



Ain Shams University
Faculty of Science
Biochemistry Department



Association between Genetic Polymorphisms and Coronary Artery Disease in Egyptian Patients

A THESIS

*Submitted for the degree of Doctor of Science
As Fulfillment for the Requirements of the Doctor Degree (Ph.D.) of Science in
Biochemistry*

By

Lamiaa Mageed Sayed Ibrahim

*Associate Researcher in the National Research Centre
(B.Sc.) Biochemistry (2005), (M.Sc.) Biochemistry (2011)
Biochemistry Department,
Faculty of Science, Ain Shams University*

Supervisors

Prof. Dr. Ibrahim Hassan Borai

Professor of Biochemistry
Biochemistry Department
Faculty of Science
Ain Shams University

Prof. Dr. Esmat Ashour Wahba

Professor of Biochemistry
Biochemistry Department
National Research Centre, Dokki, Giza,
Egypt

Assist. Prof. Dr. Nahla Samir Hassan

Assistant Professor of Biochemistry
Biochemistry Department
Faculty of Science
Ain Shams University

Prof. Dr. Olfat Gamil Shaker

Professor of Medical Biochemistry
and Molecular Biology
Biochemistry Department
Faculty of Medicine
Cairo University

Prof. Dr. Mohamed Ibrahim El-Badrawy

Heart Consultant of the National Heart Institute
The National Heart Institute
Giza, Egypt

2016

Approval Sheet of Submission

Title of PhD Thesis:

“Association between Genetic Polymorphisms and Coronary Artery Disease in Egyptian Patients”

Name of the Candidate: *Lamiaa Mageed Sayed Ibrahim*

This thesis has been approved for submission

BY SUPERVISORS

Prof. Dr. Ibrahim Hassan Borai

Professor of Biochemistry
Biochemistry Department
Faculty of Science
Ain Shams University

Prof. Dr. Esmat Ashour Wahba

Professor of Biochemistry
Biochemistry Department
Genetic Engineering and Biotechnology Division
National Research Centre

Prof. Dr. Olfat Gamil Shaker

Professor of Medical Biochemistry and Molecular Biology
Biochemistry Department
Faculty of Medicine
Cairo University

Assist. Prof. Dr. Nahla Samir Hassan

Assistant Professor of Biochemistry
Biochemistry Department
Faculty of Science
Ain Shams University

Prof. Dr. Mohamed Ibrahim El-Badrawy

Heart Consultant of the National Heart Institute
The National Heart Institute
Giza, Egypt

ACKNOWLEDGEMENT

*First and foremost, I am grateful and thanks to **ALLAH** for most merciful and most gracious who gave me the ability to carry out this work and who made all things possible.*

*I would like to acknowledge and extend my heartfelt gratitude to **Prof. Dr. Ibrahim Hassan Borai**, professor of Biochemistry, Biochemistry Department, Faculty of Science, Ain Shams University for her vital encouragement, fruitful suggestions, support, and I wish gratefully to express my appreciation to his valuable advice and guidance to complete this work.*

*I would like to express my deepest gratitude and appreciation to **Prof. Dr. Esmat Ashour Wahba**, Professor of Biochemistry, Biochemistry Department, Genetic Engineering and Biotechnology Division, National Research Centre for her valuable advice in science discussion, generous supervision, vital encouragement, support and guidance in various ways from the very early stage of this research as well as giving me extraordinary experiences throughout the work. I am grateful in every possible way for her wise opinions and critical comments throughout the whole study and work.*

*Also, I would like to acknowledge and extend my deep gratitude to **Prof. Dr. Olfat Gamil Shaker**, professor of Medical Biochemistry, Faculty of Medicine, Cairo University for her vital encouragement, fruitful suggestions, support, and I wish gratefully to express my appreciation to her valuable advice and guidance to complete this work.*

*I gratefully acknowledge and express my deepest appreciation to **Prof. Dr. Olfat Mohammed Fawzi**, Professor of Biochemistry, Biochemistry Department, Genetic Engineering and Biotechnology Division, National Research Centre for her encouragement, and support to complete this work.*

*My special thanks go to **Assistant Prof. Dr. Nahla Samir Hassan**, Assistant professor of Biochemistry, Biochemistry Department, Faculty of Science, Ain Shams University for her advice in science discussion and the great help and support to complete this work.*

*My special thanks for **Dr. Mohamed Ibrahim El-Badrawy**, Heart Consultant of the National Heart Institute, The National Heart Institute for his advice and the great help in collection of samples and his support throughout the whole study and work.*

It is pleasure to express my gratitude wholeheartedly to my family, my beloved parents, especially to my lovely Mother, is the one who sincerely raised me with her caring and gently love, and many thanks for her vital encouragement, unlimited help, guidance and thoughtful support in various ways. I am indebted to her more than she knows. Therefore, I would also thank my brother Mohammed for his encouragement, help and support. Furthermore, to my uncle Mohammed Hayaty for the great help and support.

Finally, I would like to thank my colleagues and my patients and thanks to everybody made any effort for this work to success and to be a reality, as well as expressing my apology that I could not mention personally.

ABSTRACT

Lamiaa Mageed Sayed Ibrahim

"Association between Genetic Polymorphisms and Coronary Artery Disease in Egyptian Patients"

PhD, Biochemistry Department, Faculty of Science, Ain Shams University

Aim of the study: To evaluate the level of IL-18 as a pro-inflammatory cytokine and to investigate the potential associations of the two IL-18 promoter gene polymorphisms at positions (-607C/A) and (-137G/C), ACE (I/D) and AGT (M235T) gene polymorphisms with coronary artery disease.

Subjects: A total of one hundred and twenty Egyptian patients (Sixty with CAD and sixty without CAD) and fifty healthy control subjects were included in the study.

Methods: Genotyping of IL-18 promoter gene at (-607C/A) and (-137G/C) regions, ACE (I/D) and AGT (M235T) were analyzed by Polymerase chain reaction technique (PCR), Lipid profiles (total cholesterol, triglyceride, HDL-C) were measured by enzymatic colorimetric method. Serum IL-6 and IL-18 levels were determined using ELISA.

Results: Our data indicated that IL-18 (137 GG) was significantly associated with CAD, whereas a non-significant association was observed in IL-18 (607C/A) between cases and controls. Also, there was a significant association between ACE (DD) and AGT (TT) polymorphisms and CAD. Furthermore, a significant association between lipid profiles (TC, TG and LDL-C) and risk for CAD was occurred. Elevation of serum IL-6 and IL-18 levels was observed in CAD patients.

Conclusion: Our study suggests that an association between IL-18 (137G/C) promoter gene polymorphism, ACE (I/D) and AGT (M235T) polymorphisms and susceptibility to CAD in Egyptian patients. The present study showing a strong association between dyslipidemia as an important risk factor of atherosclerosis and CAD. A markedly high level of IL-6 and IL-18 in patients with CAD, based on which suggests that IL-6 and IL-18 may be served as susceptibility biomarkers in the pathogenesis of atherosclerosis in CAD patients.

CONTENTS

Page

LIST OF ABBREVIATIONS.....	III
LIST OF TABLES.....	VI
LIST OF FIGURES.....	VIII
INTRODUCTION AND AIM OF THE WORK.....	1
- INTRODUCTION.....	1
- AIM OF THE WORK.....	5
REVIEW OF LITERATURE.....	7
1. CORONARY ARTERY SYSTEM.....	7
1.1. Morphology of the Normal Artery.....	8
1.2. Coronary Artery Disease.....	10
1.3. Pathogenesis of Atherosclerosis.....	12
1.4. Progression of Atherosclerosis	15
1.5. Prevalence of coronary Artery Disease.....	16
1.6. Risk Factors Influencing Coronary Artery Disease.....	20
1.7. Clinical Manifestations of Coronary Artery Disease.....	30
1.8. Clinical Diagnosis of Coronary Artery Disease.....	32
2. Inflammatory Biomarkers in Coronary Artery Disease.....	36
3. The Genetic Architecture of Coronary Artery Disease.....	51
3.1. Interleukin-18 (IL-18) gene.....	52
3.2. Renin-Angiotensin-Aldosterone System (RAAS).....	63
3.2.1. Angiotensin Converting Enzyme (ACE).....	64
3.2.2. Angiotensinogen Gene (AGT).....	70
SUBJECTS AND METHODS.....	77
1. Subjects.....	77
2. Methods.....	80
A) Biochemical Analyses.....	80
B) Molecular Analyses.....	94
3. Statistical Analysis.....	105
RESULTS.....	107
DISCUSSION.....	143

SUMMARY AND CONCLUSIONS.....	161
REFERENCES.....	167
ARABIC SUMMARY.....	

LIST OF ABBREVIATIONS

ACE	Angiotensin Converting Enzyme
ACS	Acute Coronary Syndrome
AGT	Angiotensinogen
AMI	Acute Myocardial Infarction
Ang I	Angiotensin I
Ang II	Angiotensin II
AR	Aortic Root
BP	Blood Pressure
CAC	Coronary Artery Calcium
CAD	Coronary Artery Disease
cAMP	Cyclic Adenosine Monophosphate
CHD	Coronary Heart Disease
CRP	C -Reactive Protein
CVDs	Cardiovascular Diseases
DBP	Diastolic Blood Pressure
DM	Diabetes Mellitus
DN	Diabetic Nephropathy
DNA	Deoxyribonucleic Acid
ECG	Electrocardiogram
EF	Ejection Fraction
EH	Essential Hypertension
ELISA	Enzyme-Linked Immunosorbent Assay
FH	Familial Hypercholesterolemia
FS	Fractional Shortening
H4TF-1	Histone 4 Transcription Factor-1
HDL-C	High Density Lipoprotein-Cholesterol
HF	Heart Failure
HRV	Heart Rate Variability
hs- CRP	High Sensitivity C -Reactive Protein
HTN	Hypertension
ICAM-1	Intracellular Cell Adhesion Molecule-1
IDL-C	Intermediate-Density Lipoprotein Cholesterol
IFN- γ	Interferon- γ

IHD	Ischemic Heart Disease
IL-1	Interleukin-1
IL-10	Interleukin-10
IL-18	Interleukin-18
IL-6	Interleukin-6
IMT	Intima Media Thickness
LA	Left Atrium
LCA	Left Coronary Artery
LDL-C	Low Density Lipoprotein-Cholesterol
Lp-PLA-2	Lipoprotein-Associated Phospholipase A2
LVEDD	Left Ventricular End Diastolic Diameter
LVEF	Left Ventricular Ejection Fraction
LVESD	Left Ventricular End Systolic Diameter
LVH	Left Ventricular Hypertrophy
LVPWT	Left Ventricular Posterior Wall Thickening
LVSWT	Left Ventricular Systolic Wall Thickening
Mcp-1	Monocyte Chemoattractant Protein-1
MI	Myocardial Infarction
MMPs	Matrix Metalloproteinases
MPO	Myeloperoxidase
MRI	Magnetic Resonance Imaging
MRP8/14	Myeloid Related Protein 8/14 Complex
MUGA	Multigated Acquisition Scans
NLRP3	Nucleotide-Binding Domain And Leucine-Rich Repeat Pyrin Containing Protein-3
NO[•]	Nitric Oxide
ox-LDL	Oxidized LDL
PAD	Peripheral Artery Disease
PAI-1	Plasminogen Activator Inhibitor-1
PCAD	Premature Coronary Artery Disease
PCR	Polymerase Chain Reaction
PCR-RFLP	Polymerase Chain Reaction Restriction Fragment Length Polymorphism
PCR-SSP	Polymerase Chain Reaction -Specific Sequence Primer
PIGF	Placental Growth Factor
PTX3	Pentraxin-3

RAAS	Renine Angiotensine Aldosterone System
RAS	Renin–Angiotensin System
RNA	Ribonucleic Acid
ROC	Receiver Operating Characteristics
ROS	Reactive Oxygen Species
RV	Right Ventricle
RV	Right Ventricular
SAA	Serum Amyloid A
SBP	Systolic Blood pressure
sCD40L	Soluble CD40 Ligand
SD	Standard Deviation
SNPs	Single Nucleotide Polymorphisms
STEMI	Segment Elevated Myocardial Infarction
T2DM	Type 2 Diabetes Mellitus
TC	Total Cholesterol
TG	Triglyceride
TNF- α	Tumor Necrosis Factor Alpha
TnI	Troponin-I
TOE	Transoesophageal Echocardiography
UA	Unstable Angina
VCAM-1	Vascular Cell Adhesion Molecule- 1
VLDL-C	Very Low Density Lipoprotein-Cholesterol

LIST OF TABLES

	Page
Table (1) The Epidemiologic Transition for Cardiovascular Disease	17
Table (2) Demographic data of patients with and without coronary artery disease	108
Table (3) Biochemical characteristics of patients with and without coronary artery disease and control groups	110
Table (4) Frequency of samples detected by ELISA for serum IL-6 Level	112
Table (5) Percent sensitivity, specificity, positive and negative predictive values (PPV, NPV), and accuracy of serum IL-6 level (pg/ml) in coronary artery disease	113
Table (6) Frequency of samples detected by ELISA for serum IL-18 Level	114
Table (7) Percent sensitivity, specificity, positive and negative predictive values (PPV, NPV), and accuracy of serum IL-18 level (pg/ml) in coronary artery disease	116
Table (8) Genotype distribution and allele frequencies of (607C/A) polymorphism in IL-18 gene for patients with and without CAD and control groups	118
Table (9) Genotype distribution and allele frequencies of (137G/C) polymorphism in IL-18 gene for patients with and without CAD and control groups	120
Table (10) Genotype distribution and allele frequencies of (I/D) polymorphism in ACE gene in patients with and without CAD and control groups	121
Table (11) Genotype distribution and allele frequencies of M235T polymorphism in AGT gene in patients with and without CAD and control groups	123

Page

Table (12)	The combined effect of ACE (I/D) and AGT (M235T) genotype frequencies in patients with and without coronary artery disease	125
Table (13)	The combined effect of ACE I/D, IL-18 607C/A, and IL-18 137G/C genotype frequencies in patients with and without Coronary artery disease	127
Table (14)	The combined effect of ACE I/D, AGT M235T, IL-18 607C/A, and IL-18 137G/C genotype frequencies in patients with and without Coronary artery disease	129
Table (15)	Clinical and Biochemical characteristics of patients with and without coronary artery disease with different 607C/A genotypes in IL-18 gene	131
Table (16)	Clinical and Biochemical characteristics of patients with and without coronary artery disease with different 137G/C genotypes in IL-18 gene	134
Table (17)	Clinical and Biochemical characteristics of patients with and without coronary artery disease with different I/D genotypes in ACE gene	137
Table (18)	Clinical and Biochemical characteristics of patients with and without coronary artery disease with different M235T genotypes in AGT gene	139

LIST OF FIGURES

	Page
Figure (1) The Anatomy of the Coronary Arteries	7
Figure (2) Morphology of Normal Artery	8
Figure (3) Atherosclerotic Lesion in a Human Artery	11
Figure (4) Normal Heart, normal artery and artery with Plaque.	13
Figure (5) Atherosclerotic Plaque Formation	14
Figure (6) Two types of vascular pathology in large and small arteries: Atherosclerosis (Left) and Arteriosclerosis (Right)	15
Figure (7) Phases of lesion development	16
Figure (8) Distribution of Major Causes of Death Including CVDs	18
Figure (9) Proportion of all deaths due to CHD, among men (A) and women (B)	18
Figure (10) Angina Pectoris or (chest pain) as a symptom of coronary artery disease	31
Figure (11) IL-6 in inflammation	47
Figure (12) IL-18 and plaque formation	49
Figure (13) IL-18 gene located on Chromosome 11 (11q23.1)	54
Figure (14) Production of IL-18 by caspase-1	55
Figure (15) IL-18 signal transduction	57
Figure (16) IL-18 gene and promoter polymorphism	60
Figure (17) Renin Angiotensin Aldosterone System (RAAS)	63
Figure (18) ACE distribution	65
Figure (19) Angiotensin converting enzyme structure	65
Figure (20) Diagram of the ACE Gene Illustrating the Insertion/Deletion Polymorphism	66

Page

Figure (21)	I and D alleles of angiotensin converting enzyme gene	66
Figure (22)	Angiotensinogen gene localization on chromosome 1 (1q42.2)	70
Figure (23)	Schematic Representation of Human AGT Gene, and Protein	72
Figure (24)	Polymorphism in the angiotensinogen gene (M235T)	73
Figure (25)	Serial dilution of IL-6 standard	87
Figure (26)	Standard curve of human serum Interleukin-6 (IL-6)	89
Figure (27)	Serial dilution of IL-18 standard	91
Figure (28)	Standard curve of human serum Interleukin-18 (IL-18)	93
Figure (29)	Biochemical characteristics of patients with and without coronary artery disease and control groups	110
Figure (30)	Serum IL-6 level in all different studied groups	112
Figure (31)	Receiver operating characteristic (ROC) curve analysis for serum IL-6 level by ELISA in coronary artery disease group versus patients without coronary artery disease	113
Figure (32)	Serum IL-18 level in all different studied groups	115
Figure (33)	Receiver operating characteristic (ROC) curve analysis for serum IL-18 level by ELISA in coronary artery disease group versus patients without coronary artery disease	115
Figure (34)	Genotype distribution and allele frequencies of (607C/A) polymorphism in IL-18 gene for patients with and without CAD and control groups	118
Figure (35)	Genotype distribution and allele frequencies of (137G/C) polymorphism in IL-18 gene for patients with and without CAD and control groups	120
Figure (36)	Genotype distribution and allele frequencies of (I/D) polymorphism in ACE gene in patients with and without CAD and control groups	122

	Page
Figure (37) Genotype distribution and allele frequencies of M235T polymorphism in AGT gene in patients with and without CAD and control groups	124
Figure (38) Clinical and Biochemical characteristics of patients with and without coronary artery disease with different 607C/A genotypes in IL-18 gene	132
Figure (39) Clinical and Biochemical characteristics of patients with and without coronary artery disease with different 137G/C genotypes in IL-18 gene	135
Figure (40) Clinical and Biochemical characteristics of patients with and without coronary artery disease with different I/D genotypes in ACE gene	138
Figure (41) Clinical and Biochemical characteristics of patients with and without coronary artery disease with different M235T genotypes in AGT gene	140
Figure (42) Agarose gel electrophoresis of IL-18 (607C/A) polymorphism	141
Figure (43) Agarose gel electrophoresis of IL-18 (137G/C) polymorphism	141
Figure (44) Agarose gel electrophoresis of ACE (I/D) polymorphism	142
Figure (45) Agarose gel electrophoresis of ACE-Specific polymorphism	142
Figure (46) Agarose gel electrophoresis of AGT (M235T) products after digestion with <i>SfaNI</i> restriction enzyme	142