The relationship between Angiotensin Converting Enzyme Gene Polymorphism and Smoking-Related Ischemic Heart Diseases

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Abstract: Background: Smoking as a major risk factor for development of ischemic heart diseases is thought to be partially genetically determined. The aim of the study is to determine the effect of ACE gene polymorphism on the development of cardiovascular diseases among smokers**. Methods:** A case-control study was conducted in Cairo university hospital, Egypt. An interviewed questionnaire was used to collect data from 200 subjects. ACE I/D polymorphism was detected using polymerase chain reaction (PCR). **Results:** The frequencies of DD, ID, and II genotypes among smokers with ischemic heart diseases were 16.3%, 77.5% and 6.2% respectively. Their frequencies among smokers with no ischemic heart diseases were 25.0%, 60.0% and 15.0% respectively; the difference between the "ischemic heart diseases" group and "non-ischemic heart diseases" group was statistically significant (P= 0.046). **Conclusion:** According to this Egyptian Study, ID genotype may be a risk factor in the occurrence of ischemic heart diseases among Egyptian smokers.
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1. Introduction

One of the most important systems in cardiovascular homeostasis and blood pressure regulation is the renin–angiotensin–aldosterone system. The key enzyme of this system is the Angiotensin-converting enzyme (ACE), it converts angiotensin I to angiotensin II and degrades bradykinin (Ljungberg et al., 2011). Studies have detected several single nucleotide polymorphisms in the gene. Accordingly, investigating the locations of functional polymorphisms became a field of interest for several researches (Sayed-Tabatabaei et al., 2006).

Debates are present regarding the association between ACE polymorphism and ischemic heart diseases (IHD). Many researches proved this association (Freitas et al., 2007) and others could not (Ulgen et al., 2007). These contradictory results may be due to Genotypic and phenotypic misclassifications, insufficient power in some studies, and the presence of interaction with other genes or environmental factors (Sayed-Tabatabaei et al., 2006).

Environmental factors especially smoking play an essential role in human health. Public health institutions now consider smoking as 'a global tobacco epidemic' (Chaouachi, 2009). Health hazards of smoking depend on several factors such as the age at which smoking began, the duration of smoking, the number of cigarettes smoked per day, smoking behavior such as the degree of inhalation, and cigarette characteristics such as tar and nicotine content or filter type (Peto, 1986). Previous studies found that the risk of hypertension was significantly increased in smokers who carried one copies of D allele. The D allele of the ACE polymorphism is associated with a significantly increased systolic blood pressures and risk of hypertension in smokers. This underlines the importance of gene environment interactions (Schut et al., 2004).

Epidemiological studies have shown a strong correlation between cigarette smoking and the development and progression of cardiovascular disease such as atherosclerosis (Burns, 2003). In addition, there were published evidences using endothelial cell culture that nicotine, which is the most widely studied compound of cigarette smoke, it did not modulate basal ACE production after 4-24 h of treatment but significantly potentiated vascular endothelial growth factor (VEGF)-induced ACE upregulation (Saijonmaa et al., 2005).

According to Ulgen et al, 'IHD is a multi-factorial disease, likely to result from interactions between many genes and some environmental exposures such as smoking' (Ulgen et al., 2007). Other researchers studied the interaction between the ACE I/D polymorphism and smoking in relation to the thickness of carotid intima media (Sayed-Tabatabaei

et al., 2004). The biological possibility of this interaction encouraged studying smoking as an effect modifier of the association between the ACE gene polymorphism and IHD mortality and morbidity (Sayed-Tabatabaei et al., 2005).

These findings triggered the present study to examine the role of the ACE gene in cardiovascular diseases particularly ischemic heart diseases and studying smoking as a potential modifier of the effect of the ACE gene.

2. Material and Methods

Study design and sampling:

A case-control study was conducted in Cairo university hospital. Two hundred subjects were selected by a systematic random sampling method (one every four subjects). Subjects were divided into 3 groups; the first group included smokers with IHD (80 subjects), the second group included smokers with no IHD (80 subjects), and the third group included non-smokers with IHD (40 subjects). Subjects of the first and third group were obtained from the cardiology department, from those who were diagnosed as having IHD. Meanwhile, subjects of the second group were obtained the outpatient clinic after confirming that they didn't suffer from any cardiovascular disease. Cases with family history of cardiovascular disease, renal disease, liver disease, and other metabolic disorders were excluded from the study. The study was conducted from November, 2008 through April, 2011.

Data collection:

Questionnaire

An interviewed questionnaire was used to collect data about personal data, smoking history and other chronic diseases.

Laboratory analysis

For DNA isolation and determination of ACE genotypes, genomic DNA was isolated from peripheral leukocytes by a salting-out technique from 5 mL EDTA blood samples. FlexiGene DNA kit, (QIAGEN)®. For the ACE I/D polymorphism, the primer pairs used and the annealing temperatures were as follows: forward 5′-CTGGAGACCACTCCCATCCTTTCT-3′, and reverse 5′-GATGTGGCCATCACATTCGTCAGAT-3′, which amplify the intron 16 region where the I/D fragment is located [12]. The DNA product was a 190 bp fragment in case of deletion (D allele), and a 490 bp fragment in the presence of insertion (I allele). Thus, there were three genotypes after electrophoresis: a 490 bp band (II), a 190 bp band (DD), or both 490 and 190 bp bands (ID). Each D/D type was subjected to a second, independent PCR amplification with a primer pair that recognizes an insertion-specific sequence forward 5′- T G G G A C C A C A G C G C C C G C C A C TA C - 3′, and reverse 5'- TCG CCA GCC CTC CCA TGC CCA TAA -3', with identical PCR conditions except for an annealing temperature of 67ºC and the absence of 5% DMSO.

The PCR product of 335- bp was amplified only in the presence of (I) allele (in the case of heterozygote) whereas there was no product in samples homozygous for (D) allele (Lindpaintner et al., 1995). All PCR products were visualized after electrophoresis on a 2% agarose gel and ethidium bromide staining.

ACE Genotyping

Agarose gel was 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 second PCR, the insertion allele (I) was detected as a 335 –bp absence of this allele which confirms DD genotype (Figure 1).

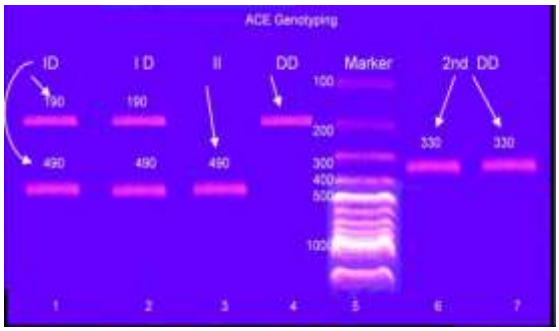

Figure 1: ACE Genotyping

Ethical considerations:

An informed consent was obtained from all subjects after full explanation for the purpose of the study. In addition, confidentiality of the obtained data was ensured.

Statistical analysis:

The obtained data was revised, entered on personal computer, analyzed using SPSS program version 18 and suitable statistical tests were performed (Chi-squared test). The significance level was set at 0.05.

Results

Demographic and smoking characteristics of the studied sample were demonstrated in table 1.

Two hundred subjects were enrolled in the study; 180 of them were males (90%), and 20 were females (10%), their age ranged from 27-65 years with mean age 51.19±7.94. The majority of the participants were married (70.5%). The table also describes the smoking habit characteristics of the studied samples; 160 of them were smokers; with smoking duration ranging

from 9-47 years (mean duration 31.25 ±8.338); and 40 subjects were non-smokers. The majority of smokers were smoking cigarettes (63.7%), 35% of them were smoking Cigarettes & Shisha. Meanwhile; only 1.3% of them (2 participants) were smoking Shisha only.

Table 1: Demographic and Smoking Characteristics of the Studied Sample (N=200)

Characteristics		N	%
Gender	Male	180	90.0
	Female	20	10.0
Age	<40	14	7.0
	40 - <50	60	30.0
	50 - <60	92	46.0
	≥60	34	17.0
Marital status	Single	32	16.0
	Married	141	70.5
	Divorced	11	5.5
	Widow	16	8.0
Smokers	Smoker	160	80.0
	Non-smoker	40	20.0
Type of smoking	Cigarettes only	102	63.7
	Shisha only	2	1.3
	Cigarettes & Shisha	56	35.0
Duration of smoking	<20 years	14	8.8
	20-<30 years	49	30.6
	30-<40 years	68	42.5
	40 years or above	29	18.1

* Mean age 51.19 ±7.94
** Mean duration of smoking 31.25 ±8.338

The majority of participants (66%) had ID genotype; participants with DD genotype represent 25% of the total participants, whereas the participants with II genotype represent only 9.0% of them (Table 2).

Table 2: Distribution of ACE Genotypes among the Studied Sample

ACE Genotypes	N	%
D/D	50	25.0
I/D	132	66.0
I/I	18	9.0
Total	200	100.0

Participants were divided into 3 groups according to their smoking habit and the presence of IHD. Table 3 showed that the ACE genotype of 77.5% of participants suffering from IHD was "ID" genotype compared to 60% of participants not suffering from IHD. The difference was statistically significant (P= 0.046). On the other hand, the homozygous "DD" and "II" genotypes were less prevalent among participants suffering from IHD compared to participants not suffering from IHD (16.3% versus 25% and 6.2% versus 15% respectively). The differences were statistically significant (p=0.046) (table 3).

The mean age of 80 smokers suffering from IHD was 51.8 ± 7.7. On the other hand, the mean age of the other 80 smokers not suffering from IHD was 49.07 ± 8.1. There was no statistically significant difference between smokers with IHD and without IHD regarding their age and marital status (table 3).

Table 3: ACE Genotype and Demographic Differences in Relation to IHD among Smokers (N=160)

	IHD		no IHD		X^2	p-value
	n	%	n	%		
ACE Genotypes						
DD	13	16.3	20	25.0	6.149	0.046
ID	62	77.5	48	60.0		
II	5	6.2	12	15.0		
Age (in years)						
<40	4	5.0	9	11.3		
40 - <50	26	32.5	27	33.7	2.666	0.446
50 - <60	36	45.0	34	42.5		
≥60	14	17.5	10	12.5		
Mean ± SD	51.8 ± 7.7		49.07 ± 8.1			
Marital status						
Single	15	18.7	17	21.3		
Married	54	67.5	57	71.2	1.892	0.595
Divorced	6	7.5	4	5.0		
Widow	5	6.3	2	2.5		
Total	80	100	80	100		

Table 4: Relation between Duration of Smoking and IHD among Smokers (N=160)

	IHD*		no IHD**		X^2	p-value
	n	%	n	%		
<20 years	3	3.8	11	13.7		
20-<30 years	21	26.2	28	35.0		
30-<40 years	37	46.3	31	38.8	8.894	0.031
≥40 years	19	23.7	10	12.5		
Total	80	100	80	100		

*Mean ± SD = 33.1±7.6
**Mean ± SD = 29.37±8.6

Table 4 shows that only 3.8% of participants suffering from IHD have duration of smoking less than 20 years compared to 13.7% of participants not suffering from IHD. On the other hand, 23.7% of participants suffering from IHD have duration of smoking of 40 years or more compared to only 12.5% of participants not suffering from IHD. The difference between the IHD group and non-IHD group as regard duration of smoking was statistically significant (P=0.03). Results also show that the mean duration of smoking of participants suffering from IHD was higher than participants not suffering from IHD.

Table 5: Relation between smoking and ACE genotyping among participants with IHD (N=120)

	Smokers		Non-smokers		X^2	p-value
	n	%	n	%		
ACE Genotypes						
DD	13	16.2	17	42.5		
ID	62	77.5	22	55.0	10.03	0.007
II	5	6.3	1	2.5		
Total	80	100	40	100		

The interaction between ACE genotyping and smoking as predictors of IHD was investigated in the

present study; the first group (smokers suffering from IHD) was compared with the third group (non-smokers suffering from IHD) regarding their ACE genotypes (table 5); results revealed that, among the 120 cases of ischemic heart diseases, the ACE genotypes (DD, ID, II) of smokers (n=80) were 13(16.2%), 62(77.5%), and 5(6.3%) respectively while for non-smokers group (n=40) were 17(42.5%), 22(55.0%), and 1(2.5%) respectively. The difference between the smokers group and non-smokers group regarding the ACE genotypes was statistically significant (p=0.007).

4. Discussion

Many environmental as well as genetic factors influence the occurrence of ischemic heart diseases (Egred et al., 2005). The present study demonstrated the possible relation between the ACE gene polymorphism and smoking-related diseases.

Studying the distribution of ACE Genotypes among the studied sample revealed that the majority of participants had ID genotype; whereas frequency of II genotype were the lowest. This finding is consistent with the results of Settin et al who studied the frequency of ACE genotype in Egyptian patients with myocardial infarction; they found a higher frequency of ID than DD genotype, and a lower frequency of the II genotype (Settin et al., 2009). In addition, Zintzaras et al conducted a similar study and found that ID genotype had the highest occurrence, whereas the frequency of II genotype was the lowest (Zintzaras et al., 2008).

Studying the relation between ACE genotyping and ischemic heart diseases among smokers revealed that:

- The heterozygous "ID" genotype was more prevalent among participants suffering from IHD. This indicates that "ID" genotype may be considered as a risk factor in the occurrence of IHD among Egyptian smokers. This finding supports the results of Sayed-Tabatabaei et al who found that the interaction between ACE ID polymorphism and smoking may be crucial in the cardiovascular mortality particularly at younger age; however, they claimed that the ACE ID polymorphism alone is not a strong risk factor for the development of IHD (Sayed-Tabatabaei et al., 2005). However, Schut et al studied the relation between the ACE I/D polymorphism and risk of hypertension in current, former and non-smokers. They found a significant increase in the systolic blood pressure in DD carriers compared with II carriers among smokers (Schut et al., 2004). Also Hibi et al found the smoking-associated effects of ID polymorphism of the ACE gene in the severity of coronary atherosclerosis in Japanese patients with acute coronary syndromes. These smoking-associated effects of the ACE *DD* genotype suggest that heritable and environmental factors interact and lead to the manifestations of CAD. From the standpoint of prevention, young persons screened for the ACE *I*/*D* gene polymorphism and found to bear the *DD* genotype should be advised by physicians that they are at particularly high risk for CAD and that continued smoking could significantly shorten their lives (Hibi et al., 1996). On the contrary, Tascilar et al in their study found that ACE I/D polymorphism had no effect on the risk of hypertension (Tascilar et al., 2009).
- The homozygous "DD" and "II" genotypes were less prevalent among participants suffering from IHD compared to participants not suffering from IHD This means that "DD" and "II" genotypes may not be a risk factor in the occurrence of IHD among smokers. This finding confirms the results of Shafiee et al who studied the relevance of ACE polymorphism for coronary artery diseases (CAD) in the Iranian population. Their results showed that there was no increased risk of CAD in association with "DD" genotype and that it may not be as a risk factor (Shafiee et al., 2010).

The present study results demonstrated the relation between demographic characteristics and IHD among smokers; the age and the marital status did not appear to be predictors of ischemic heart diseases among smokers. This finding disagrees with the results of Sayed-Tabatabaei et al who found a significant interaction between ACE genotypes and age in smokers (Sayed-Tabatabaei et al., 2005).

Comparing the duration of smoking of participants with and without IHD revealed that the IHD was more prevalent among smokers with longer duration of smoking. This finding confirms the results of Streppel et al who studied the mortality and life expectancy in relation to long-term cigarette, cigar and pipe smoking, their results showed that the duration of cigarette smoking was strongly associated with mortality from cardiovascular diseases (Streppel et al., 2007). In addition, Mannan et al found that the risk of CVD was associated with smoking and that risk varies with the intensity and duration of smoking (Mannan et al., 2010).

Studying the interaction between ACE genotyping and smoking as predictors of IHD indicates that the "ID" genotype was more prevalent among smokers suggesting an interaction between ID" genotype and smoking in the occurrence of ischemic heart diseases. On the other hand, the homozygous "DD" genotype was less prevalent among smokers compared to non-smokers. This means that "DD" genotype is not a risk factor in the occurrence of IHD among smokers. These findings agree with the results of an Egyptian research study that tested the recent Egyptian 2010,

2011 association of ACE gene I/D polymorphism with myocardial infarction. They concluded that the angiotensin-converting enzyme gene I/D polymorphism are probably a risk factor for IHD among Egyptian cases, particularly if integrated with other environmental and genetic risk factors (Settin et al., 2009).

5. Conclusion

In this Egyptian study, the heterozygous "ID" genotype was found to be more prevalent among Egyptian smokers suffering from IHD which means that "ID" genotype may be a risk factor in the occurrence of IHD among smokers. On the other hand, the homozygous "DD" and "II" genotypes were less prevalent among participants suffering from IHD compared to smokers not suffering from IHD, indicating that "DD" and "II" genotypes may not be a risk factor in the occurrence of IHD among smokers.

Among the cases of ischemic heart diseases, "ID" genotype was more prevalent among smokers compared to non-smokers , which means that there may be an interaction between ID" genotype and smoking in the occurrence of ischemic heart diseases.

List of abbreviations

ACE: Angiotensin-converting enzyme; bp: Base pairs; D/D: Deletion/ deletion; DNA: Deoxyribonucleic acid; DMSO: Dimethyl sulfoxide; EDTA: Ethylenediamine; tetraacetic acid; I/I: Insertion/ insertion; I/D: Insertion/deletion; IHD: Ischemic heart diseases; PCR: Polymerase Chain Reaction

Competing interests

The authors of this study declare that they have no competing interests.

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References

Burns D. Epidemiology of smoking-induced cardiovascular disease. Prog Cardiovasc Dis 2003; 46:11–29.

Chaouachi K. Hookah (Shisha, Narghile) Smoking and Environmental Tobacco Smoke (ETS). A Critical Review of the Relevant Literature and the Public Health Cons. Int. J. Environ. Res. Public Health 2009; 6:798-843.

Egred M, Viswanathan G, Davis GK. Myocardial infarction in young adults. Postgrad Med J 2005; 81:741-745.

Freitas SR, Cabello PH, Moura-Neto RS, Dolinsky LC, Bóia MN. Combined analysis of genetic and environmental factors on essential hypertension in a brazilian rural population in the Amazon region. Arq Bras Cardiol 2007; 88(4):447-451.

Hibi K, Ishigami T, Kimura K, Nakao M, Iwamoto T, Tamura K, Nemoto T, Shimizu T, Mochida Y, Ochiai H, Umemura S, Ishii M. Angiotensin-converting enzyme gene polymorphism adds risk for the severity of coronary atherosclerosis in smokers. Hypertension1997; 30:574–579.

Lee YJ, Tsai JCR. ACE gene insertion/deletion polymorphism associated with 1998 World Health Organization definition of metabolic syndrome in Chinese type 2 diabetic patients. Diabetes Care 2002; 25(6):1002–1008.

Lindpaintner K, Pfeffer MA, Kreutz R, Stampfer MJ, Grodstein F, LaMotte F, Buring J, Hennekens CH. A prospective evaluation of an angiotensin-converting enzyme gene polymorphism and the risk of ischemic heart disease. N Engl J Med 1995; 332:706-712.

Ljungberg L, Alehagen U, Länne T, Björck, H, De Basso R, Dahlström U, Persson K. The association between circulating angiotensin-converting enzyme and cardiovascular risk in the elderly. Journal of Renin-Angiotensin-Aldosterone System 2011; 12(3):281-289.

Mannan HR, Stevenson C, Peters A, Walls H, Mcneil J. Framingham risk prediction equations for incidence of cardiovascular disease using detailed measures for smoking. Heart International 2010; 5(2):49-57.

Peto R. Influence of dose and duration of smoking on lung cancer rates. In: Zarridge DG, Peto R. (Eds) Tobacco: a Major International Health Hazard. Lyon, France: International Agency for Research on Cancer 1986: 23-33.

Saijonmaa O, Nyman T, Fyhrquist F. Regulation of angiotensin-converting enzyme production by nicotine in human endothelial cells. Am J Physiol Heart Circ Physiol 2005; 289:H2000-2004.

Sayed-Tabatabaei FA, Oostra BA, Isaacs A, Van Duijn CM, Witteman JC. ACE polymorphisms. Circ Res 2006; 98:1123-1133.

Sayed-Tabatabaei FA, Schut AF, Hofman A, Bertoli-Avella AM, Vergeer J, Witteman, JC, van Duijn CM. A study of gene-environment interaction on the gene for angiotensin converting enzyme: a combined functional and population based approach. J

Med Genet 2004; 41:99-103.
Sayed-Tabatabaei FA, Schut AF, Vasquez AA, Bertoli-Avella AM, Hofman A, Witteman JC, van Duijn CM. Angiotensin converting enzyme gene polymorphism and cardiovascular morbidity and mortality: the Rotterdam Study. J Med Genet 2005; 42:26-30.
Schut AF, Sayed-Tabatabaei FA, Witteman JC, Bertoli-Avella AM, Vergeer J, Pols HA, Hofman A, Deinum J, Van Duijn CM. Smoking-dependent effects of the angiotensin-converting enzyme gene insertion/deletion polymorphism on blood pressure. J Hypertens 2004; 22:313–319.
Settin A, ElBaz R, Abbas A, Abd-Al-Samad A, Noaman A. Angiotensin-converting enzyme gene insertion/deletion polymorphism in Egyptian patients with myocardial infarction. Journal of Renin-Angiotensin-Aldosterone System 2009; 10(2):96-100.
Shafiee SM, Firoozrai M, Salimi S, Zand H, Hesabi B, Mohebbi A. Angiotensin converting enzyme DD genotype not associated with increased risk of coronary artery disease in the Iranian population. Pathophysiology 2010; 17(3):163-167.
Streppel MT, Boshuizen HC, Ocké, MC, Kok FJ, Kromhout, D. Mortality and life expectancy in relation to long-term cigarette, cigar and pipe smoking. The Zutphen Study Tob Control 2007; 16(2):107-113.
Tascilar N, Dursun A, Ankarali H, Mungan G, Ekem S, Baris S. Angiotensin-converting enzyme insertion/deletion polymorphism has no effect on the risk of atherosclerotic stroke or hypertension. J Neurol Sci 2009; 285(1-2):137-141.
Ulgen MS, Ozturk O, Alan S, Kayrak M, Turan Y, Tekes S, Toprak N. The relationship between angiotensin-converting enzyme (insertion/deletion) gene polymorphism and left ventricular remodeling in acute myocardial infarction. Coron Artery Dis 2007; 18(3):153-157.
Zintzaras E, Raman G, Kitsios G, Lau J. Angiotensin–Converting Enzyme Insertion/Deletion Gene polymorphism Variant as a Marker of Coronary Artery Disease. Arch Intern Med 2008; 168(10):1077-1089.

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