions mediated through ACE activity has generated a lot of data showing its association with several diseases (Table 1). In the present review, its role in deciding on the health status is discussed in the light of previous studies on several health indicators.

**Growth and ACE polymorphism**

Intrauterine environment has obvious effects on the gene expression of ACE, which ultimately influences the period of gestation and birth weight of the newborn. Kajanti et al. (2004) noticed the association of I/I genotype with shorter gestation duration and higher birth weight, which indirectly means less chances for development of coronary heart diseases, type 2 diabetes, insulin resistance and metabolic syndrome in adults (22-24). So, a concept was developed on I allele to be responsible for higher birth weight. This concept remained acceptable until Hindmarsh et al. (2007) have claimed that there is no association of ACE genotype of the newborn or his/her parents with birth weight (25). However, I/I genotype exhibited advantageous role in the early growth of babies, showing more gain in weight, body mass index and mid-darm circumference in one fiscal year as compared to the babies with D/D genotype, the majority of which showed no change or catch-down. I/D genotype was distributed equally across all the categories. These effects were more prominent in males. So, I/I genotype has positive effects on the early growth after birth.
Childhood Hypertension

Sanjeev Gulati

Introduction

Hypertension is one of the commonest diseases with an estimated worldwide prevalence of 1 billion. Data from the 3rd National Health and Nutritional Assessment Survey reveals that in US, one-third of people were unaware of this problem and another one third had blood pressure control below established goals(1). To add to this is the observation in the 7th Joint National Committee on Prevention, Detection, Evaluation, and Treatment of High Blood Pressure report that each increment of 20 mm Hg in systolic or 10 mm Hg diastolic pressure doubles the risk of cardiovascular disease(2). There are no similar data in children, where the age, gender and height need to be taken into account while interpreting blood pressure values.

Essential hypertension has been found to be associated with family history of hypertension, IUGR, oligonephronia, obesity and elevated uric acid levels. According to studies in adult populations, the effective treatment of hypertension reduces the risk of coronary heart disease, stroke, renal disease, and congestive heart failure(3).

Prevalence of Primary and Secondary Hypertension

The prevalence of hypertension in children is reported to be 1-3%. In recent years, the prevalence of hypertension in school-aged children appears to be increasing, perhaps as a result of the increased prevalence of obesity. School-based blood pressure screening and measurement of height and weight in 5102 children, revealed a prevalence of 4.5%, which is higher than that reported earlier(4). The majority of these children have mild hypertension, most often primary (essential). A small group of children have much higher blood pressures usually due to a secondary cause. The prevalence of persistent secondary hypertension in children is 0.1% and renal disease predominates in this group(5). Education, anticipatory guidance, early detection, accurate diagnosis and effective therapy may help improve the long-term outcomes of children and adolescents with hypertension.

Hypertension—Techniques and Case Definitions

All measurements used in constructing the task force tables were made with a standard mercury sphygmomanometer(6). Despite the availability of electronic monitors, the mercury instrument remains the method of choice. The aneroid sphygmomanometer requires periodic calibration. Automated oscillometric devices are useful but expensive and require maintenance and calibration. However, these automated devices can be very helpful in evaluating infants and small
**Etiology**

Hypertension is usually described as primary (essential) or secondary due to a definable cause.

The younger the patient and more severe the hypertension, the more likely that a secondary cause will be found.

Most acute hypertension in childhood is due to glomerulonephritis. *Chronic hypertension is commonly associated with renal parenchymal disease and only a small proportion have renovascular hypertension, pheochromocytoma or coarctation of the aorta* (Table I). Late in the first decade and into the second decade of life, primary hypertension begins to predominate(11). Coarctation of the aorta accounts for one third cases of hypertension in neonatal period and infancy. Renovascular causes are amongst the curable forms of hypertension(12). In Asia, aortoarteritis is common and accounts for most patients with malignant hypertension(13).

**Comorbid Factors**

Children with blood pressure who are in higher percentiles tend to track in those percentiles in adulthood. Obesity is a common cofactor in the development of essential hypertension. Obesity contributes to blood pressure control through high sodium intake and insulin resistance. The “syndrome X” of hypertension, obesity, hyperlipidemia and diabetes mellitus is a major cause of long-term cardiovascular morbidity and mortality. Sleep disorders including sleep apnea are associated with hypertension; approximately 15% of children snore, and 1-3% have sleep-disordered breathing. Repetitive loud snoring followed by silent periods of apnea usually represents the syndrome of obstructive sleep apnea. Because of the associations of hypertension with sleep disorders, particularly among overweight children, a history of sleeping patterns should be obtained(7).

**Clinical Presentation**

Young infants may present in acute distress with signs and symptoms of congestive heart failure. In contrast, after infancy hypertension is usually silent. Patients with severe hypertension may have headache, vision changes, nosebleeds, or nausea and should be treated immediately. Presence of edema, oliguria, hematuria suggests rapidly progressive renal failure. Presence of joint pains, rash and systemic symptoms suggests connective tissue disorders. Hypertensive emergencies are defined as the presence of severe hypertension associated with life-threatening or organ-threatening complications, including encephalopathy (seizures, stroke, focal deficits), acute heart failure, pulmonary edema, dissecting aortic aneurysm or acute renal failure.

Attention should to be paid on physical examination to anthropometric measurements, height, weight and body mass index. It

<table>
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<tr>
<th>Age group</th>
<th>Common causes</th>
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<tr>
<td>Newborns</td>
<td>Renal artery thrombosis, renal artery stenosis, congenital malformation, coarctation of aorta, bronchopulmonary dysplasia</td>
</tr>
<tr>
<td>Infancy-6 yr</td>
<td>Renal parenchymal disease, coarctation of aorta, renal artery stenosis</td>
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<td>6-10 yr</td>
<td>Essential hypertension, renal artery stenosis, renal parenchymal disease.</td>
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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).

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 nonapeptide belonging to a class of active peptides (kinins) that are released from tissue to produce a variety of effects, including arterial vasodilation and vasoconstriction. 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).
DISCUSSION

We examined ACE gene polymorphism, one of the important genes in rennin-angiotensin system in hypertension, type 2 diabetes 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

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REFERENCES

Cambien R, Poirier O, Lecerf L, Evans A, Cambou JP,
Protein family review

Angiotensin-I-converting enzyme and its relatives  
Genome Biology 2003, 4:225

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Summary

Angiotensin-I-converting enzyme (ACE) is a monomeric, membrane-bound, zinc- and chloride-dependent peptidyl dipeptidase that catalyzes the conversion of the decapeptide angiotensin I to the octapeptide angiotensin II, by removing a carboxy-terminal dipeptide. ACE has long been known to be a key part of the renin angiotensin system that regulates blood pressure, and ACE inhibitors are important for the treatment of hypertension. There are two forms of the enzyme in humans, the ubiquitous somatic ACE and the sperm-specific germinal ACE, both encoded by the same gene through transcription from alternative promoters. Somatic ACE has two tandem active sites with distinct catalytic properties, whereas germinal ACE, the function of which is largely unknown, has just a single active site. Recently, an ACE homolog, ACE2, has been identified in humans that differs from ACE in being a carboxypeptidase that preferentially removes carboxy-terminal hydrophobic or basic amino acids; it appears to be important in cardiac function. ACE homologs (also known as members of the M2 gluzincin family) have been found in a wide variety of species, even in those that neither have a cardiovascular system nor synthesize angiotensin. X-ray structures of a truncated, deglycosylated form of germinal ACE and a related enzyme from Drosophila have been reported, and these show that the active site is deep within a central cavity. Structure-based drug design targeting the individual active sites of somatic ACE may lead to a new generation of ACE inhibitors, with fewer side-effects than currently available inhibitors.

Gene organization and evolutionary history

Angiotensin-I-converting enzyme (ACE, also known as peptidyl-dipeptidase A or kininase II) was first isolated in 1956 and shown to be a chloride-dependent metalloenzyme that cleaves a dipeptide from the carboxyl terminus of the decapetide angiotensin I to form the potent vasopressor (blood vessel constrictor) angiotensin II [1]. In addition, it inactivates the vasodilator bradykinin by sequential removal of two carboxy-terminal dipeptides. Indeed, it is a broad-specificity dipeptidyl carboxypeptidase and may also act on non-vasoactive peptides. There are two forms of ACE in humans, encoded by a single gene located on chromosome 17 at q23; it is 21 kb in length and contains 26 exons and 25 introns. The longer form, known as somatic ACE (sACE), is transcribed from exons 1-12 and 14-26, whereas the shorter form, known as germinal or testicular ACE (gACE), is transcribed from exons 13-26. The promoter for sACE is in the 5′ flanking region of the first exon, whereas that for gACE is located within intron 12 [2].

Somatic ACE consists of an intracellular domain, a transmembrane domain and two similar extracellular domains, the amino or N domain and the carboxy or C domain (Figure 1). The structure of the ACE gene is the result of gene duplication; the N and C domains are similar in sequence, and the homologous exons encoding the N and C domains (exons 4-11 and 17-24, respectively) are very similar in size and have similar codon phases at exon-intron boundaries. Each of the domains contains a catalytically active site characterized by a consensus zinc-binding motif (HEXXH in the single-letter amino-acid code, where X is any amino acid) and a glutamine nearer the carboxyl terminus that also binds zinc; ACE and its homologs (see below) therefore make up the M2 gluzincin family [3].
Protein family review

Angiotensin-I-converting enzyme and its relatives
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Angiotensin-I-converting enzyme (ACE) is a monomeric, membrane-bound, zinc- and chloride-dependent peptidyl dipeptidase that catalyzes the conversion of the decapeptide angiotensin I to the octapeptide angiotensin II, by removing a carboxy-terminal dipeptide. ACE has long been known to be a key part of the renin angiotensin system that regulates blood pressure, and ACE inhibitors are important for the treatment of hypertension. There are two forms of the enzyme in humans, the ubiquitous somatic ACE and the sperm-specific germinal ACE, both encoded by the same gene through transcription from alternative promoters. Somatic ACE has two tandem active sites with distinct catalytic properties, whereas germinal ACE, the function of which is largely unknown, has just a single active site. Recently, an ACE homolog, ACE2, has been identified in humans that differs from ACE in being a carboxypeptidase that preferentially removes carboxy-terminal hydrophobic or basic amino acids; it appears to be important in cardiac function. ACE homologs (also known as members of the M2 gluzincin family) have been found in a wide variety of species, even in those that neither have a cardiovascular system nor synthesize angiotensin. X-ray structures of a truncated, deglycosylated form of germinal ACE and a related enzyme from Drosophila have been reported, and these show that the active site is deep within a central cavity. Structure-based drug design targeting the individual active sites of somatic ACE may lead to a new generation of ACE inhibitors, with fewer side-effects than currently available inhibitors.

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ACE Gene Polymorphism in Children with Nephrotic Syndrome in the Indonesian Population

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Keywords: ACE, polymorphism, steroid responsiveness, Indonesia, nephrotic syndrome

Background: The angiotensin converting enzyme (ACE) gene carries insertion (I) and deletion (D) polymorphism within its intron 16. The presence of D-allele in the ACE gene has been reported as a probable genetic risk factor for idiopathic nephrotic syndrome (INS), especially the subtype of focal segmental glomerulosclerosis (FSGS). The D-allele may be related to poor responsiveness to steroid therapy. To clarify the relationship between the D-allele and INS, we studied the prevalence of the D-allele in the Javanese-Indonesian patients. Additionally, we also analyzed relationship between each genotype and steroid sensitivity among the MCNS patients.

Methods: Eighty-five Javanese-Indonesian patients under 15 years of age with INS were enrolled in this study: 16 patients with FSGS and 69 patients with minimal change nephrotic syndrome (MCNS). As controls, 68 healthy adult Javanese-Indonesians with no history of kidney disease volunteered to participate in this study. Genotypes based on the polymorphisms (I/D) were determined by using a PCR method. As for the steroid responsiveness, the information of 14 out of 16 FSGS patient (87.5%) and 69 out of 69 MCNS patients (100%) was available.

Results: The genotype frequencies in the FSGS patients were II 37% (6/16), ID 44% (7/16) and DD 19% (3/16), and the D-allele frequency was 41% (13/32). The genotype frequencies in the MCNS patients were II 56% (39/69), ID 38% (26/69) and DD 6% (4/69), and the D-allele frequency was 25% (34/138). The genotype frequencies in the controls were II 60% (41/68), ID 31% (21/68), and DD 9% (6/68), and the D-allele frequency was 26% (33/136). None of the FSGS patients were sensitive to steroid, while almost all MCNS patients (66/69) were sensitive to steroid. The genotype frequencies among steroid-sensitive MCNS patients were consistent with those of the controls, suggesting that there was no relationship between each genotype and steroid sensitivity.

Conclusions: In the Javanese-Indonesian population, none of the comparisons showed any significant differences in the genotypic distribution and allelic frequencies among the three groups, FSGS, MCNS and controls, although D-allele tended to exist more frequently in FSGS patients than in the MCNS patients and controls. In addition, the D-allele frequency was not related to steroid sensitivity in the MCNS patients.

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INTRODUCTION

Angiotensin converting enzyme (ACE) is a key enzyme that converts inactive angiotensin I into a vasoactive and aldosterone-stimulating peptide angiotensin II. In some cases, the increase of ACE protein is responsible for the elevation of angiotensin II level. Elevated angiotensin II level makes deleterious effects on renal hemodynamics and induces the expression of other growth factors, leading to glomerulosclerosis (7).

The ACE gene carries insertion (I) and deletion (D) polymorphism, and the DD-genotype is reportedly related to an increase in the ACE protein expression (9). Therefore, it has been thought that the DD genotype may link to the ACE-related pathophysiology of renal diseases (4,6). Of the ACE I/D polymorphism impacts on the renal diseases, idiopathic nephrotic syndrome (INS) holds particular attention, especially the focal segmental glomerulosclerosis (FSGS). Hori et al. (4) reported that the frequency of DD genotype was higher in FSGS patients than in controls. Lee et al. (6) reported that FSGS patients with DD genotype showed a lower responsiveness to corticosteroid therapy and a higher incidence of chronic renal failure than those with other genotypes.

Although there are many reports from other populations, there is no studies on the relationships between ACE I/D polymorphism and renal diseases have been reported from the Indonesian population. Here, we determined the distribution of the ACE I/D polymorphism among INS patients and healthy individuals in the Javanese-Indonesian population, and compared our results with the data reported from other populations.

SUBJECTS AND METHODS

Subjects

A total of 85 Javanese-Indonesian patients with INS who visited Sardjito General Hospital in Yogyakarta, Indonesia, were enrolled in this study: 16 patients with FSGS (male/female: 9/7, age: 1.5~15) and 69 patients with minimal change nephrotic syndrome (MCNS) (male/female: 38/31 age: 1.6~13). Javanese-Indonesian is defined as native inhabitants of Java Island. FSGS and MCNS were diagnosed based on renal biopsy findings. As controls, 68 non-related healthy individuals living in Java Island volunteered to participate in this study. DNA analysis was performed after obtaining informed consent from the patients and/or the patient’s parents. The ethical committee, Gadjah Mada University School of Medicine, approved this study plan.

Among the 16 FSGS patients, 10 were dependent on steroid and 4 resistant to steroid. The other two patients were omitted from the analysis for steroid responsiveness, because their information was not available. Among the 69 MCNS patients, 66 were sensitive to steroid and 3 dependent on steroid. Steroid sensitive condition is defined as excreting protein-free urine (less than 100 mg/m² per day) for at least 3 consecutive days during 8 weeks of initial therapy (6,7). Meanwhile, steroid dependent condition is defined as the tendency to relapse while on or during tapering of steroid dose, or within 14 days of steroid withdrawal. In this study, steroid-responsive patients included steroid-sensitive and steroid-dependent patients. Steroid resistant condition is defined as excreting urinary protein exceeding 1,000 mg/m² per day without any clinical improvement (7).

ACE Gene Insertion/Deletion Polymorphism Genotyping

Genomic DNA was extracted from whole blood using SepaGene Kit (Sanyo Junyaku Co., Ltd., Tokyo, Japan). PCR was carried out according to the method of Rigat B et al (8). The sequences of the forward and reverse primers were: 5'-CTG GAG ACC ACT CCC ATC CTT TCT-3' and 5'-GAT GTG GCC ATC ACA TTC GTC AGA T-3', respectively (8).
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ACE Gene Insertion/Deletion Polymorphism Among Patients with Type 2 Diabetes, and Its Relationship with Metabolic Syndrome at Sardjito Hospital Yogyakarta, Indonesia


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ABSTRACT

**Aim:** to know the frequencies of insertion/deletion polymorphism in the angiotensin-converting enzyme (ACE) gene among patients with type 2 diabetes and its relationship with metabolic syndrome at Sardjito Hospital Yogyakarta.

**Methods:** we examined 69 patients with type 2 diabetes at Sardjito Hospital Yogyakarta, divided 2 groups based on ATP III criteria of metabolic syndrome. To determine the ACE genotype of the patients, a genomic DNA fragment on intron 16 of the ACE gene was amplified by polymerase chain reaction (PCR) using a forward primer 5'-CTG GAG ACC ACT CCC ATC TTT TCT-3' and reverse primer 5'-GAT GTG GCC ATC ACA RTC GTC AGA T-3'. II genotype 1 band on 490 bp (homozigot), DD genotype 1 band on 190 bp (homozigot) and ID genotype 2 band (heteroduplex) on 490 bp and 190 bp were separately detected on a 3% agarose gel containing ethidium bromide.

**Results:** of 69 patients with type 2 diabetes, there were 51 females (73.91%) and 18 males (26.09%). Subjects with metabolic syndrome were 49 patients (71.02%) while without metabolic syndrome were 20 patients (28.98%). Subjects with II, DD, ID genotype were 57.97%, 23.19% and 18.84% respectively. The male subjects with II, DD, ID genotype were 55.56%, 27.78% and 16.67% respectively, and the female subject II, DD ID genotype were 58.82%, 21.57% and 19.61% respectively. The association between ACE I/D polymorphism and metabolic syndrome in type 2 diabetes, was not significant (p=0.204).

**Conclusion:** the frequency of ACE I/D polymorphism among type 2 diabetes are 57.97% II, 23.19% DD, 18.84% ID. There is no association between metabolic syndrome and the component of metabolic syndrome and varians of the ACE gene among the type 2 diabetes patients.

**Key words:** insertion/deletion polymorphism of the ACE gene, metabolic syndrome.

INTRODUCTION

The ACE studies in recent years showed as candidate for a variety of diseases. The renin-angiotensin system (RAS) has long been known to be an important regulator of blood pressure and renal electrolyte homeostasis, and this system has also been implicated in the pathological changes of organ damage through modulation of gene expression, growth, fibrosis, and inflammatory response.

A polymorphism in the ACE gene has been described consisting of an insertion or deletion (I/D) of a 287-bp fragment in intron 16. The polymorphism ACE/ID is strongly associated with the level of circulating enzyme. This enzyme plays a key role in the production of angiotensin II and in the catabolism of bradykinin, two peptides involved in the modulation of vascular tone and in the proliferation of smooth muscle cells. The DD genotype is associated with higher levels of circulating ACE than the ID and II genotypes and studies showed that DD genotype was significantly more frequent in patients with myocardial infarction and hypertension.

Angiotensin II can also increase insulin sensitivity due to an enhanced blood flow to insulin sensitive tissues and subject with DD genotype is more insulin sensitive. Studies about polymorphism ACE/ID has controversial result. Some studies showed no association between the DD genotype and hypertension, other DD genotype studies were associated with an increased susceptibility to type 2 diabetes and dislipidemia.

There is a controversial result about study in understanding the association between ACE I/D
ACE Gene Insertion/Deletion Polymorphism Among Patients with Type 2 Diabetes, and Its Relationship with Metabolic Syndrome at Sardjito Hospital Yogyakarta, Indonesia


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Angiotensin-converting enzyme gene polymorphism in hypertensive rural population of Haryana, India

Sumeet Gupta, Bimal K Agrawal, Rajesh K Goel, and Prabodh K Sehajpal

Abstract

Background:
Essential hypertension is a complex genetic disorder influenced by diverse environmental factors. Of the various physiological pathways affecting the homeostasis of blood pressure, the renin-angiotensin system (RAS) is known to play a critical role. Angiotensin-I converting enzyme (ACE) is a significant component of RAS and an insertion/deletion (I/D) polymorphism in its gene has been implicated in predisposition to hypertension.

Objective:
The present study is aimed to determine the association, if any, of ACE I/D polymorphism with essential hypertension in a rural population of Haryana, India.

Materials and Methods:
The blood samples were collected from the patients visiting M. M. Institute of Medical Sciences, Mullana, Haryana. DNA from the patients (106) and control (110) specimens were isolated, amplified by PCR and analyzed employing agarose gel electrophoresis.

Results:
There was no significant difference in the distribution of DD, II and I/D genotypes of ACE polymorphism in essential hypertensive patients (28.8, 25.5, and 46.2%) and their ethnically matched normal control (24.5, 30, and 45.5), respectively. The two groups also presented with very similar allelic frequencies and were also found to be in Hardy-Weinberg equilibrium.

Conclusions:
The present study demonstrates that ACE I/D polymorphism is not a risk factor for essential hypertension in the hitherto unstudied rural population of Haryana.

Keywords: Angiotsin-I converting enzyme, insertion/deletion polymorphism, essential hypertension, North Indian population

INTRODUCTION
Cardiovascular diseases are becoming a major health burden in developing countries. About 2.6 million Indian people are estimated to die due to coronary heart disease (CHD) alone by the year 2020.[1] Hypertension is one of the important risk factor for the development of CHD. It is a multifactorial and polygenic disorder in which the interaction between several candidate genes and environmental factors play a role. The renin angiotensin system (RAS) is an important regulatory mechanism for maintaining normal blood pressure, fluid and electrolyte balance and its encoding components have been proposed as independent factors for hypertension and other cardiovascular diseases.[2,3]

Angiotensin-I-converting enzyme (ACE) is a zinc metallopeptidase widely distributed on the surface of endothelial and epithelial cells and participates in producing arteriolar constriction and a rise in systolic and diastolic blood pressure. The ACE is encoded by a 21 kb gene that consists of 26 exons and is located...
history and body mass index in the hypertensive patients shows statistically significant difference from the control population [Table 1] and it does suggest that genetic factors and body mass index do influence the ability to develop this disease in the studied rural population of Haryana. These observations are in line with earlier report providing evidence that heritable factors in combination with a number of recognized environmental risk factors are important determinants of the pathogenesis and natural history of essential hypertension.[20]

It is important to ascertain gene(s) that are involved in hypertension. This would help in identifying individuals at an increased risk of developing this disease and to initiate appropriate actions in them to avoid development or delay the onset of disease. Genome wide scan and candidate gene approach are two strategies used in dissecting complex genetic diseases.[21] The former, links specific chromosomal region with inheritance of the disease, is technically cumbersome and requires sophisticated infrastructure. The candidate gene approach targets selected gene with defined polymorphism(s) for their association with the disease. The polymorphism could exist as single nucleotide change, insertion/deletion of nucleotide sequence or repetitive DNA elements. A gene and its selected polymorphism preferably should have the following features to make them a candidate target:

- The gene product must be functionally relevant to hypertension
- Polymorphism within the gene must alter its function
- Hypertension needs to link to the chromosomal region harboring the candidate gene.

Available studies demonstrate that the ACE I/D polymorphism fulfills above mentioned criterions in the context of hypertension[7,22–24] and was therefore investigated in the present study.

The frequencies of different genotypes were found to be similar in patient and the control population [Table 2]. The frequencies of both the alleles (I/D) are quite high in the control and cases, thus obviating the possibility that the frequency of the rare allele is a cause for concern in the studied sample. Lack of association between ACE I/D polymorphism and essential hypertension have been reported by investigators in Indian and other populations of the world.[25–28,40] Ethnic background is known to influence the ACE I/D polymorphism globally.[29,30] A significant association of the ACE high producing D allele with hypertension in African, Americans, Chinese, and Japanese populations have already been reported.[8,22–24] However, two studies from Australia[31] and Pakistan[32] recorded the association of I allele with hypertension. The association of I allele with hypertension in Pakistan population was attributed to limited number of individuals studied[32] and to the presence of high levels of inbreeding.[25]

The frequency of D allele of ACE I/D polymorphism in different hypertensive populations of India varied within 0.522 to 0.409 [Table 3]. The highest frequency was reported in a Sikh group from Punjab that also showed an association between the D allele and the hypertension. Similar observations have also been made on populations from other states of India.[30] The frequency of D allele in the studied patient and control populations were well within the reported range for the North Indian populations [Table 3]. Contrary to the earlier findings, no association between D allele and essential hypertension was observed in the rural population of Haryana. We believe the number of patients studied in other Indian populations showing positive associations with D allele [Table 3] were very small to allow any meaningful conclusion.

Table 3
The genotypic distribution and allele frequencies of the ACE I/D polymorphism in essential hypertension in different population of the world

Identifying association between a gene and a complex genetic disease is difficult. One possible reason for this is the involvement of a large number of genes in the etiology of essential hypertension. Furthermore, these genes may interact with each other in different combinations to give rise to a similar disease phenotype. The magnitude of this problem makes the frequency of any polymorphism contributing to a disease phenotype marginally higher in disease group compared with unaffected controls.[33] Linkage
ACE I/D genotype, adiposity, and blood pressure in children

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Abstract

Background: Angiotensin converting enzyme (ACE) is a possible candidate gene that may influence both body fatness and blood pressure. Although several genetic studies have been conducted in adults, relatively few studies have examined the contribution of potential candidate genes, and specifically ACE I/D, on adiposity and BP phenotypes in childhood. Such studies may prove insightful for the development of the obesity-hypertension phenotype early in life. The purpose of this study was to examine differences in body fatness and resting blood pressure (BP) by ACE I/D genotype, and determine if the association between adiposity and BP varies by ACE I/D genotype in children.

Methods: 152 children (75 girls, 77 boys) were assessed for body composition (% body fat) using dual energy x-ray absorptiometry and resting BP according to American Heart Association recommendations. Buccal cell samples were genotyped using newly developed PCR-RFLP tests for two SNPs (rs4341 and rs4343) in complete linkage disequilibrium with the ACE I/D polymorphism. Partial correlations were computed to assess the associations between % body fat and BP in the total sample and by genotype. ANCOVA was used to examine differences in resting BP by ACE I/D genotype and fatness groups.

Results: Approximately 39% of youth were overfat based on % body fat (>30% fat in girls, 25% fat in boys). Body mass, body mass index, and fat-free mass were significantly higher in the ACE D-carriers compared to the II group (p < 0.05). BP was not significantly different by ACE I/D genotypes. In the total sample, correlations between adiposity and BP ranged from 0.30 to 0.46, and were not significantly different between genotypes. When grouped by genotype and body fat category, the overfat D-carrier subjects had significantly higher SBP and MAP compared to the normal fat D-carrier and normal fat II groups (p < 0.05).

Conclusion: ACE D-carriers are heavier than ACE II children; however, BP did not differ by ACE I/D genotype but was adversely influenced in the overfat D-carriers. Further studies are warranted to investigate the genetics of fatness and BP phenotypes in children.

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adjusted SBP than the other ACE genotypes [13]. In a case-control study of hypertension in adolescents, a significant dominant effect of ACE D alleles on SBP was found in boys only [36]. These results mirror those in adults, which show the association between BP and the ACE I/D genotype may be sex dependent [31,38]. Unfortunately, our sample was not large enough to conduct sex-specific analyses.

Another purpose of this paper was to examine if the ACE I/D genotype modified the relationship between adiposity and BP. Our results showing a moderate correlation between adiposity and BP and differences between overweight and normal fat youth confirm previous work [15,39]. However, there remains unexplained phenotypic variation and considerable variation in BP among individuals with similar levels of adiposity. To our knowledge, this is the first study to examine if the association between adiposity and BP is modified by ACE genotype (or any other candidate gene) in children, although a recent study of 292 eight-year-old children found that the magnitude of the association between adiposity and insulin resistance and triglycerides was stronger in ACE DD subjects compared to II or ID subjects [40]. Altered levels of ACE caused by obesity have been previously suggested as a potential pathway through which obesity leads to the elevation of BP in adults [41]. A case-control study in adults found that the DD genotype had 2.5-fold odds of hypertension compared to the II genotype [42]. However, the additive effects of the ACE D allele and BMI increased the proportion of hypertensive individuals from 40% in non-obese II and ID individuals to 60% in the non-obese DD group and 86% in the obese DD group [42]. Our results offer some confirmation of these findings in that youth who possessed the D-allele and were overweight had significantly higher BP compared to the normal fat youth in either genotype. Thus, it appears that obesity may enhance the expression of ACE I/D genotype differences and lead to elevated BP and perhaps the metabolic syndrome.

**Conclusion**

The role of ACE I/D genotype on adiposity and BP phenotypes of children are important to consider in the context of complex, multi-factorial phenotypes. First, these traits are not monogenic, and therefore other candidate genes influence these traits as well. Second, adverse exposure to other environmental factors may also be important to consider. However, we did show that BP was adversely influenced in the overfat D-carriers. Finally, it is possible that ACE I/D may influence these traits differently at various lifestages. Given the paucity of data in the area of genetics and pediatric health and the relative importance of understanding the role of the genome in human health and disease, additional study is warranted in this emerging field of study.

**Competing interests**

The authors declare that they have no competing interests.

**Authors' contributions**

JCE has made substantial contributions to all aspects of this paper including acquisition of funding; conception and design, acquisition of data, analysis and interpretation of data; and writing the manuscript. MAS has made contributions to the analysis and interpretation of data, and assisted in drafting the manuscript and revising it critically for important intellectual content. KAH was responsible for Acquisition of funding, coordinating data collection, and provided critical feedback to the manuscript for important intellectual content. All authors read and approved the final manuscript.

**Acknowledgements**

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**References**

10.  Lagou V, Manios Y, Moran CN, Bailey ME, Grammatikaki E, Oikonomou E, Ioannou E, Moschonis G, Wilson RH, Pitsiladis YP: Developmental changes in adiposity in toddlers and preschoolers in...
the GENESIS study and associations with the ACE I/D polymorphism. *Int J Obes (Lond)* 2007, 31(7):1052-1060.


The ACE insertion/deletion polymorphism has no influence on progression of renal function loss in autosomal dominant polycystic kidney disease

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1Department of Nephrology and 2Department of Human and Clinical Genetics, Leiden University Medical Centre, Leiden, The Netherlands

Abstract

Background. Autosomal dominant polycystic kidney disease (ADPKD) shows a variable clinical course that is not fully explained by the genetic heterogeneity of this disease. We looked for a possible genetic modifier, the ACE I/D polymorphism, and its influence on progression towards end-stage renal failure (ESRF).

Methods. Forty-nine ADPKD patients who reached ESRF <40 years, and 21 PKD1 patients who reached ESRF >60 years or were not on dialysis at 60 years of age were recruited. Clinical data were provided by questionnaires. Blood was collected for the determination of the ACE insertion/deletion (I/D) polymorphism genotype. The ACE genotype was also determined in a general, control PKD1 group (n = 59).

Results. Patients who reached ESRF <40 years had significantly more early onset hypertension than patients reaching ESRF >60 years (80% vs 21%; P <0.001). The ACE genotype distribution showed no differences between the groups of the rapid progressors (DD 20%, ID 56%, II 24%), the slow progressors (DD 29%, ID 52%, II 19%) and the general PKD1 control population (DD 31%, ID 47%, II 22%).

Conclusion. There is no relationship between progression towards ESRD and the ACE I/D polymorphism in ADPKD patients.

Keywords: ACE insertion/deletion polymorphism; autosomal dominant polycystic kidney disease; progression

Introduction

Autosomal dominant polycystic kidney disease (ADPKD) is the most frequent inherited renal disorder. Its clinical course is highly variable. End-stage renal failure (ESRF) in ADPKD can be reached as early as childhood or not at all [1]. Part of this variation can be attributed to genetic heterogeneity [2]. In the majority of cases (85%) the PKD1 gene, located on chromosome 16, is mutated. However, when the disease is caused by a mutation in the PKD2 gene, located on chromosome 4, the disease generally runs a milder course: the mean age at which ESRF is reached is 54 years in PKD1 patients and 73 years in PKD2 patients [3]. Nevertheless, the clinical variability cannot be explained fully by these two different genes. Considerable interfamilial variability between either PKD1 or PKD2 families is frequently observed. Different mutations in the PKD gene may have different effects on renal function. A possible genotype–phenotype relationship though, has not been found so far. A possible genotype–phenotype relationship certainly cannot explain the diversity in clinical course that can be seen between members of the same family, known to carry the same mutation [4,5]. Environmental and other genetic modifying factors can to a large extend be held responsible for this intrafamilial variability. Factors such as hypertension, haematuria, urinary-tract infections in men, or more than four pregnancies are known to have a negative influence on progression [6]. The influence of genetic modifiers on progression has not been clarified yet.

The renin–angiotensin system (RAS) activity has been implicated in the pathogenesis of hypertension [7,8]. In view of the effect of hypertension on progression towards ESRF, the genetic polymorphisms of the RAS have recently received great interest, in particular the ACE insertion/deletion (I/D) polymorphism [9]. Although this polymorphism is located in an intron of the ACE gene (chromosome 17), it is associated with a 50% variability in serum ACE levels. Individuals homozygous for the deletion (DD) have the highest ACE levels, individuals homozygous for the insertion (II) the lowest. Several reports have been published trying to relate this polymorphism with hypertension [7,10]. Such an association was demonstrated in two recent reports in a population of young men [11,12].
The ACE I/D polymorphism was also shown to have prognostic value in various cardiovascular disorders [13,14]. Enhanced progression in renal disease could also be linked to DD genotype, as shown for IgA nephropathy and focal segmental glomerulosclerosis [15,16]. Therefore the ACE I/D genotype may also explain some of the variation in progression seen in ADPKD patients. We performed a case-control study, comparing the differences in frequencies of the ACE I/D genotype between PKD1 patients who reached ESRF above the age of 60 years of age and ADPKD patients who reached ESRF under 40 years of age. These groups of rapid and slow progressors were also compared with a control, general PKD1 population.

Subjects and methods

Patients

After the protocol was approved by the Medical Ethics Review Committee of the LUMC, nephrologists of 47 (small) dialysis centres in our country were asked for their co-operation. We received a positive answer from 37 centres, and with their consent we asked RENINE (a central database for patients on dialysis in the Netherlands) to identify patients eligible for this study. Patients with the diagnosis of PKD who became dialysis dependent under 40 years of age and patients who became dialysis dependent above 60 years of age were identified to the nephrologist of the participating centres. The participating centres were applied with questionnaires and material necessary for blood sampling. The forms contained questions about age at ESRF, the existence of hypertension before ESRF (defined as diastolic blood pressure \(>95\) mmHg and systolic blood pressure \(>165\) mmHg, or treatment with antihypertensive medication), haematuria, urinary-tract infections, kidney stones, cerebrovascular accidents, pregnancies, nephrectomies, important other complications before reaching ESRF, and family history. Blood was collected in heparinized tubes and sent to our hospital for DNA isolation and determination of the ACE genotype.

Collected data

Blood samples were received from 49 patients who had reached ESRF \(<40\) years (rapid progressors), and from 21 patients who had reached ESRF \(>60\) years (slow progressors). We were unable to retrieve data from five patients (three rapid progressors and two slow progressors), whereas DNA isolation was impossible in three rapid progressors. In both groups, only two patients were from the same family. Patients were diagnosed as having ADPKD by the presence of cysts in both kidneys and a positive family history for ADPKD. In all patients with a negative family history for ADPKD, ultrasound investigation showed enlarged kidneys with numerous cysts throughout the kidneys and liver.

We presumed that the group of rapid progressors consisted of PKD1 patients. Because PKD2 patients are known to be slow progressors, we needed to ensure that all patients in the group of slow progressors were PKD1 patients. We were able to link all the slow progressors to the PKD1 gene.

To collect a general PKD1 control population, we randomly took samples from our collection of genetically identified PKD1 families and determined the ACE genotype.

Families who had members included in our study population were excluded.

ACE I/D genotyping

DNA was isolated from peripheral blood leukocytes, using standard techniques [17]. The ACE gene I/D polymorphism was detected by performing the polymerase chain reaction (PCR) as described by Rigat et al. [18]. The PCR product is a 190-bp fragment in the absence of the insertion and a 490 bp fragment in the presence of the insertion. To prevent mistyping of ACE heterozygotes we used a specific primer for the insertion [19], whenever a DD genotype was found. In case of an I-allele, a fragment of 408 bp was present, in case of a true DD homozygote no such band was present. In both cases the PCR products were visualized after electrophoresis in 2% agarose gels.

Statistical analysis

Chi square tests according to Pearson were performed to compare the frequency of ACE genotype between the groups. A \(P\) value \(<0.05\) was considered significant.

Results

Clinical characteristics of rapid and slow progressors

Although more women were present, the gender distribution was not significantly different between the two groups (Table 1). In the rapid progressors hypertension was present in 80% before the age of 40, when all had reached ESRF, whereas in the slow progressors only 21% had hypertension before they reached the same age (Table 1). This difference was significant (\(P<0.001\)).

Four patients with rapid progression had major complications that might have had a deleterious effect on the disease progression. Two patients were on non-steroidal anti-inflammatory drugs, one because of gout and the other because of Bechterew’s disease. Another patient had undergone a nephrectomy at the age of 6 for unknown reasons. In one patient pyocystis occurred for which the kidney was removed, followed by hypotensive periods after which dialysis became necessary. Exclusion of these patients from our data set had no influence on the results. Other complications were nephrolithiasis in one patient and an intracranial haem-

<table>
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<th>Table 1. Clinical characteristics of ADPKD patients who reached ESRF (&lt;40) years and PKD1 patients who reached ESRF (&gt;60) years</th>
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<td>ESRF (&lt;40) years</td>
</tr>
<tr>
<td>--------------------</td>
</tr>
<tr>
<td>Number</td>
</tr>
<tr>
<td>Male/female</td>
</tr>
<tr>
<td>Hypertension*</td>
</tr>
</tbody>
</table>

*Hypertension before reaching ESRF in the \(<40\) group and hypertension before the age of 40 years in the \(>60\) group. Percentages are shown in parentheses.
Hypertension in Children: An Overview

Andres Pinto, D.M.D.; Rosie Roldan, D.M.D., M.D.; Thomas P. Sollecito, D.M.D.

Abstract: Hypertension in children is an increasing concern for health care professionals. Updated guidelines for the treatment of hypertension in children and adolescents were published in 2004. This report reviews the epidemiology and management of pediatric hypertension and suggests an oral health protocol to apply to hypertensive children in the dental setting. A web search was performed using Medline, PubMed, ISI Citation Index, and Cochrane evidence-based databases for articles regarding hypertension in children published in English between 1998 and 2004. Relevant articles describing the epidemiology, classification, pathophysiology, and management of pediatric hypertension are discussed, and recommendations for dental treatment of pediatric patients are suggested. The incidence of pediatric hypertension can reach 5 percent. Data on the prevalence of pediatric hypertension in the dental setting is scarce. However, using the prevalence in the general population, at least fifty young patients will be hypertensive in a busy general or pediatric practice. Dental students and residents should have the opportunity to screen for hypertension during their training and familiarize themselves with the appropriate techniques in children. Oral health professionals should become aware of the implications of hypertension in children.

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Key words: pediatrics, hypertension, oral health, children

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Hypertension is defined as a blood pressure reading that exceeds a threshold that separates individuals at risk for adverse outcomes from those with no increased risk.1,2 A sustained elevation in blood pressure increases the risk of an adverse outcome, such as stroke and myocardial events.3-5 Many efforts have focused on the primary prevention and control of hypertension in adults.3 Nevertheless, the increasing incidence of hypertension in younger age groups has drawn attention to the severity and complications of the disease in children and adolescents.6,7 Public health implications of hypertension in children are overwhelming because many of these individuals will eventually face medical sequelae into adulthood.3,9 Management and screening of pediatric patients with elevated blood pressure should form part of the dental school curriculum because the incidence of the disease is climbing. This article provides an overview of pediatric hypertension and offers suggestions for oral health management of hypertensive pediatric patients.

Epidemiology and Classification

The National Health and Examination Survey (NHANES III) reported an average rise of 1.4 mmHg in systolic and 3.3 mmHg in diastolic measurement in children between the years of 1988 to 1994 and 1999 to 2000.6-14 This seemingly innocent variation in systolic blood pressure will affect the epidemiology of systemic disease in young adults within a decade.11-13 Evidence of ethnic disparities in the prevalence of hypertension in children follows the proven disparity in the presence of cardiovascular disease among ethnic groups.13-17 Although the prevalence of pediatric hypertension in the United States has been calculated to be between 1 and 5 percent,3,6 this number is expected to increase due to the close association between hypertension and obesity.3,18 Obesity has become an epidemic in children, reaching almost 16 percent in recent years.17,18 A direct relationship between weight status and systolic blood pressure has also been reported.18 Slight elevations in pressure (1 to 2 mm Hg) in childhood will elevate the risk of developing hypertension as an adult by 10 percent.6,17

The updated classification of hypertension in children was published in August 2004 by the National Blood Pressure Education Program, Working Group on Children and Adolescents. Hypertension in children is defined as systolic and/or diastolic based on repeated measurements (more than three occasions) above the 95th percentile for age, sex, and height (Table 1). Staging of hypertension in pediatrics follows the percentile classification.14 Measurement-
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High frequency of the D allele of the angiotensin-converting enzyme gene in Arabic populations
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* Corresponding author

Abstract
Background: The angiotensin-converting enzyme (ACE) gene in humans has an insertion-deletion (I/D) polymorphic state in intron 16 on chromosome 17q23. This polymorphism has been widely investigated in different populations due to its association with the renin-angiotensin system. However, similar studies for Arab populations are limited. This study addresses the distribution of the ACE gene polymorphism in three Arab populations (Egyptians, Jordanians and Syrians).

Findings: The polymorphisms of ACE gene were investigated using polymerase chain reaction for detection of an I/D mutation. The results showed a high frequency of the ACE D allele among the three Arab populations, Egyptians (0.67), Jordanians (0.66) and Syrians (0.60), which is similar to those obtained from previous studies for Arab populations.

Conclusion: The relationship between ACE alleles and disease in these three Arab populations is still not known, but the present results clearly suggest that geographic origin should be carefully considered in the increasing number of studies on the association between ACE alleles and disease etiology. This study adds to the data showing the wide variation in the distribution of the ACE alleles in different populations and highlights that great care needs to be taken when interpreting clinical data on the association of the ACE alleles with different diseases.

Background
Angiotensin-converting enzyme (ACE), a key enzyme of the rennin-angiotensin system, is localized in the kidney [1]. The ACE catalyzes the conversion of angiotensin I to the biologically active peptide, angiotensin II, which is involved in the control of fluid-electrolyte balance and systemic blood pressure [2]. The ACE gene is mapped to chromosome 17q23 and it has been widely investigated. The insertion/deletion (I/D) polymorphism of ACE was discovered by Rigat et al. [3] and it is characterized by the presence (insertion) or absence (deletion) of a 287 bp AluYa5 element inside intron 16 producing three genotypes (II homozygote, ID heterozygote and DD homozygote) [3]. Although the I/D polymorphism is located in a non-coding region (i.e. intron) of the ACE gene, several investigators have found that the D allele is related to increased activity of ACE in serum [3,4]. The highest serum ACE activity was seen in the DD genotype while the lowest was seen in the II genotype [3]. Several investigations suggested the genetic predisposition of the ACE I/D polymorphism with several diseases including coronary heart diseases [5], stroke [6], hypertension [7] and diabe-
tes mellitus [8]. However, conflicting results have been reported regarding the association between ACE polymorphism and disease [9,10]. Moreover, various reports were published suggesting inter-ethnic variations in the frequency of allelic forms of the ACE genes [11,12].

In this study we aim to investigate the distribution of ACE gene I/D polymorphism in three Arab populations (Egyptians, Jordanians and Syrians). The three Arab populations have a mixed genetic background with an ethnic heterogeneity. Most of the three populations are of Mediterranean or Arabic origin that migrated from the Arabian Peninsula and surrounding areas.

Method

The human population samples used for this study have been described previously and were available from previous studies [13]. The samples studied were collected from unrelated individuals from three Arab populations: (Egyptians, Jordanians and Syrians) under institutionally approved internal review board protocols with informed consent. DNA was prepared from blood leukocytes by standard methods. A total of 164 Egyptians from Ismailia, and Sinai, 60 Jordanians and 70 Syrians were analyzed. The Egyptian samples were from Ismailia (112 subjects), and the Sinai (52 subjects). The specific segment of ACE gene was amplified by polymerase chain reaction (PCR) using the following primers [14]: ACE-F (5-CTGGAGAC-CACTCCCATCCTTTCT-3) and ACE-R (5-GATGT-GGCCATCACATTGCAGAT-3). PCR amplification was carried out in 25 μl reactions containing 20–100 ng of template DNA, 40 pM of each oligonucleotide primers, 200 μM dNTPs, 50 mM KCl, 1.5 mM MgCl2, 10 mM Tris-HCl (pH 8.4) and Taq DNA polymerase (1.25 Units). The reaction were subjected to 32 cycles: an initial denaturation of 60 s at 94°C, 30 s denaturation at 94°C, 45 s at the annealing temperature 58°C, extension at 72°C for 45 s. Following the amplification cycles, a final extension was performed at 72°C for 10 min. For analysis, 20 μl of each sample was fractionated on a 2% agarose gel with 0.05 μg/ml ethidium bromide. PCR products were directly visualized using UV fluorescence. The homozygous individuals for the D allele (DD genotype) were identified by the presence of a single 190 bp PCR product. The homozygous for the I allele (II genotype) were identified by the presence of a single 490 bp PCR product. The heterozygous individuals (ID genotype) were identified by the presence of both 190 and 490 bp PCR products. Because the D allele in heterozygous samples is preferentially amplified, all samples that were typed initially as a DD genotype were reanalyzed using an insertion-specific primer pair, as reported by Lindpaintner et al. [15], except that the annealing temperature was 67°C. A 335 bp band was obtained only in the presence of the I allele and no bands were detected for samples with DD genotype.

Statistical analysis was performed using SPSS version 15 statistical package for windows. Allele and genotype frequencies were calculated by direct counting; the Hardy-Weinberg equilibrium was assessed by an exact test provided by the Arlequin program [16].

Results and discussion

As shown in Table 1, 16 individuals from Egypt living in Ismailia were homozygous for the II genotype, 40 were heterozygous for the ID genotype and 56 were homozygous for the DD genotype, giving a D allelic frequency of 0.679. Among 52 Egyptians living in Sinai that

<table>
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<th>Population</th>
<th>N</th>
<th>ACE Genotype</th>
<th>Number Observed (and Expected)</th>
<th>I</th>
<th>D</th>
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<th>Expected</th>
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Angiotensin-converting enzyme gene polymorphism in patients with Essential hypertension

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Abstract:

Several genetic investigations have been attempted to elucidate the association of angiotensin-converting enzyme (ACE) gene polymorphism and essential hypertension. This study was conducted to investigate the frequency of ACE gene insertion/deletion (I/D) polymorphism in patients with essential hypertension (EH). The study included one hundred patients with essential hypertension and seventy age and sex matched healthy individuals as a control group. The patients and control group were subjected to routine investigations, assay of serum cholesterol, triglycerides, high density lipoprotein-cholesterol (HDL-C), low density lipoprotein-cholesterol (LDL-C) and assay of ACE gene I/D polymorphism using real-time polymerase chain reaction (PCR). The results of the study showed that the frequency of DD,ID and II genotypes were 42%, 44% and 14 % respectively in hypertensive group and 30%, 50% and 14 % respectively in control group with significantly higher frequency of DD genotype in patients as compared to the control group (p<0.05). There was a significant association between DD genotype and hypertension, as there was significant increase in both systolic and diastolic blood pressure in patients with DD genotype as compared to other genotypes. Serum cholesterol , HDL-C and LDL –C levels showed significant increase in patients as compared to the control group (P <0.001, P <0.001 and P <0.001: respectively ) Also, serum Cholesterol and LDL-C levels showed significant increase in patients with DD and ID genotypes as compared to II genotype , while triglycerides and HDL-C didn’t show differences between the three genotypes. It was concluded that the DD genotype of ACE gene showed significantly higher frequency among patients with essential hypertension as compared to the normal subjects and that DD genotype was associated with significantly higher blood pressure as compared to ID and II genotypes. Also, DD genotype was associated with significantly higher serum cholesterol and LDL-C as compared to II genotype. This polymorphism in the ACE gene may contribute to the pathogenesis and severity of essential hypertension and may help in selection of anti-hypertensive drugs.

Introduction:

Hypertension is a common risk factor for coronary artery and cerebrovascular diseases that are the major causes of morbidity and mortality, accounting for more than 12 million deaths annually worldwild (Caulfield et al., 2002). Essential hypertension is a multifactorial trait involving interactions among genetic, environmental and demographic factors (Kato,2002). Several genetic investigations have been attempted to elucidate
which results in enhanced conversion of angiotensin I to II, which stimulate cholesterol biosynthesis in macrophage (Batalla et al., 2000). A previous study reported the lack of association between ACE gene polymorphism and serum cholesterol, HDL-C, LDL-C and triglycerides in a group of patients with hypertension (Pereira et al., 2002). On the other hand, Kawamoto et al., (2002) found significant association between DD genotype and total cholesterol in a group of patients with hypertension and carotid atherosclerosis.

**Conclusion:**

ACE gene DD genotype showed significantly higher frequency in patients with essential hypertension and was associated with higher blood pressure as compared to the other genotypes ID and II. Also, DD genotype was associated with higher serum cholesterol and LDL-C as compared to II genotype in patients with essential hypertension. This variation in ACE gene may contribute to the development and severity of hypertension and may help in selection of anti-hypertensive drugs.

**References:**

13. Lin M., Tseng C. H., Tseng C.C. Huang C. Chong C. and Tseng
ASSOCIATION BETWEEN ACE GENE INSERTION (I) / DELETION (D) POLYMORPHISM AND PRIMARY HYPERTENSION IN TURKISH PATIENTS OF TRAKYA REGION

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ABSTRACT
Hypertension is immensely common in Turkish subjects in Trakya region. The renin-angiotensin system (RAS) helps maintain blood pressure and salt homeostasis and appears important in the pathogenesis of hypertension. Angiotensin I-converting enzyme (ACE) is a key component of RAS. Insertion/Deletion (I/D) polymorphism of the ACE gene has been implicated in the pathogenesis of cardiovascular diseases. In addition to this, the association between ACE I/D polymorphism and hypertension is controversial, when numerous studies have addressed the role of ACE I/D polymorphism in the development of hypertension, there were different studies showed that no correlation has been found between ACE I/D polymorphism and in the development of hypertension. The objective of our study was to investigate the relation between the ACE gene I/D polymorphism and primary hypertension in Turkish subjects in Trakya region. We analyzed the ACE gene I/D polymorphism in 79 patients with primary hypertension as a primary hypertensive group and 38 age matched healthy individuals as a control group by using a polymerase chain reaction assay, and agarose gel electrophoresis system. The genotype distributions were not different between the patients and normal control groups in the men. But the frequency of ACE Deletion/Deletion (DD) genotype in patients with primary hypertension (35.5%) was significantly higher than in controls (21.4%) in the women. This result suggested that ACE DD genotype may be associated with primary hypertension in the women, not in the men, and showed the possibility of ACE DD genotype as a potent risk factor for primary hypertension for the women not for the men.

Introduction
Primary hypertension is of unknown etiology; its diverse hemodynamic and pathophysiologic derangements are unlikely to result from a single cause (22, 8, 2). Heredity is a predisposing factor (11), but the exact mechanism is unclear. Environmental factors (eg. high salt intake, obesity, stress) seem to act only in genetically susceptible persons (5). The renin-angiotensin system (RAS) may be the most important of the endocrine systems that affect the control of blood pressure (10, 17, 15). This system has been implicated in the pathological changes of organ damage through modulation of gene expression, proliferation, fibrosis, and inflammatory response (19, 9, 18). Angiotensin I-converting enzyme (ACE) is a component of RAS. ACE is a key enzyme in the generation of angiotensin (AT)-II (a potent vasoconstrictor and aldosterone-stimulating peptide) from AT-I (a vasoinactive decapeptide). The ACE gene consists of 26 exons and 25 introns and spans
21 kb on chromosome 17q23 (7, 13). The polymorphism consists of the presence (I allele) or absence (D allele) of a 287 bp Alu repeat sequence resulting in 3 genotypes (DD and II homozygote, and ID heterozygote) (21). An I/D (region in intron 16) polymorphism of the ACE gene correlates with circulating ACE plasma activity (21); higher plasma ACE activity is observed in subjects with ACE-D (16). An increase in plasma ACE activity may increase blood pressure through increased production of angiotensin II. Studies have demonstrated that ACE insertion (I) / deletion (D) polymorphism is not only associated with diabetes (4, 6), coronary heart disease (23), and diabetic nephropathy (24), but have also demonstrated that ACE I/D polymorphism is associated with hypertension (3, 12). In primary hypertension no early pathologic changes occur. Ultimately, generalized arteriolar sclerosis develops; it’s particularly apparent in the kidney (nephrosclerosis) and is characterized by medial hypertrophy and hyalinization. Nephrosclerosis is the hallmark of primary hypertension, left ventricular hypertrophy and, eventually, dilation develops gradually. This study was designed to investigate whether ACE I/D polymorphism is associated with primary hypertension in Turkish subjects in Trakya region.

Materials and Methods

Materials
All reagents for PCR Amplification and Gel Electrophoresis were purchased from Fermentas Life Sciences (ELIPS), Istanbul, Turkey. All other chemicals were bought from Sigma or Merck, Darmstadt, Germany, and were of the highest purity available.

Methods

Patients
Approval for the study was obtained from the Ethics Committee of Trakya University School of Medicine. The study included 117 Turkish individuals from Trakya region. Untreated 79 patients with mild to moderate primary hypertension (48 males and 31 females); mean age 43.52±8.06 years, and 38 age-matched healthy individuals as a control group (24 males and 14 females); mean age 38.40±6.53 years. Patients who were found to have renal disease, secondary hypertension or already on anti-hypertensive treatment were excluded from the study.

Blood Pressure
Blood pressure was measured in the morning follow 12 hours fasting by using manual sphingomanometre after resting for 5 minutes in seated position.

Presence of hypertension was defined as systolic blood pressure (SBP) ≥ 140 mm Hg and/or diastolic blood pressure (DBP) ≥ 90 mm Hg.

DNA Extraction
DNA was extracted from whole blood containing ethylenediamine-tetracetic acid (EDTA) as an anticoagulant by a standard salting-out procedure, followed by phenol/chloroform extraction and resuspended in a TE (10 mmol/L Tris / 1 mmol/L EDTA pH 7.6) buffer (1). DNA purity and quantity were assessed by absorbance values in spectrophotometre and checked by 0.5% agarose gel electrophoresis.

Determination of genotypes

Amplification of Genomic DNA by Polymerase Chain reaction (PCR)
To determine the ACE genotype of the patients and control groups, a genomic DNA fragments on intron 16 of the ACE gene was amplified by PCR in a 50 μl PCR reaction mixture containing 200 ng of DNA, deoxynucleotide triphosphates (0.2 mM of each), upstream and downstream oligonucleotide primers (20 pmol), 75 mM Tris-HCl (Ph 8.8), 20 mM (NH₄)₂SO₄, 0.01% Tween 20, and 2.5 U of Taq DNA polymerase (Fermentas Life Sciences). The PCR primers with the sequences reported by Rigat B et al. were used (20): ACE-1 24-Mer MBI (5’-CTGGAGACCACTCCCATCCTTTCT-3’) and ACE-2 25-Mer MBI...
The reaction products were electrophorized on 2% agarose gels and stained with ethidium bromide. Under ultraviolet light two bands, insertion (I; 490 bp) and deletion (D; 190 bp) were visible (Figure). The 335 bp fragment was identified on 3% agarose gels and stained with ethidium bromide. The reaction yields no product in the sample of DD genotype.

Statistical Analysis

Chi square tests according to Pearson were performed to compare the frequency of ACE genotype between the groups. A $P$ value <0.05 was considered significant.

Results and Discussion

At baseline the two groups were similar with regard to sex, age, and body mass index (Table 1). Primary hypertension patients had higher systolic and diastolic blood pressures.

Group 1, normal controls; group 2, patients group. Data are expressed as means ± SEM. BMI, body mass index; SBP, systolic blood pressure; DBP, diastolic blood pressure.

Table 2 shows the ACE genotype distributions of normal controls and patients with primary hypertension.

Several studies have shown a high prevalence of the DD genotype among patients with primary hypertension (6). On the other hand, researches have shown no differences in the allele frequencies and genotype distributions of ACE gene polymorphisms between the control and hypertension group (14). In our study, the overall frequencies of the genotypes II, ID, and DD were 31.9, 36.2, and 31.9, respectively in men, and 17.8, 51.1, and 31.1 respectively in women. The individual allele frequencies for I and D were 50.0% for each in men, while in women it was 43.3, 56.7, and 1 min at 74°C (extension). Only the (I) allele produces a 335 bp amplicon.

Detection of ACE Polymorphism by Electrophoresis

The reaction products were electrophorized on 2% agarose gels and stained with ethidium bromide. Under ultraviolet light two bands, insertion (I; 490 bp) and deletion (D; 190 bp) were visible (Figure). The 335 bp fragment was identified on 3% agarose gels and stained with ethidium bromide. The reaction yields no product in the sample of DD genotype.

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TABLE 1
Clinical characteristics of the control and primary hypertensive patients

<table>
<thead>
<tr>
<th></th>
<th>Group 1 (n=38)</th>
<th>Group 2 (n=80)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sex (M:F)</td>
<td>24:14</td>
<td>48:31</td>
</tr>
<tr>
<td>Age (years)</td>
<td>38.40±6.53</td>
<td>43.52±8.06</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>26.81±3.59</td>
<td>28.78±3.37</td>
</tr>
<tr>
<td>SBP (mmHg)</td>
<td>117.11±9.98</td>
<td>155.59±11.31</td>
</tr>
<tr>
<td>DBP (mmHg)</td>
<td>74.63±7.00</td>
<td>98.25±6.47</td>
</tr>
</tbody>
</table>

TABLE 2
Distributions of ACE genotype and allele frequencies in the controls and primary hypertension patients subjects

<table>
<thead>
<tr>
<th>Variable</th>
<th>Normal control (n=38)</th>
<th>Primary hypertensive (n=79)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Genotypes</td>
<td></td>
<td></td>
</tr>
<tr>
<td>II</td>
<td>12 (31.6)</td>
<td>19 (24.1)</td>
</tr>
<tr>
<td>ID</td>
<td>15 (39.5)</td>
<td>34 (43.0)</td>
</tr>
<tr>
<td>DD</td>
<td>11 (28.9)</td>
<td>26 (32.9)</td>
</tr>
<tr>
<td>Alleles</td>
<td></td>
<td></td>
</tr>
<tr>
<td>I</td>
<td>39 (51.3)</td>
<td>72 (45.6)</td>
</tr>
<tr>
<td>D</td>
<td>37 (48.7)</td>
<td>86 (54.4)</td>
</tr>
</tbody>
</table>

Percentages are shown in parentheses.

respectively, and 47.4%, 52.6% respectively in the entire sample. The observed genotype frequencies are in agreement with frequencies predicted by Hardy-Weinberg equilibrium.

The frequencies of the genotypes II, DI, and DD were 29.2, 39.6 and 31.2 respectively in male patient group, whereas the frequencies of the genotypes II, DI, and DD were 37.5, 29.2 and 33.3 respectively in male control group. Statistical analyses have shown no statistically significant difference between the frequencies of the genotype of the two groups.

The frequencies of the genotypes II, DI, and DD were 16.1, 48.4 and 35.5 respectively in female patient group, whereas the frequencies of the genotypes II, DI, and DD were 21.4, 57.2 and 21.4 respectively in female control group. Analyses of this data have shown that the frequencies of the DD genotype in female patient group were statistically significantly higher than that in female control group.

This study have verified a relationship between the ACE DD genotype and the development of primary hypertension in female population of Trakya, but this relationship was not reflected to be true for the male population.

REFERENCES
Association between the Angiotensin-converting Enzyme Gene Insertion/Deletion Polymorphism and Essential Hypertension in Young Pakistani Patients

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Several studies have demonstrated the importance of angiotensin-converting enzyme (ACE) insertion (I)/deletion (D) polymorphisms in the pathogenesis of hypertension. This study sought to determine the association between the ACE I/D polymorphism and essential hypertension in young Pakistanis. The frequency of the ACE I/D polymorphism was established by a comparative cross-sectional survey of Pakistani patients suffering from essential hypertension and ethnically matched normotensive controls. Samples were collected from tertiary care hospitals in northern Pakistan. Hypertensive individuals were defined as those with a systolic blood pressure > 140 mmHg and/or diastolic blood pressure > 90 mmHg on three separate occasions, or those currently receiving one, or more, anti-hypertensive agents. DNA samples obtained from hypertensive (n = 211) and normotensive (n = 108) individuals were typed by PCR. The frequency of the ACE I/I genotype was significantly higher in hypertensive patients, aged 20-40 years, than in normotensive controls of the same age group ($\chi^2 = 4.0$, $P = 0.041$). Whereas no overall significant differences were observed between the I/I, I/D and D/D ACE genotypes (One way ANOVA, $F = 0.672$; $P = 0.413$). The association between the ACE I/I genotype and essential hypertension in individuals aged < 40 years suggests that ACE has a role in early onset essential hypertension in Pakistan.

Keywords: ACE I/D polymorphism, Angiotensin-converting enzyme, Cross-sectional survey, Essential hypertension, Pakistani population

Introduction

Cardiovascular diseases are fast emerging as a major health burden for developing economies like Pakistan (Nistar, 2002). Hypertension is a major modifiable risk factor of morbidity and mortality from cardiovascular causes. It is a multifactorial and polygenic disorder in which the interaction between several candidate genes and environmental factors play a role. The rennin angiotensin system is an important regulatory mechanism for maintaining normal blood pressure and fluid and electrolyte balance, and angiotensin-converting enzyme (ACE) is a key enzyme in this system, which catalyzes the conversion of angiotensin I to angiotensin II, a potent vasopressor (Erdos, and Skidgel, 1987).

ACE plasma level variability has been reported to be associated with the insertion (I)/deletion (D) polymorphism of an Alu repeat sequences in intron 16 of the ACE gene (Rigat et al., 1990). Various studies have shown association between this polymorphism and several cardiovascular diseases like myocardial infarction (Ludwig et al., 1995), cardiomyopathy (Raynolds et al., 1993) and hypertension (Duru et al., 1994; Barley et al., 1996; Jeng et al., 1997). Moreover, studies have been carried out on the association between the ACE I/D polymorphism and hypertension in various populations, and both positive (Duru et al., 1994; Barley et al., 1996; Jeng et al., 1997) and negative (Higashimori et al., 1993; Vassilikioti et al., 1996; Chiang et al., 1997) associations have been reported. It has been postulated that the association between the ACE I/D polymorphism and hypertension might be related to gender and ethnicity (Barley et al., 1996; Sagnella et al., 1999).

However, to date no study of this type has been conducted in Pakistan. The present study was initiated to determine whether the presence or absence of the ACE I-allele polymorphism is associated with essential hypertension in the Pakistani population.
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