ACE Gene DD Genotype Association with Obesity in Pakistani Population

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Abstract: *The renin-angiotensin system (RAS) has an established role in pathogenesis of metabolic etiologies. Angiotensin converting enzyme (ACE) is an important component of RAS that may influence metabolic outcomes in adipose tissue. The deletion "D allele", of ACE gene I/D (insertion/deletion) polymorphism has been shown to be associated with rise in the serum level of ACE. This study is designed to correlate the association between ACE gene I/D polymorphism and obesity in adult population of Pakistan. Our study included 535 individuals; 147 normal with body mass index (BMI) 19-24.9, 183 overweight (BMI 26-29.9) and 205 obese (BMI > 30). The individuals were genotyped for ACE gene I/D polymorphism. The ratio of ACE gene II and ID genotypes were not significantly different among normal, overweight and obese individuals. However, the DD genotype in normal, overweight and obese individuals was 12.9%, 18.0% and 28.8% respectively. DD genotype is significantly high (P = 0.002) in obese than in overweight and normal individuals. Thus the results of this study may suggest a possible association of the D allele in adipogenesis and adipocyte metabolism by affecting the ACE plasma level.*

Keywords: *Renin-angiotensin system, Angiotensin converting enzyme, Insertion/deletion polymorphism, Body mass index.*

Introduction

Obesity has become a global problem with high rise in south Asian countries including Pakistan, where drifty transitions in life style have accelerated gene-environment imbalances. Many genes have been reported to have a complicated effect on obesity [21]. The components of rennin-angiotensin system (RAS) are expressed in metabolically active adipose tissue, skeletal muscle and pancreas [10] and may represent an important link between obesity and its complications.

An important molecule of RAS is Angiotensin II which regulates blood pressure and salt and water balance. Previous studies show that Ang II has an adipogenic affect on adipocyte growth and development through interacting with its receptor [7, 11, 19]. Ang II precursor, angiotensinogen, is expressed and nutritionally and hormonally regulated in adipose tissue

[8]. Furthermore, it has been observed that treating cultured adipocytes with Ang II increases triglyceride storage and the activities of lipogenic enzymes [9].

Angiotensinogen (AGT), the precursor of angiotensin, is synthesized primarily by the liver and is secreted into the circulation, where it is cleaved by renin to the decapeptide angiotensin I. Angiotensin I is subsequently converted to the octapeptide angiotensin II by angiotensin-converting enzyme (ACE) [15]. Though there are many different types of polymorphisms in the ACE gene but the 287 bp insertion/deletion (I/D) polymorphism in intron 16 of ACE gene is an important precursor for non communicable diseases (NCDs) including diabetes, obesity and hypertension. There are three distinguished genotypes of ACE gene I/D polymorphism; I/I, I/D and D/D based on presence or absence of 287bp *alu* repeat. The DD homozygote individuals have a higher concentration of serum Ang II [16]. Angiotensin II functions as a vasoconstrictor and inactivates bradykinin which is a vasodilator [20].

We have previously shown that ACE gene I/I genotype has a significant association ($P < 0.05$) with essential hypertension in young Pakistani patients [12]. In another study we reported the high frequencies of ACE gene I allele in male diabetic nephropathy patients of Pakistan [13]. Here we demonstrate whether or not the ACE gene I/D polymorphism is associated with development of obesity in Pakistani population.

Materials and Methods

Subjects

Our study included 535 individuals out of which 147 were normal (BMI 19-24.9) and 183 were overweight (BMI 26-29.9) and 205 were obese (BMI > 30). 5ml blood samples were collected with informed consents. Comparisons were made among individual with the II, ID, and DD genotypes of ACE gene in obese, overweight and normal individuals.

Methods

DNA was isolated from peripheral blood samples of the subjects using standard organic method [17]. ACE gene I /D polymorphism were investigated by polymerase chain reaction [2]. Amplification was done in a final volume of 15µl containing 20ng genomic DNA, 1X PCR buffer (*Bioline*), 0.45mM $MgCl_2$, 200µM dNTPs (*Promega*), 1µM each forward and reverse primer, 1U Taq DNA polymerase (Institute of Biomedical and genetic engineering, Islamabad, Pakistan). Amplification was done with initial denaturing at 94^oC for 3 min, 35 cycles each consisting of denaturing at 94^oC for 45 sec, annealing at 58^oC for 45 sec, extension at 72^oC for 45 sec and final extension for 10 min at 72^oC. Amplified DNA products were run on 2% (w/v) agarose gel containing 0.5µg/ml ethidium bromide at constant power supply of 200 volts. I allele was resolved as 490bp fragment and D allele as 190bp fragment; size depicted by 100bp ladder (*Promega*).

Statistical analysis

Finally, statistical analysis was done by SPSS for windows, version 10.0 (SPSS Inc., Chicago, USA).

Results

The statistical results showed that the ratio of ACE gene “II genotype” is 0.33, 0.32, and 0.26 in normal, over-weight and obese individuals respectively whereas; the ratio of “ID genotype” is 0.53, 0.49 and 0.44 in normal, over-weight and obese individuals respectively (Table 1). Therefore, these results show that the ratio of II and ID genotypes were not significantly

different among normal, overweight and obese individuals. However the ratio of DD genotype was higher in obese individuals i.e. 0.28 than in normal and overweight individuals i.e. 0.12 and 0.18 respectively (Table 1). This difference of DD genotype was statistically significant with *P* value 0.002 ($P < 0.05$) among obese, overweight and normal individuals (Fig. 1).

Table 1. Distribution of ACE II, DD and ID genotypes in Pakistani individuals

Groups	**II**	**DD**	**ID**	**TOTAL**
Normal (BMI = 19-24.9)	49 (0.33)	19 (0.12)	79 (0.53)	147
Over Weight (BMI = 25-29.9)	60 (0.32)	33 (0.18)	90 (0.49)	183
Obese (BMI ≥ 30)	55 (0.26)	59 (0.28)	91 (0.44)	205

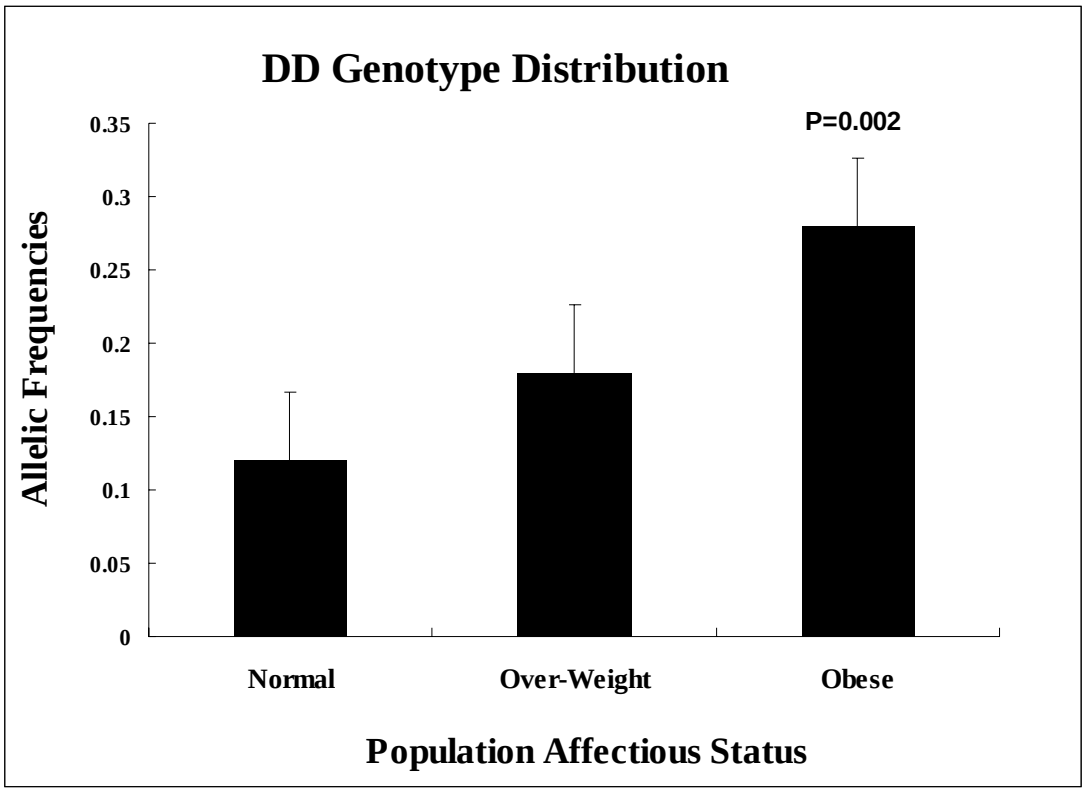

Fig. 1 Comparison of DD genotype distribution observed in normal, over-weight and obese individuals. DD genotype was significantly high in obese individuals compared to normal individuals (*P* = 0.002)

Graphical representation of distribution of ACE II, DD and ID genotypes in Pakistani individuals in the form of a bar graph is shown in Fig. 2. One Way Analysis of variance (ANOVA) was carried out for comparing means by using multiple F Tests. Comparison of

mean ± SEM DD genotype distribution in normal, over- weight and obese individuals is shown in Fig. 2 and it was seen that DD genotype was significantly high ($P < 0.05$) in obese individuals as compared to normal individuals.

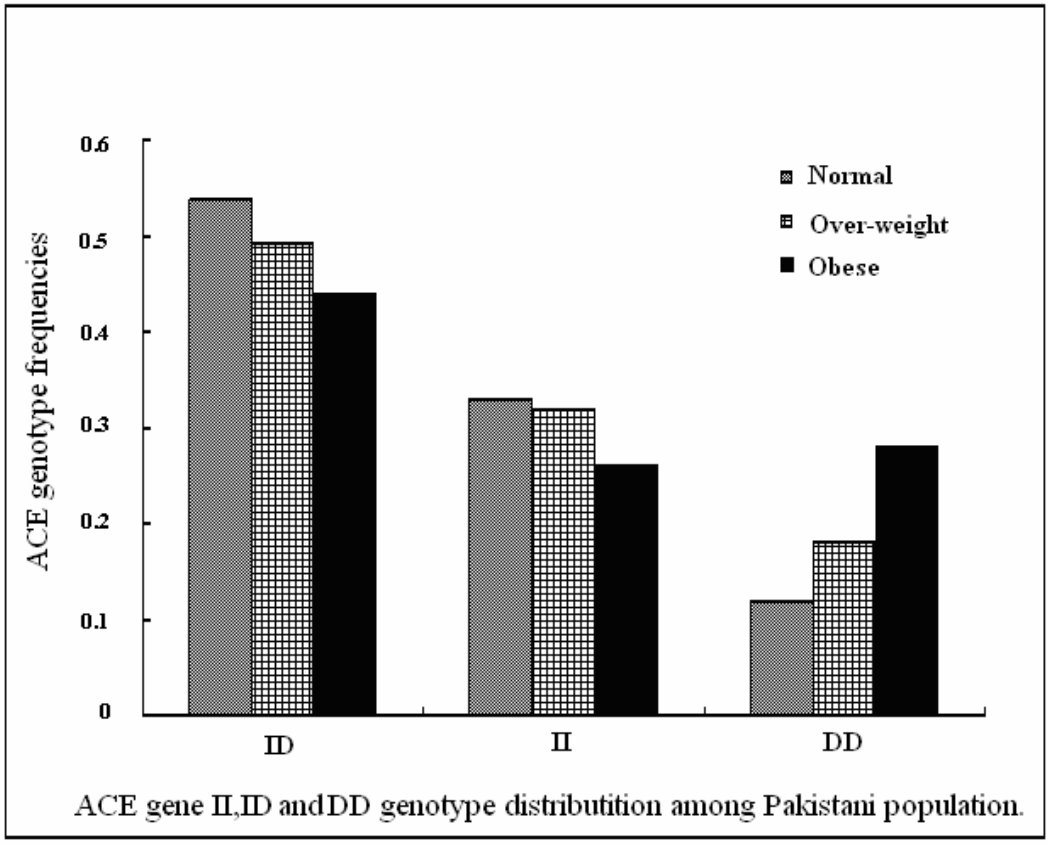

Fig. 2 Distribution of ACE ID, II and DD genotypes in Pakistani individuals

Discussion

In this study we investigated the association of obesity with ACE gene DD genotype in Pakistani population. We collected samples from federal capital of Pakistan where a pool of different ethnic groups of the country live. In our study a significant association ($P < 0.05$) was found between the ACE gene DD genotype and obesity. This correlates with the finding that subjects with the DD genotype have enhanced ACE expression, which results in higher levels of Ang II, resulting in its enhanced modulation and interaction with genes involved in adipogenesis including FAS, apoE and effecting adipocyte lipid metabolism.

A recent study in Czech population showed that a strong significant effect of DD genotype was observed in the phenotypes of extreme obesity with the highest carbohydrate intake where ACE I/D polymorphism could represent a gene modulator of carbohydrate intake in morbidly obese Czech population [22].

Age and gender dependent associations between the ACE gene deletion polymorphism (DD genotype) and hypertension have been demonstrated in the Caucasian and Chinese [1, 3, 5, 18]. It has been observed that a large number of patients with obesity have a higher prevalence of developing hypertension [14]. Since Ang II is classically known to regulate the blood pressure [6] there may be a direct relationship in hypertension and obesity as Ang II is expressed in adipocytes.

The association of the ACE DD genotype with obesity in the Pakistani population is based on a limited number of individuals (n = 535) and a more extensive survey is required to confirm this finding, which may have possible diagnostic and therapeutic implications. Other hunger and satiety signals such as ghrelin and peptide Y are potentially attractive therapeutic strategies for treatment of obesity and its complications. These recent discoveries should lead to novel strategies for treatment of obesity and hypertension.

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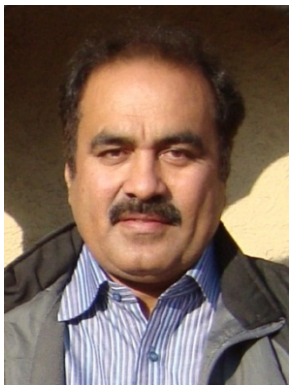

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