RELATIONSHIP BETWEEN THE ACE GENE POLYMORPHISM AND SMOKING-RELATED DISEASES

By

Soheir Mahmoud Mahmoud Ibrahim

B. Sc. Al- Azhar University, 1993

A thesis submitted in Partial Fulfillment
Of
The Requirement for the Master Degree
in
Environmental Science

Department of Environmental Basic Science
Institute of Environmental Studies and Research
Ain Shams University

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APPROVAL SHEET

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Abstract

Background: The aim of the present study was to determine the effect of Angiotensin-converting enzyme (ACE) gene polymorphism on the development of cardiovascular diseases among smokers**. Methods:** Using polymerase chain reaction (PCR), I/D polymorphism was determined in the ACE gene in 160 smokers & 40 non-smokers. **Results:** the frequency of ACE genotype DD, ID, and II among smokers with ischemic heart disease (IHD) were 13(16.3%), 62(77.5%) and 5(6.2%) respectively. The DD, ID, and II genotypes among smokers with no IHD were 20(25.0%), 48(60.0%) 15(15.0%) respectively; the difference between the IHD group and non-IHD group was statistically significant (P= 0.046). Among the 120 cases of (IHD), 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). **Conclusion:** "ID" genotype may be a risk factor in the occurrence of IHD among smokers. In addition, there may be an interaction between "ID" genotype and smoking in the occurrence of ischemic heart disease.

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The Candidate

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List of Abbreviations

A	Adenine
ACE	Angiotensin-converting enzyme
ACEi	ACE inhibitors
AGT	Angiotensinogen
$AGTR_1$	Angiotensin II receptors type 1
$AGTR_2$	Angiotensin II receptors type 2
Ang I	Angiotensin I
Ang II	Angiotensin II
AT1	Angiotensin II type 1
AT2	Angiotensin II type 2
AT1R	Angiotensin II type 1 receptor
AT2R	Angiotensin II type 2 receptor
ARBs	Angiotensin II receptor blockers
Bp	Base pairs
CAD	Coronary artery disease
COPD	Chronic obstructive pulmonary disease
CHD	Coronary heart disease
CVDs	Cardiovascular diseases
°C	Degree Celcius
C	Cytosine
CYP11B2	Cytochromp11B2
D	Deletion
D/D	Deletion/ deletion
AD	Alzheimer's disease
DNA	Deoxyribonucleic acid
dNTPs	Deoxynucleotide 5'-triphosphate
DMSO	Dimethyl sulfoxide
EDTA	Ethylenediamine tetraacetic acid
EH	Essential hypertension

EMSS	Exhaled Main-Stream Smoke
ETS	Environmental Tobacco Smoke
gDNA	genomic DNA
G	gram (s)
HF	Heart failure
HPMC	Human peritoneal mesothelial cells
I	Insertion
I/I	Insertion/ insertion
I/D	Insertion/deletion
IgG	Immunoglobulin G
IHD	Ischemic heart disease
Kg	Kilo Gram
Kb	Kilobase pair
Mg	Milligram (s)
mg/dl	Milligram per deciliter (s)
Ml	Milliliter (s)
mM	Millimolar
MI	Myocardial infraction
L	Liter (s)
LEG	Low exposure-gene
NO	Nitric oxide
PCR	Polymerase Chain Reaction
PGI2	Prostaglandin I2
PDI	Peritoneal Dialysis International
Bp	Base pairs
Pmol	Picomol
RAAS	Renin-angiotensin-aldosterone system
RAS	Renin-angiotensin system
ROS	Reactive oxygen species
LVH	Left ventricular hypertrophy
sACE	somatic Angiotensin-converting enzyme

SSS	Side-Stream Smoke
Ta	Annealing Temperature
TBE	Tris borate buffer
TGF	Transforming growth factor
VEGF	Vascular endothelial growth factor
WHO	World Health Organization
w/v	Weight/volume

INTRODUCTION

The renin–angiotensin–aldosterone system (RAAS) is one of the most important systems in cardiovascular homeostasis and blood pressure regulation. Angiotensin-converting enzyme (ACE) is a key enzyme in the renin–angiotensin–aldosterone system, converting angiotensin I to angiotensin II and degrading bradykinin **(**Ljungberg, et al, 2011). Recently, many single nucleotide polymorphisms were detected in the gene and the search for the locations of functional polymorphisms became a topic of extensive investigation (Sayed-Tabatabaei, et al, 2006).

The association between ACE polymorphism and cardiovascular diseases is controversial. Several studies confirm the association of ACE insertion/deletion (I/D) dimorphism and the risk of developing cardiovascular diseases (CVDs), such as essential hypertension (EH)' and myocardial infarction (MI) (Freitas, et al, 2007). On the other hand, other studies could not confirm the association of ACE I/D with MI and coronary heart disease (CHD) (Ulgen, et al, 2007). Genotypic and phenotypic misclassifications, insufficient power in some studies, and the presence of interaction with other genes or environmental factors are possible explanations for the contradictory findings (Sayed-Tabatabaei, et al, 2006).

Environmental factors especially smoking play an essential role in human health. It is now seen by public health officials as a global tobacco epidemic (Chaouachi, 2009). Hazards of smoking, depend on several factors including the age at which smoking began, duration of smoking, number of cigarettes

smoked per day, degree of inhalation, and cigarette characteristics such as tar and nicotine content or filter type (Ezzati and Lopez, 2003). Smoking leads to cardiovascular illnesses such as high blood pressure, and coronary artery disease .In fact, studies show that smokers are two to three times more likely to have a stroke or heart attack than non-smokers, and the risk of cardiovascular disease is highest for smokers with high blood pressure than non-smokers (Schut, et al, 2004).

According to (Ulgen, et al, 2007), CHD is a multi-factorial disease, likely to result from interactions between many genes and some environmental exposures such as smoking. There is also published evidence of an interaction between the ACE I/D polymorphism and smoking in relation to carotid intima media thickness (Sayed-Tabatabaei, et al, 2004). In addition, it has been previously reported that nicotine, which is the main component of cigarette smoke, affects ACE expression (Zhang, et al, 2001) and regulates ACE that plays an important role in the pathophysiology of atherosclerosis and hypertension (Saijonmaa, et al, 2005). The biological plausibility of such an interaction prompted to study smoking as an effect modifier of the association between the ACE gene polymorphism and CVD mortality and morbidity (Sayed-Tabatabaei, et al, 2005).

OBJECTIVE OF THE STUDY

The objective of the present study was to determine the effect of ACE gene polymorphism on the development of ischemic heart disease among smokers.

REVIEW OF LITERATURE

Chapter 1

Smoking as an Environmental Hazard

Environmental factors especially smoking play an essential role in human health. Hazards of smoking, which depend on several factors including the age at which smoking began, duration of smoking, number of cigarettes smoked per day, degree of inhalation, and cigarette characteristics such as tar and nicotine content or filter type (Ezzati and Lopez, 2003).

Smoking is seen by public health officials as a global tobacco epidemic. Cigarette Environmental Tobacco Smoke (ETS) is classically understood as a combination of Side-Stream Smoke (SSS) and Exhaled Main-Stream Smoke (EMSS) (Chaouachi, 2009). Although it contains the same toxic and carcinogenic constituents as the mainstream smoke inhaled by smokers, side stream smoke contains more unburnt hydrocarbons and is a more potent mutagen on a weight basis (Kobayashi et al, 1997). Some of the corresponding cigarette studies have served as the scientific basis for stringent legislation on indoor smoking across the world (Chaouachi, 2009).

Health hazards of smoking

There are 1.3 billion cigarette smokers, 82 percent in developing countries, and if current practices continue, there will be an estimated one