

## Correlation between Gene Polymorphism of the Angiotensin-I-converting Enzyme and Type-II diabetes in Egyptians

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**Abstract:** Non-insulin dependent diabetes mellitus is often associated with some complications such as nephropathy, retinopathy and neuropathy. Genes of the renin angiotensin system are potential candidate genes for diabetic complications. This study was conducted to study the association between ACE gene insertion/deletion (I/D) polymorphism and T2DM in Egyptians one hundred twenty four (124) patients with T2DM and (108) one-hundred eight control subjects from different parts of Egypt. Genotyping for the ACE I/D polymorphisms was performed by PCR using specific primers. *P*- Value and odds ratio were used for association studies and to assess the differences in the values among the groups. The distribution of the genotypes in the patients was as follows: 34/124 (27.4%) were homozygous for deletion allele (DD genotype), 77/124 (62.1%) were heterozygous (ID genotype), and 13/124 (10.5%) were homozygous for insertion allele (II genotype). Among the control subjects, 24/108 (22.2%) were homozygous for deletion (DD genotype), 70/108 (64.8%) were heterozygous (ID genotype), and 14/108 (13%) were homozygous for insertion (II genotype). The prevalence of the D-allele in T2DM patients (58.5%) was not significantly different from that in the controls (54.6%). Thus, ACE I/D dimorphism cannot be considered a risk factor for T2DM in the Egyptian population.

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### 1. Introduction

The ACE-(I/D) polymorphism in diabetes mellitus, The variance in plasma ACE levels is explained for 47 % by a polymorphism in the ACE gene. This polymorphism consists of a 287 base pair insertion (I) or deletion (D) of intron 16 of the ACE gene and influences plasma ACE and tissue ACE activity (*Rigat et al., 1990*). It is a common polymorphism, with marked ethnical differences in distribution (*Johanninget al., 1995*). In healthy Caucasian populations the D allele frequency ranges between 0.50 and 0.63 (*Barley et al., 1994*). The D-allele is associated with the highest plasma ACE levels. There is evidence to suggest that renal (*Mizuir et al., 2001*) and vascular tissue ACE also relate to ACE genotype (*Danser et al., 1992*), (*Costerousse et al., 1993*).

Interestingly, patients with diabetic nephropathy carrying the DD genotype showed a more rapid decline in kidney function, compared to patients with the ID and II genotype (*Parving et al., 1996*), also found in non-diabetic kidney disease (*Broekroelofs et al., 1998*).

Many association studies on effects of the ACE genotype on the progression of diabetic nephropathy have been performed. If an association is present, it is always with the DD-genotype and a poor prognosis, but there are also many studies that could not confirm

this. These inter study discrepancies may be accounted for by methodological flaws, such as inhomogeneous patient groups, selection bias by competing risks of the DD-genotype on cardiovascular mortality, lack of adjustment for diabetes duration, glycemic control (HbA1c) or the presence of diabetic complications, and by the weaknesses of association studies in general. One prospective study found an association mainly in patients with poor glycemic control (*Hadjadj et al., 2001*).

The association between the D-allele and an increased propensity to end-organ damage is attributed to an increased AngII formation in subjects with the DD genotype, as supported by studies in non-diabetic healthy subjects. These studies suggest that DD homozygotes have an increased conversion of AngI to AngII. Thus, ACE levels and the ACE gene polymorphism might play a role in the conversion of AngI to AngII. The functional significance of the elevated plasma ACE levels in physiology and pathophysiology, however, is uncertain so far. In a pharmacological setup in normal volunteers, our group (*van der Kleij et al., 2002*) and others (*Ueda et al., 1995*) found evidence that elevated ACE levels in the DD-genotype can have functional consequences, as in the DD genotype infusion of pharmacological doses of AngI leads to an enhanced response of blood pressure and renal function as compared to the II genotype,

consistent with enhanced conversion of AngI. In line with these findings, an attenuated response of renal vascular resistance and plasma flow after captopril administration in healthy volunteers with the DD genotype was reported, suggesting that the ACE genotype might affect the activity of tissue RAAS (*Mizuri et al., 1997*). but data on the impact of the ACE genotype on the renal hemodynamic effects of RAAS blockade are not consistent (*van der Kleij et al., 1997*).

We also found an effect of sodium intake on the impact of the ACE genotype on the antiproteinuric response to ACE inhibition in non-diabetic renal patients, consistent with interaction between sodium status and ACE genotype (*van der Kleij FG. et al., 1997*). Interestingly, the enhanced response of blood pressure and renal hemodynamics to AngI in DD homozygotes that was reported by our group was blunted by a low sodium diet, supporting the possible gene-environment interaction between the ACE (I/D) genotype and sodium intake (*van der Kleij et al., 2002*). Failure to account for the role of sodium intake may partly explain the discrepancies between studies on the impact of ACE genotype on therapy response to RAAS blockade. The enhanced AngI response in DD homozygotes opens the possibility that the elevated ACE levels modulate RAAS-function. In diabetic patients the functional consequences of higher ACE levels in the DD genotype are unknown. This question is even more interesting when one takes in mind that diabetes as such is associated with elevated ACE levels (*Lieberman J. et al., 1980 Van Dyk et al., 1994, Eur J Clin Invest., 1994*).

Several reports mentioned elevated plasma ACE levels in DM compared to healthy controls (*Toop et al., 1986*). Whether this has functional consequences by increased AngII formation is unknown. It has to be noted here that the mechanisms and functional consequences of diabetes-associated increases in plasma ACE levels, may not be similar to those of genetically determined elevations in plasma ACE levels. It has been suggested that high ACE levels in diabetes merely reflect increased shedding of ACE from cell membranes, due to impaired endothelial anchoring of this ecto-enzyme, as a feature of endothelial dysfunction (*Van Dyk et al., Eur Clin Invest., 1994*).

whereas the genetically elevated plasma ACE level in DD genotype reflects elevated tissue ACE as well (*Costerousse et al., 1993*). In this respect, there are reports on a familial increase in plasma ACE-level due to increased endothelial shedding of ACE. In 26 these subjects, plasma ACE levels are up to 5 times the normal levels, whereas no increased cardiovascular morbidity or mortality was observed (*Eyries et al., 2001*).

Studies on functional effects were not performed, but this observation strongly suggests that the functional effects of elevated plasma ACE-levels based on increased ACE expression (i.e. the ACE (I/D) polymorphism, with cellular and circulating ACE increased) might differ from elevations in circulating ACE levels that are due to an increased endothelial ACE shedding.

## 2. Patients and Methods

### 1-Subject

The group of Egyptian subjects which Their ages ranges from (30 – 77)years were invited to participate in this study and divided into 2 groups. This study was approved by the ethic committees of the Faculty of Medicine Kasr Elaini Hospital, Cairo University from April, 2007 through April, 2009 and informed consent was obtained from all subjects after full explanation for the purpose of the study. According to the criteria in each group, Egyptian subjects are divided as followings:

#### Group 1: Control Subjects

One hundred and eight Egyptian healthy subjects (mean age:  $51.9 \pm 0.53$  years, range: 27-77 years) including 93 males (86.1%) and 15 females (13.9%) recruited into this group. A subject with family history of heart disease, history of cardiovascular diabetes, hypertension, renal disease, liver disease, and other metabolic disorders were excluded. Control healthy subjects were collected from out clinic of KasrElaini Hospital, Cairo University.

#### Group 2: Type 2 diabetic Subjects

One hundred and twenty four type 2 DM subjects with plasma glucose level  $>126$  mg/dl and whether with history of hypertension and dislipidemia were recruited into this group. A subject with family history of heart disease, history of cardiovascular disease, renal disease, liver disease, and other metabolic disorders were excluded. The definition of hypertension was a current use of antihypertensive medication or blood pressure of systolic  $\geq 140$  mmHg and/or diastolic  $\geq 90$  mmHg, and dyslipidemia was definition from use of drug to lowering cholesterol and follow diagnosis by physicians. they were (96 males and 28 females) patients with a mean $\pm$ SD age of  $55.4 \pm 8.81$  (range: 30 – 77) years completed the study.

## Identification of Gene Polymorphisms

### Genomic DNA extraction

Blood samples (3 mL) were collected in EDTA tubes from patient and control subjects, and genomic DNA was isolated from whole blood samples using DNA kit (Quiamp® blood kit (QIAGEN, Hilden, Germany). DNA was extracted from 200  $\mu$ L of the whole blood according to the manufacturer's protocol. The isolated DNA was stored at  $-20$  °C.

### Oligonucleotides:

Oligonucleotides primers to amplify an intron 16 of Ace gene was described by *Alvarez et al. (1998)*.

The oligonucleotide primer was synthesized by Sigma GenosysAustralia y.Ltd

Table(1) Oligonucleotides primers

|   |
|---|
| ACE (I/D) polymorphism                                    |
| Forward: 5'-CTG GAG ACC ACT CCC ATC CTT TCT-3'            |
| Reverse: 5'-GAT GTG GCC ATC ACA TTC GTC AGA T-3'          |
| I allele specific primers                                 |
| forward primer (5'-CGG GAT GGT CTC GAT CTC-3')            |
| reverse primer (5'- GAT GTG GCC ATC ACA TTC GTC AGA T-3') |

#### Procedure for ACE genes polymorphism

The ACE (insertion/deletion) polymorphism of ACE gene was amplified by polymerase chain reaction (PCR). The oligonucleotide primers were listed in table (1). The reaction of the ACE polymorphism was performed in final volume of 25  $\mu$ l. Nevertheless, the temperature profile was optimized in this study. The microcentrifuge tube contained 5  $\mu$ l of gDNA, 1 unit Taq DNA polymerase, 1X PCR buffer, 0.1 mM of dNTPs and 10 pmol of each nucleotide primer. The mixture was mixed and covered with a drop of mineral oil. PCR reaction was performed in 0.6 microcentrifuge tube and conducted in an automated Perkin-Elmer Thermal Cycler model 2400. PCR condition of ACE (I/D) polymorphism was modified from previous studied by (*Alvarez et al., 1998*), which performed on an initial cycle of denaturation at 95°C for 1 minute, followed by 30 cycles of denaturation at 94°C for 1 minute, annealing at 63°C for 1 minute, and primer extension at 72°C for 1 minute. Finally, final extension at 72°C for 10 minute to promote completion of partial extension products and complete the annealing of single-stranded complementary products and followed by chilling to 4°C for stopping of the reaction. The previous study reports that ID genotypes amplified as DDs. This led to conclude that amplification of the I allele was sometimes suppressed in an ID heterozygote so that the latter can be mistyped as DD. Because the ACE D allele was preferentially amplified, and about 5-10% were found mistyping in probability. Thus, each DD genotype was confirmed through a second PCR with primers specific for the insertion sequence, *Shanmugam Sell Saha,1993, Chiang et al., 1998 (Keavney et al., 2000)*. The I allele specific forward primer (5'-CGG GAT GGT CTC GAT CTC-3') inside the *Alu* sequence and reverse primer in the primary PCR (5'- GAT GTG GCC ATC ACA TTC GTC AGA T-3') were used for confirmed DD genotype

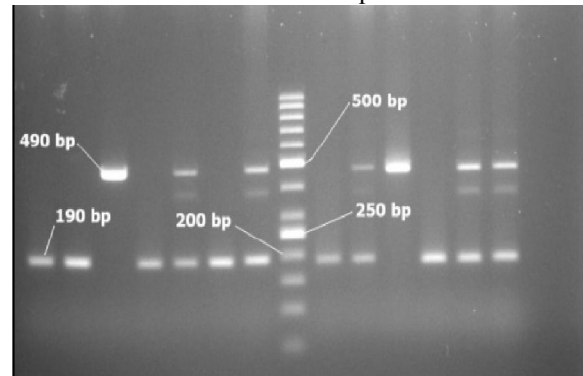
The ACE gene was detected by 3% agarose gel electrophoresis. Five  $\mu$ l of PCR product of ACE gene was mixed with 2  $\mu$ l of loading dye and loaded in each well of agarose gel compared with 50 bp DNA marker. The agarose gel was run for 40 minutes at 100

volts, then the insertion allele size was 490 bp and the deletion allele size was 190 bp. The insertion/ deletion fragments were observed by submerged gel in ethidium bromide solution for 1 minute and visualized on UV - transilluminator. Photograph was taken by Gel Doc EQ system to collected data for analysis.

### 3. Result

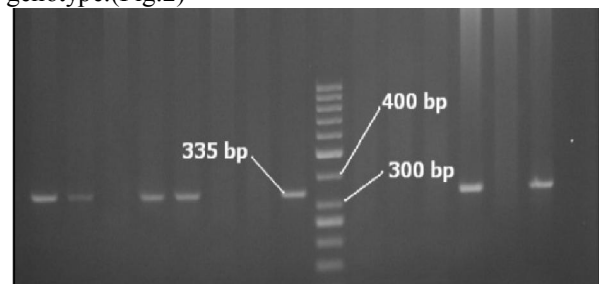
#### Analysis of insertion/deletion polymorphism of ACE gene

All subjects were genotyped by PCR for the insertion/deletion (I/D) polymorphism of the ACE gene. The amplified DNA was detected by 1.5% agarose gel electrophoresis. The presence of a 287 bp fragment (*Alu* sequence) within intron 16 is defined as the Insertion (I) allele and the absence of this fragment is identified as the Deletion (D) allele (Figure 1). The PCR product of insertion allele size was 490 bp and the deletion allele size was 190 bp.



**Figure (1):** ACE Genotyping. agarose gel stained with ethidium bromide and photographed under ultraviolet transillumination. The insertion allele (I) was detected as a 490-bp band, and the deletion allele (D) was detected as a 190-bp band. The result is three genotypes: II, ID, and DD.

In order to avoid misclassification of ID genotypes into DD genotypes, a second PCR was performed using an I-specific primer to confirm DD genotype.(Fig:2)



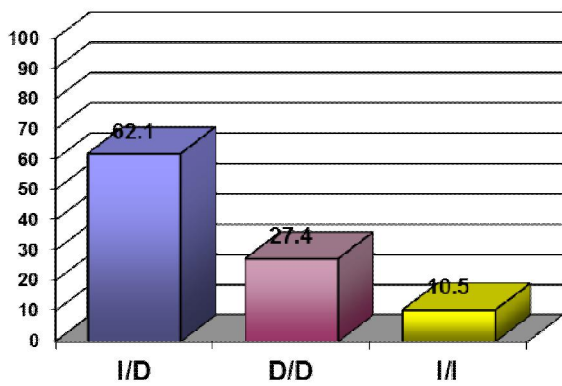
**Fig(2):** ACE Genotyping. agarose gel stained with ethidium bromide and photographed under ultraviolet Transillumination.The insertion allele (I) was detected as a 335 -bp absence of this allele confirm DD genotype.

**IV-1-ACE insertion/deletion polymorphism in the diabetic pts:**

Genotyping the diabetic pts for ACE insertion/deletion polymorphism revealed that 77 pts (62.1%) carry the I/D genotype, whereas only 13 pts carried the I/I genotype (10.5%) and 34 pts carried the DD genotype (27.4%), (Table 2, Figure 3).

**Table (2):** Percentage of genotypes of ACE Insertion/Deletion Polymorphism in diabetic pt

| ACE Genotype | Number | Percentage |
|--------------|--------|------------|
| I/D          | 77     | 62.1%      |
| D/D          | 34     | 27.4%      |
| I/I          | 13     | 10.5%      |



**Figure (3):**Percentage of genotypes of ACE Insertion/Deletion Polymorphism in diabetic pts

**IV-5-ACE insertion/deletion polymorphism in non-diabetic control group:**

Genotyping this group (108) for ACE insertion/deletion polymorphism revealed that 70 (64.8%) carry the I/D genotype, whereas 14 carried the I/I genotype (13.0%) and 24 carried the DD genotype (22.2%),(table 3 fig. 4).

**Table (3):** Percentage of genotypes of ACE Insertion/Deletion Polymorphism in non- diabetic control group

| ACE Genotype | Number | Percentage |
|--------------|--------|------------|
| I/D          | 70     | 64.8%      |
| D/D          | 24     | 22.2%      |
| I/I          | 14     | 13.0%      |

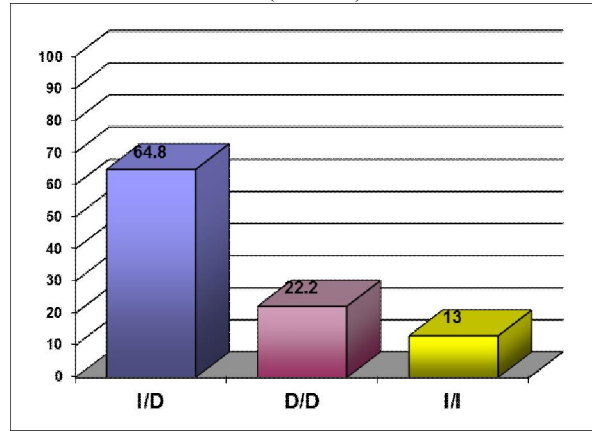
The genotype distribution and allele frequencies of ACE I/D polymorphism in all subjects were presented in table( 4).

Genotype frequencies of insertion/deletion (I/D) polymorphism of ACE gene were analyzed in 108 healthy control and 124 T2DM patients. The distributions of three genotype frequencies of II, ID and DD in healthy control were 13.0%, 64.8% and

22.2%, respectively (Table 3), T2DM: 10.5%, 62.1% and 27.4%, respectively (table 2). Statistical analysis indicated that genotype distributions in healthy control and T2DM were not significantly different for I/D polymorphism among the two groups (Table 4).

Allelic frequencies of ACE I/D gene were calculated and displayed in table (4). By using the gene counting method, estimated allelic frequencies of I and D allele in ACE gene were determined. The allelic frequencies showed no significantly different among the two groups (Table 4).

The frequency of D allele of T2DM was 58.5% which was higher from 54.6% in healthy control subjects. However, no statistical significant differences were observed between them (Table 4).



**Figure (4):** Percentage of genotypes of ACE Insertion/Deletion Polymorphism in non- diabetic control group

**Table (4):** Study of the genotype frequencies and allelic frequencies of ACE I/D Polymorphism in diabetic and control group

|            | Control (n =108) | DM (n =124)   | p-value |
|------------|------------------|---------------|---------|
| II %       | 14<br>12.96%     | 13<br>10.48%  | 0.878   |
| ID %       | 70<br>64.81%     | 77<br>62.09%  | 0.565   |
| DD %       | 24<br>22.22%     | 34<br>27.42%  | 0.457   |
| Allele I % | 98<br>45.40%     | 103<br>42.50% | 0.445   |
| Allele D % | 118<br>54.60%    | 145<br>58.50% | 0.086   |

**IV-8-Assessment the risk of ACE I/D polymorphism genotype and allele frequencies between healthy control subjects and T2 DM patients**

To study the association of ACE polymorphism on T2DM subjects, the odds ratio was used to evaluate for the risk assessment of ACE I/D polymorphism on

healthy control compared with T2DM patients. If the odds ratio is more than 1, there will be a positive correlation between the ACE I/D genotypes or allele frequencies with T2DM. In contrast, when the odds ratio is less than 1, there is a negative correlation. The odds ratio of ACE I/D polymorphism genotype and allele frequencies between healthy control subjects and T2DM patients in this study were summarized in table (5). The odds ratio of II, ID and DD genotypes were **0.789**, **0.889** and **1.32**, respectively. Furthermore, the odds ratio of D allele was **1.162** when compared with T2DM patients. The results showed that there was no association between this I/D polymorphism and T2DM, but the prevalence of DM intended to increase among individuals with D allele.

**Table (5):**The odds ratio of ACE I/D Polymorphism genotype and allele frequencies between healthy control and T2DM patients

|                 | Control<br>(n =108) | DM<br>(n =124) | Odds ratio   |
|-----------------|---------------------|----------------|--------------|
| <b>II</b>       | <b>14</b>           | <b>13</b>      | <b>0.789</b> |
| <b>%</b>        | <b>12.96%</b>       | <b>10.48%</b>  |              |
| <b>ID</b>       | <b>70</b>           | <b>77</b>      | <b>0.889</b> |
| <b>%</b>        | <b>64.81%</b>       | <b>62.09%</b>  |              |
| <b>DD</b>       | <b>24</b>           | <b>34</b>      | <b>1.32</b>  |
| <b>%</b>        | <b>22.22%</b>       | <b>27.42%</b>  |              |
| <b>Allele I</b> | <b>98</b>           | <b>103</b>     | <b>0.855</b> |
| <b>%</b>        | <b>45.40%</b>       | <b>42.50%</b>  |              |
| <b>Allele D</b> | <b>118</b>          | <b>145</b>     | <b>1.162</b> |
| <b>%</b>        | <b>54.60%</b>       | <b>58.50%</b>  |              |

#### 4. Discussion

Diabetes mellitus is one of the leading causes of death worldwide, account for at least 5.2% of cardiovascular disease in the USA. Hyperglycemia is well established as a risk factor for diabetes microvascular and macrovascular complications, but it has not been consistent and shown to be a CAD risk factor in DM patients. However, among patients with type 2 diabetes, cardiovascular disease (CVD) accounts for 70-80% of mortality, with around 15% of patients dying from stroke. Coronary heart disease (CHD) rates are 2-6 folds higher than in the non-diabetic population. Coronary artery disease (CAD) is a complex genetic disease with many genes involved including environmental influences and important gene-environment interaction. These factors may differ in each race or ethnic group.

RAS is a hormone system that helps regulating blood pressure and fluid and electrolyte balance, and has been extensively studied as an important mediator of atherosclerosis as RAS plays a central role in the pathogenesis of cardiovascular disease. (Yan *et al.*, 2005), found significant differences of distributions of

ACE and AT1R polymorphisms between Chinese She and Han populations even though it was in the same country. Therefore, it is interesting to investigate the association between these genes and CAD in each ethnic group for therapy and prevention in future aspect, especially for diabetes patient who suffer from CAD. Despite remarkable successes in the treatment and prevention of CAD in the past decades, it is still the leading cause of death and premature disability in developed and developing countries. Therefore, the role of genetics in the development and progression of CAD, and the component of a genetic evaluation for CAD, including genetic role assessment, risk factor modification, early detection strategies, and genetic counseling and education should be concerned.

Genetic and environmental factors are important determinants of the pathogenesis and progression of almost all diseases. Variations in genes can alter the function of the constituents within a metabolic pathway, resulting in variable susceptibility to the development and progression of atherosclerosis. Considerable evidence implicates the RAS in vascular biology, and furthermore the ACE I/D polymorphism has a major influence on plasma and tissue ACE levels, which in turn could affect generation of Ang II, the direct effector molecule for the AT1R. RAS system has become increasingly established in many types of cardiovascular disease. Established indications for pharmacologic inhibition of the RAS include hypertension, left ventricular dysfunction, acute myocardial infarction (MI), diabetic nephropathy, and Atherosclerosis (Pfeffer. *et al.*, 2001). Angiotensin converting enzyme and angiotensin II type 1 receptor are the candidate genes that have been associated with CAD or MI. Both genes play an important role in the regulation of blood pressure, fluid balance, and electrolyte homeostasis. The direct relationship between blood pressure and the incidence of CAD events is well accepted. However, many of these associations are controversial with various results in literature. ACE and AT1R gene polymorphisms have been described as genetic factors for cardiovascular disease and diabetes. (Rigat *et al.*, 1990) and (Tiret *et al.*, 1992) originally detected the I/D polymorphism in the ACE gene locus using an ACE cDNA probe that spanned the complete endothelial ACE-mRNA sequence. They reported that marked difference in serum ACE levels of healthy subjects were found among the three genotypes. Subjects with the D allele had higher serum ACE levels than I allele. (Cambien. *et al.*, 1992). also reported the DD genotype, which is associated with higher levels of circulating ACE than the ID and II genotypes, and is significantly more frequent in patients with MI. (Ruiz 1994)., reported that the D allele of the ACE gene is a strong and independent

risk factor for CAD in NIDDM patients. The PCR technique was used to determine the I/D polymorphism of ACE gene and DD genotype was confirmed with I-specific primer to protect mistyping of ID as DD genotype. However, in this study, I-specific primer could not distinguish ID genotype from DD genotype.

In this study, we studied I/D polymorphism of ACE gene in T2DM compared with healthy control subjects. The ACE polymorphisms among 2 groups were not significantly different ( $p=0.457$ ). This study corresponded to the report of (Nitiyanant 1997), which showed I and D allele frequency in diabetic patients similar in healthy subjects ( $p=0.69$ ) and (Chuang et al., 1997).found no difference in genotype frequencies of ACE among normal, hypertensive, NIDDM, and NIDDM with CAD subgroups and (Degirmenci et al., 2005).found the distribution of ACE I/D genotypes and allelic frequencies between T2DM patients was not significant differences from healthy controls. However, (Chiu et al., 1997).reported that possession of the I (rather than the D) allele was associated with insulin resistance. Thomas et al. showed the ACE I allele was significant in each group comprising subjects with type 2 diabetes/ glucose intolerance, and the I allele was associated with higher fasting plasma glucose levels (Thomas et al., 2001). Similarly, (Panahloet al., 1995).reported that individuals with DD genotype were more insulin sensitive than those with the I allele. The explanation may be about Ang II production in which Ang II increase insulin sensitivity in both glucose-tolerant and NIDDM patients, subjects with the I allele have lower plasma ACE levels, which produces the lower Ang II levels associated with insulin resistance, as observed in Chiu et al. study (Chiu et al., 1997). In contrast, (Feng et al., 2002) and (Hsieh et al., 2000) indicated that the frequency of ACE DD genotype was markedly higher in patients with type 2 diabetes. Therefore, in this study we investigated I and D allele between healthy controls compared with T2DM patients. There were no statistically significant difference in both frequencies of genotypes and alleles in the I/D polymorphism between healthy control subjects and T2DM patients. The crude odds ratio of I allele was 0.855 (95% CI: 0.526-1.174), while the D allele was 1.162 (95% CI: 0.852- 1.901). It seem likely that the D allele was intend to associated with diabetes because D allele in T2DM patients had a trend to be higher than in healthy control (58.5% vs 54.6%). However, the result showed no significant different of I and D allele with diabetes. This finding was similar to the report from Japan (Doi et al., 1996). The conventional risk factor, such as BMI showed significant difference between healthy control subjects compared with T2DM patients.

To study the influence of I/D polymorphism as the risk factor for CAD in T2DM patients. Many reports showed the positive results of D allele associated with CAD (Cambien et al., 1992, Ruiz et al., 1994, Alvarez et al., 1998, Oren et al., 1999, Fatini et al., 2000, Sekuri et al., 2005, Berdeli et al., 2005). Ruiz et al., 1994 indicated that the D allele is a strong and independent risk factor for CAD in NIDDM patients.

Furthermore, the crude odds ratio of D allele was 0.927 (95% CI: 0.622-1.380) showed no significant association of D allele to the CAD in T2DM patients. Similar to the result of (So et al., 2006).who found the ACE DD polymorphism was not independent predictor for cardiovascular end point. From this study, we concluded that D allele of ACE gene was not associated with CAD in T2DM patients rather than the other conventional risk factors, such as, BMI, age, and smoking which were found to be independent predictors for the prevalence of CAD in T2DM patients.

To study the influence of I/D polymorphism on hypertension, previous reports (Oren et al., 1999, Higaki et al., 2000, Yoo 2005). suggested that DD genotype or D allele associated with hypertension. Recent study from (Suehiro et al., 2004).found that D allele had a higher expression of the ACE mRNA and may affect the RAS in local regions, suggesting that the ACE DD genotype plays a role in the pathogenesis of essential hypertension. However, existing data about the association of ACE I/D polymorphism with blood pressure was conflicting, mainly due to racial difference and environmental exposure status. In this study, we found that among the 124 T2DM 82 T2DM with hypertension and 56 patients with dislipidemia, showed no significant differences between hypertensive and normotensive among these patients. In other words, DD genotype in T2DM and T2DM with complications showed insignificant differences between hypertensive and normotensive patients in each groups. This study found the contradictory result concerning the association between ACE I/D polymorphism and hypertension from (Yoo 2005 ). (Higaki et al., 2000).and (Oren et al., 1999).but our study was in concordance with the reports of (Napolset al., 2007). (Mondorf et al., 1998). (Chuang et al., 1997) who found ACE I/D polymorphism was not significant association with essential hypertension. However, (Mondorf et al., 1998).found the highest ACE levels in the DD genotype and lowest levels in the II genotype. Although it was unlikely that the ACE deletion determines ACE plasma levels, their report suggested that the presence of a regulatory site, which is inherited together with I/D genotype in a linked manner. A combined segregation and linkage analysis of French families (Tiret et al., 1992). suggested that

the I/D polymorphism functions as a marker for major gene or genes within or close to the ACE gene because of a variant of ACE gene, in strong linkage disequilibrium with the I/D polymorphism. The concentration ratio of Ang II/ Ang I vary in different organ. Therefore, the ACE concentration may be important for physiologic and pathologic effects, depending on the target organ. The association of I/D polymorphism of the ACE gene with hypertension or NIDDM or CAD is conflicting, as reported from different populations. The relationship between diabetes, hypertension, and CAD is complex. Our data did not support the notions that whether I nor D allele of the ACE gene is a marker for insulin resistance, hypertension and CAD in Thai T2DM patients. By the association studies in our population, we found that I/D polymorphism of the ACE gene did not play a role in the development of NIDDM, hypertension and CAD in T2DM patients. Taken together, multiple factors other than I/D polymorphism of the ACE gene might be more important for the development of hypertension, NIDDM, and CAD in Egyptian population. For interpreting each finding, it is important to consider ethnic differences in allele frequencies, as the D allele is less common in Asian populations than in European Caucasians. As mentioned above, both environmental and genetic factors contribute the risk of CAD, and common polymorphism at several genes have been described as genetic risk factors for MI (*Alvarez. et al., 1998, Gardemann A., 1998*). and CHD or CAD (*Amant et al., 1997, Pastinen et al., 1998, Fatini et al., 2000, Sekuri et al., 2005*). In the meanwhile, other controversial results have been reported in other population which similar to our study. In summary, there was no relationship between the effects of genetic variants of ACE polymorphism on CAD and hypertension in type 2 diabetes patients. This study of ACE polymorphism demonstrate the importance of using a homogeneous population in the selection of the study samples, making possible the identification of more exact distributions of the ACE genotype among Egyptian and other racial populations. The results of the I/D and polymorphism obtained in various populations are not yet conclusive. Further study is needed with large scale population in future. Studies in other racial or ethnic groups will be of great interest.

### Conclusion

The ACE I/D polymorphism were not associated with type 2- diabetes.

It is possible that other genetic loci rather than this proposed to be associated with accelerated atherosclerosis may be important as risk for CAD.

### References

1. Alvarez R, Reguero J, Batalla A, *et al.*(1998): Angiotensin-converting enzyme and angiotensin II receptor 1 polymorphisms: association with early coronary disease. *Cardiovasc Res*.40:375-9.
2. Amant C, Hamon M, Bauters C, *et al.*(1997):The angiotensin II type 1 receptor gene polymorphism is associated with coronary artery vasoconstriction. *JACC*. 29:486-80.
3. Barley J, Blackwood A, Carter ND, Crews DE, Cruickshank JK, Jeffery S *et al.*(1994): Angiotensin converting enzyme insertion/deletion polymorphism: association with ethnic origin. *J Hypertens* 12(8):955- 957.
4. Berdeli A, Sekuri C, Cam FS, *et al.*(2005): Association between the eNOS (Glu298Asp) and the RAS genes polymorphisms and premature coronary artery disease in a Turkish population. *Clin Chim Acta*. 351:87-94.
5. Broekroelofs J, Stegeman CA, Navis G, Tegzess AM, de Zeeuw D, de Jong PE. (1998):Risk factors for long-term renal survival after renal transplantation: a role for angiotensin-converting enzyme (insertion/deletion) polymorphism?*J Am Soc Nephrol* 9(11):2075-2081.
6. Cambien F, Poirier O, Lecerf L, *et al.*(1992): Deletion polymorphism in the gene for angiotensin-converting enzyme is a potent risk factor for myocardial infarction. *Nature* 359:641-4.
7. Chiang F-T, Hsu K-L, Chen W-M, *et al.*( 1998): Determination of angiotensin-converting enzyme gene polymorphisms: Stepdown PCR increase detection of heterozygotes.*Clinical Chemistry*. 44(6):1353-6.
8. Chiu KC, McCarthy JE. (1997):The insertion allele at the angiotensin I-converting enzyme gene locus is associated with insulin resistance. *Metabolism*. 46:395-9.
9. Chuang LM, Chiu KC, Chiang FT, *et al.*(1997):Insertion/deletion polymorphism of the angiotensin I-converting enzyme gene in patients with hypertension, non-insulin-dependent diabetes mellitus, and coronary heart disease in Taiwan. *Metabolism*. 46:1211-4.
10. Costerousse O, Allegrini J, Lopez M, Alhenc-Gelas F. (1993): Angiotensin I-converting enzyme in human circulating mononuclear cells: genetic polymorphism of expression in T-lymphocytes. *Biochem J* 290 ( Pt 1):33-40.
11. Danser AH, Koning MM, Admiraal PJ, Sassen LM, Derkx FH, Verdouw PD *et al.*(1992) :Production of angiotensins I and II at tissue sites in intact pigs. *Am J Physiol* 263(2 Pt 2):H429-H437.

12. Danser AH, Koning MM, Admiraal PJ, Sassen LM, Derkx FH, Verdouw PD *et al.*(1992): Production of angiotensins I and II at tissue sites in intact pigs.*Am J Physiol* 263(2 Pt 2):H429-H437.
13. Degirmenci I, Kebapci N, Basaran A, *et al.* (2005): Frequency of angiotensin-converting enzyme gene polymorphism in Turkish type 2 diabetic patients. *Int J Clin Pract.* 59:1137-42
14. Doi Y, Yoshizumi H, Yoshinari M, *et al.*(1996): Association between a polymorphism in the angiotensin-converting enzyme gene and microvascular complications in Japanese patients with NIDDM. *Diabetologia.* 39:97-102.
15. Eyries M, Michaud A, Deinum J, Agrapart M, Chomilier J, Kramers C *et al.*(2001): Increased shedding of angiotensin-converting enzyme by a mutation identified in the stalk region.*JBiolChem* 276(8): 5525-5532.
16. Fatini C, Abbate R, Pepe G, *et al.*(2000): Searching for a better assessment of the individual coronary risk profile: The role of angiotensin-converting enzyme, angiotensin II type 1 receptor and angiotensinogen gene polymorphisms. *EHJ.* 21:633-8.
17. Feng Y, Niu T, Xu X, *et al.*(2002): Insertion/deletion polymorphism of the ACE gene is associated with type 2 diabetes. *Diabetes.* 51:1986-8.
18. Gardemann A, Nguyen QD, Humme J, *et al.*(1998): Angiotensin II type 1 receptor A1166C gene polymorphism: Absence of an association with the risk of coronary artery disease and myocardial infarction and of a synergistic effect with angiotensin-converting enzyme gene polymorphism on the risk of these diseases.*EHJ.* 19:1657-65.
19. Hadjadj S, Belloum R, Bouhanick B, Gallois Y, Guilloteau G, Chatellier G *et al.*(2001): Prognostic value of angiotensin-I converting enzyme I/D polymorphism for nephropathy in type 1 diabetes mellitus: a prospective study.*J Am SocNephrol* 12(3):541-549.
20. Higaki J, Baba S, Katsuya T, Sato N, Ishikawa K, *et al.*(2000):Deletion allele of angiotensin-converting enzyme gene increases risk of essential hypertension in Japanese men. *Circulation.* 101:2060-5.
21. Hsieh M-C, Lin S-R, Hsieh T-J, *et al.*(2000): Increased frequency of angiotensin-converting enzyme DD genotype in patients with type 2 diabetes in Taiwan.*Nephrol Dial Transplant.* 15:1008-13.
22. Johannig GL, Johnston KE, Tamura T, Goldenberg RL. (1995): Ethnic differences in angiotensin converting enzyme gene polymorphism. *J Hypertens* 13(6):710-711.
23. Keavney B, McKenzie C, Parish S, *et al.*(2000): Large-scale test of hypothesized associations between the angiotensin-converting enzyme insertion/deletion polymorphism and myocardial infarction in about 5000 cases and 6000 controls. *Lancet* 355:434-42.
24. Lieberman J, Sastre A. (1980):Serum angiotensin-converting enzyme: elevations in diabetes mellitus.*Ann Intern Med* 93(6):825-826.
25. Mizuiri S, Hemmi H, Inoue A, Takano M, Kadomatsu S, Tanimoto H *et al.*(1997): Renal hemodynamic changes induced by captopril and angiotensin-converting enzyme gene polymorphism. *Nephron* 75(3):310-314.
26. Mizuiri S, Hemmi H, Kumanomidou H, Iwamoto M, Miyagi M, Sakai K *et al.*(2001): Angiotensin-converting enzyme (ACE) I/D genotype and renal ACE gene expression. *KidneyInt* 60(3):1124-1130.
27. Mondorf UF, Russ A, Wiesemann A, *et al.* (1998): Contribution of angiotensin I converting enzyme gene polymorphism and angiotensinogen gene polymorphism to blood pressure regulation in essentialhypertension.*AJH.* 11:174-83.
28. Nitiyanant W, Ploybutr S, Tunlakit M, *et al.* (1997): Angiotensin converting enzyme gene polymorphism in healthy Thais and patients with non-insulin dependent diabetes mellitus.*J Med Assoc Thai.* 80:747-52.4.
29. Oren I, Brook JG, Gershoni-Baruch R, Kepten I, Tamir A, Linn S, *et al.*(1999): The D allele of the angiotensin-converting enzyme gene contributes towards blood LDL-cholesterol levels and the presence of hypertension. *Atherosclerosis.* 145:267-71.
30. Panahloo A, Andres C, Mohamed-Ali V, *et al.* (1995): The insertion allele of the ACE gene I/D polymorphism: a candidate gene for insulin resistance? *Circulation.* 92:3390-3.
31. Parving HH, Jacobsen P, Tarnow L, Rossing P, Lecerf L, Poirier O *et al.*(1996): Effect of deletion polymorphism of angiotensin converting enzyme gene on progression of diabetic nephropathy during inhibition of angiotensin 313(7057):591-594.
32. Pastinen T, Perola M, Niini P, *et al.*(1998):Array-based multiplex analysis of candidate genes reveals two independent and additive genetic risk factors for myocardial infarction in the Finnish population. *Hum Mol Genet.* 7:1453-62.
33. Pfeffer M. (2001):Enhancing cardiac protection after myocardial infarction: rationale for newer clinical trails of angiotensin receptor blockers. *AHJ*139:S23-8.



34. Rigat B, Hubert C, Alhenc-Gelas F, Cambien F, Corvol P, Soubrier F. (1990): An insertion/deletion polymorphism in the angiotensin I-converting enzyme gene accounting for half the variance of serum enzyme levels. *J Clin Invest* 86(4):1343-1346.
35. Ruiz J, Blanche H, Cohen N, et al.(1994): Insertion/deletion polymorphism of the angiotensin-converting enzyme gene is strongly associated with coronary heart disease in non-insulin-dependent diabetes mellitus. *Proc Natl Acad Sci USA*. 91:3662-5.
36. Sekuri C, Cam FS, Ercan E, et al.(2005):Renin-angiotensin system gene polymorphisms and premature coronary heart disease. *JRAAS*. 6:38-42.
37. Shanmugam V, Sell K, Saha B. (1993):Mistyping ACE heterozygotes. *PCR MethodsAppl*. 3:120-1.
38. So W, Ma R, Ozaki R, et al.(2006):Angiotensin-converting enzyme (ACE) inhibition in type 2, diabetic patients-interaction with ACE insertion/deletion polymorphism.*Kidney International*. 69:1438-43.
39. Suehiro T, Morita T, Inoue M, et al.(2004): Increased amount of the angiotensin- enzyme (ACE) mRNA originating from the ACE allele with deletion.*Humconverthing Genet*. 115:91-6.
40. Thomas GN, Tomlinson B, Chan JCN, et al. (2001): Renin-angiotensin system gene polymorphisms, blood pressure, dyslipidemia, and diabetes in Hong Kong Chinese: a significant association of the ACE insertion/deletion polymorphism with type 2 diabetes.*Diabetes care*. 24:356-61.
41. Tiret L, Rigat B, Visvikis S, et al.(1992): Evidence from combined segregation and linkage analysis that a variant of the angiotensin I-converting enzyme (ACE) gene controls plasma ACE levels.*Am J Hum Genet*. 51:197-205.
42. Toop MJ, Dallinger KJ, Jennings PE, Barnett AH. (1986): Angiotensin-converting enzyme (ACE): relationship to insulin-dependent diabetes and microangiopathy.*Diabet Med* 3(5):455-457.
43. Ueda S, Elliott HL, Morton JJ, Connell JM. (1995): Enhancedpressor response to angiotensin I in normotensive men with the deletion genotype (DD) for angiotensin-converting enzyme. *Hypertension* 25(6):1266-1269.
44. van der Kleij FG, de Jong PE, Henning RH, de Zeeuw D, Navis G. (2002): Enhanced Responses of Blood Pressure, Renal Function, and Aldosterone to Angiotensin I in the DD Genotype Are Blunted by Low Sodium Intake.*J Am SocNephrol* 13(4):1025-1033.
45. van der Kleij FG, de Jong PE, Henning RH, de Zeeuw D, Navis G. (2002): Enhanced Responses of Blood Pressure, Renal Function, and Aldosterone to Angiotensin I in the DD Genotype Are Blunted by Low Sodium Intake.*J Am SocNephrol* 13(4):1025-1033.
46. van der Kleij FG, Navis GJ, Gansevoort RT, Heeg JE, Scheffer H, de Zeeuw D et al.(1997): ACE polymorphism does not determine short-term renal response to ACE-inhibition in proteinuricpatients. *Nephrol Dial Transplant* 12 Suppl 2:42-46.
47. Van Dyk DJ, Erman A, Erman T, Chen-Gal B, Sulkes J, Boner G. (1994):Increased serum angiotensin converting enzyme activity in type I insulin-dependent diabetes mellitus: its relation to metabolic control and diabetic complications. *Eur J Clin Invest* 24(7):463-467.
48. Yan C, Zhan J, Feng W. (2005):Gene polymorphisms of angiotensin II type 1 receptor and angiotensin-converting enzyme in two ethnic groups living in Zhejiang Province, China. *JRAAS*. 6:132-7.
49. Yoo J-H. (2005):Deletion polymorphism in the gene for angiotensin-converting enzyme is associated with essential hypertension in men born during the Pacific War.*Mechanisms of Ageing and Development*. 126:899-905.

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