Association of Apolipoprotein A5 gene 1131T/C Polymorphism with Lipid metabolism and Insulin Resistance in Patients with Metabolic Syndrome

Thesis

Submitted for Partial Fulfillment of the Master Degree in Clinical and Chemical Pathology

BY

Mariam Saad Abdel Hameed Helal

M.B.B.Ch Faculty of Medicine; Cairo University

Supervised by

Prof. Dr. Amal AbdelWahab Mohamed

Professor of Clinical and Chemical Pathology
Faculty of Medicine
Cairo University

Prof. Dr. Salwa Tawfik Tabozada

Professor of Nutrition National Research Center

Dr. Mona Mohamed Fathy

Lecturer of Clinical and Chemical Pathology
Faculty of Medicine
Cairo University

National Research Center 2011

Abstract

Background and objectives:

Metabolic syndrome (MetS) is a cluster of the most dangerous risk factors for type 2 diabetes mellitus and cardiovascular disease (CVD). The -1131T/C is a naturally occurring variant of the apolipoprotein A5 (APOA5) gene, which has been shown to associate with Hypertriglyceridaemia and correlate with impaired glucose homeostasis. These observations prompted us to explore the frequency of APOA5 1131T/C polymorphism among patients with MetS and to assess its effects on lipid metabolism and insulin resistance.

Patients and Methods:

The study was conducted on 90 subjects divided into 2 groups: 60 MetS patients and 30 healthy controls. Fasting glucose, lipid profile, C-peptide (by ELISA method to calculate modified HOMA-IR) and APOA5 1131T/C polymorphism (PCR-RFLP) were done to all participants

Results:

The homozygous variant (CC) of APOA5 gene was present only in MetS group compared to controls with a frequency of 13.3% vs 0 %(P=0.011), while the wild genotype (TT) was more frequent among the controls (86.7% vs 56.7%, P=0.011). The mutant genotypes (TC,CC) were associated with higher risk for MetS (OR 4.97). The C allele was significantly increased in cases compared to controls (28.3% vs. 6.7%. P= 0.001) and was associated with increased risk of MetS, OR=5.535. Cholesterol, triglycerides, HDL, glucose levels and modified HOMA-IR showed significantly higher values in the TC/CC genotypes compared to TT genotype among MetS patients.

Conclusions:

Our findings strongly suggest that APOA5 gene T1131C polymorphism is associated with a higher risk for MetS, and C allele carriers (TC/CC) are more susceptible to dyslipidemia and insulin resistance among those patients.

Key words:

Apolipoprotein A5, Metabolic syndrome, Insulin resistance

ACKNOWLEDGEMENT

First of all, I would like to thank "GOD" For his grace and mercy, and for giving me the effort to complete this work.

I would like to express deep thanks and sincere gratitude to **Prof. Dr. Amal Abdel Wahab**, Prof. of Cinical Chemical pathology, Cairo University, She kindly offered me her valuable meticulous scientific help, precious time, effort and constant support.

My deepest appreciation and thanks are offered to **Prof. Dr. Salwa Tabozada**, Prof. of Nutrition, Department of Food Technology and Nutrition, National Research Center, for her kind supervision, valuable suggestions, advices and continuous support.

I am heartily thankful to my dear **Dr. Mona Fathy**, lecturer of Clinical Chemical pathology, Cairo University, for her encouragement, guidance and support from the initial to the final level which enabled me to develop an understanding of the subject.

My sincere thanks to **Dr.Maha Ibrahim**, researcher of clinical Lchemical pathology, Department of Food Technology and Nutrition, National Research Center, for her valuable advice, help and continuous supervision.

My sincere thanks to **Dr.Wael Aref**, lecturer of Internal medicine, Cairo University, for his help.

To the metabolic syndrome patients, for whose benefit the present work was conducted, I wish it would help them a step forward.

Finally, I take this opportunity to express my profound gratitude to my beloved parents, my lovely sincere husband and my dear son for their moral support and patience during completion of this work.

CONTENTS

List of tables	
List of figures	
List of abbreviations	
Introduction&aim of work	.1
Review of literature	
Chapter 1:Metabolic syndrome	.3
Chapter 2:Insulin resistance and C-peptide	.24
Chapter 3:Apolipoprotein A5	.43
Subjects and methods	.55
Results	.70
Discussion	.86
Summary and conclusion	93
Recommendations	95
References	.96
Arabic summary	.112

List of Tables

No		Page
Table(1)	Showing restriction enzyme used in the study.	67
Table(2)	Showing identified bands after UV transillumination.	68
Table(3)	Comparison of age, gender and anthropometric measures	70
	among cases and control.	
Table(4)	Comparison values assayed in all studied groups.	71
Table(5)	Genotype frequency of ApoA5gene among MetS patients	72
	control subjects.	
Table(6)	Allele frequency of ApoA5gene among MetS patients and	74
	control subjects.	
Table(7)	Comparison of assayed analytes among metabolic	76
	syndrome patients according to genotype.	
Table(8)	Show correlation between studied parameters in cases.	79
Table(9)	Comparison of the main daily nutrients intake of the	81
	control subjects and patients with MetS and RDA.	
Table(10)	Individual data of patients' group	83
Table(11)	Individual data of controls' group	85

List of Figures

NO		page
Figure(1)	Definitions of the Metabolic Syndrome.	13
Figure(2)	Signs and symptoms of metabolic syndrome(IR).	14
Figure(3)	Schematic of components of the MetS.	15
Figure(4)	Conditions associated with metabolic syndrome.	21
Figure(5)	Structure of insulin receptor.	25
Figure(6)	Diagram of insulin signal transduction pathways.	26
Figure(7)	Pathophysiology of metabolic syndrome.	26
Figure(8)	Showing the strucrure of pro insulin molecule.	36
Figure(9)	Schematic presentation of the molecular mechanism of C-peptide activity on endothelial cells and microvascular	39
	blood flow	
Figure(10)	Postulated extracellular effects of apoA5 on TG-rich lipoprotein metabolism.	46
Figure(11)	Schematic representation of the ApoA5 gene and relative single nucleotide polymorphisms (SNPs) positions	47
Figure(12)	Calibration curve of assayed C-peptide.	61
Figure(13)	Restriction enzyme analysis of Apo A5 gene polymorphism TT,TC&CC genotypes	69
Figure(14)	Median values of different analytes in the two studied groups	71
Figure(15)	Median values of modified HOMA in the two studied groups.	72
Figure(16)	Frequency distribution of apo A5 1131 genotypes among cases	73
Figure(17)	Frequency distribution of apo A5 1131 genotypes among the controls.	73
Figure(18)	Frequency distribution of TT genotype among cases and controls	74
Figure(19)	Frequency distribution of TC genotype among cases and controls.	74
Figure(20)	Frequency distribution of apo A5 1131 alleles among cases.	75
Figure(21)	Frequency distribution of apo A5 1131 alleles among the controls.	75
Figure(22)	Frequency distribution of C allele among cases and controls	76
Figure(23)	Frequency distribution of T allele among cases and controls	76

Figure(24)	Box and wisker plot of cholesterol among cases according	77
	to genotype	
Figure(25)	Box and wisker plot of TG among cases according to	77
	genotype	
Figure(26)	Box and wisker plot of HDL among cases according to	77
	genotype	
Figure(27)	Box and wisker plot of LDL among cases according to	78
	genotype	
Figure(28)	Comparison between FBG median values among cases	78
	according to genotype	
Figure(29)	Comparison between modified HOMA median values	78
	among cases according to genotype.	
Figure(30)	Scatter plot showing correlation between TG and HOMA	80
Figure(31)	Scatter plot showing correlation between FBG and HOMA.	80

List of Abbreviations

ACE Angiotensin converting enzyme

AD Alzheimer disease

AHA American Heart Association

AKT Protein kinase B

aPKC Atypical protein kinase C

APOA5 Apolipoprotein A5

ASCVD Atherosclerotic cardiovascular diseases

ATP Adenosin Triphosphate

ATP III Adult Treatment Panel

BMI Body mass index

CCA Common carotid artery

CE Cholesterol esters

CETP Cholesterol ester transfer protein

CHD Coronary heart disease

CLIA Chemiluminescence Immunoassay

CM Chylomicrons

C-peptide Connecting peptide

CVD Cardio vascular diseases

EDTA Ethylene Diamine Tetra Acetate

EGIR The European Group for the Study of Insulin Resistance

ELISA Enzyme-linked immunosorbent assays

FFAs Free fatty acids

FPG Fasting plasma glucose

FSIVGTT Frequently sampled intravenous glucose tolerance test

G/I ratio Glucose/insulin ratio

GFR Glomerular filtration rate

GIR Glucose infusion rate

GLUT 4 Glucose transporter 4

GPIHBP1 Glycosylphosphatidylinositol high-density lipoprotein

binding protein1

GSK3 Glycogen synthase kinase-3

HbA1c Glycated haemoglobin

HDL High Density Lipoprotein

HGP Hepatic Glucose Production

HIV Human Immunodeficiency Virus

HOMA Homeostasis Model Assessmen

HPA-axis Hypothalamic-pituitary-adrenal axis

hs-CRP High-sensitivity C-reactive protein

HSPG Heparan Sulfate Proteoglycan

IDF International Diabetes Federation

IGF Insulin-like growth factor

IGT Impaired glucose tolerance

IL-6 Interleukin 6

IMT Intima-media thickness

IR Insulin resistance

IRS Insulin receptor substrate

IST Insulin suppression test

LADA latent autoimmune diabetes of adults

LDL low density lipoprotein

LH Lutinizing Hormone

LPL Lipoprotein Lipase

MAPK Mitogen-Activated pathway kinase

MetS Metabolic Syndrome

NAFLD Non-alcoholic fatty liver disease

NASH Non-alcoholic steatohepatitis

NCEP National Cholesterol Education Program

NEFAs Non-Esterified fatty acids

NF-κB Nuclear Factor-κB

NIDDM Non-Insulin dependent diabetics

NO Nitric oxide

OGTT Oral Glucose Tolerance Test

PAI-1 Plasminogen activator inhibitor-1

PCOS Polycystic ovary syndrome

PCR Polymerase chain reaction

PDK Phosphoinositide-dependent protein kinase

PPAR Peroxisome proliferator-activated receptor alpha

PUFA Poly unsaturated fatty acid

QUICKI Quantitative insulin sensitivity check index

RAAS Renin-Angiotensin-Aldosterone System

RDA Recommended Daily Allowances

RFLP Restriction Fragment Length Polymorphism

RIA Radioimmunoassay

RLU Relative light units

ROS Reactive oxygen species

SHBG Sex hormone-binding globulin

S_I Index of insulin sensitivity

SNPs Single nucleotide polymorphisms

SSPG Steady-state plasma glucose

SSPI Steady-state plasma insulin

TG Triglyceride

TMB Tetramethylebenzidine

TNF-α Tumor necrosis factor-alpha

U.S United States

VLDL Very low density lipoprotein

W/H Waist/Hip ratio

WC Waist circumference

WHO World Health Organization criteria

B Beta

Introduction

Metabolic syndrome is a common disease which affects adult population at a rate of 20-25%. This condition can develop in both sexes at any time of life; however, it is age-dependent (Maasz et al., 2007). Metabolic syndrome is known to mean a major risk for several cardio- and cerebrovascular diseases as well (Todd et al., 2009). This syndrome as defined by The National Cholesterol Education Program (NCEP)and Adult Panel (ATP) Ш includes abdominal Treatment obesity, dyslipidemia ,hypertriglyceridemia and low levels of high density lipoprotein cholesterol(HDL-C), elevated blood pressure, insulin resistance and hyperglycemia (Reaven 2004). Development of metabolic syndrome depends on environmental factors including the nutritional habits, but can also be attributed to genetic susceptibility (Maasz et al., 2007). At the level of the adipocyte, impaired insulin action leads to increased rates of intracellular hydrolysis of triglycerides and is associated with complex alterations in plasma lipids (Li et al., 2009).

The recently identified apolipoprotein A5 gene (APOA5) is located approximately 27 kb downstream from the APOA1-APOC3-APOA4 gene cluster. APOA5gene is composed of 4 exons and encodes 366 amino acids. The mature APOA5 protein is expressed in the liver only and secreted into the plasma as a regulator of the triglyceride levels (*Hubacek et al. 2005*). APOA5is located on TG rich particles (chylomicrones and very low density lipoproteins – VLDL) and high density lipoprotein (HDL) particles. In its mechanism of action APOA5 was found to bind to and enhances the activity of lipoprotein lipase (LPL) (*Fruchart-Najib et al. 2004*). Recently, *Dorfmeister et al.* (2008) have demonstrated that recombinant APOA5 interacts with high affinity with the LDL receptor family members (*Hubacek et al. 2009*). In comparison to other apolipoproteins, the plasma concentration of APOA5 is low in human- about 100 μg/L-(*O'Brien et al. 2005*).

The T-1131C form, as a naturally occurring variant in the promoter region of APOA5gene, was claimed to be associated with elevated triglyceride levels and hyperinsulinemia (*Maasz et al., 2007*). So APOA5 T-1131C variant may confer risk for metabolic syndrome.

Aim of the work

The aim of this work is to explore the frequency of apolipoproteins A5 1131T/C polymorphism among patients with metabolic syndrome and to assess its effects on lipid metabolism and insulin resistance.

Review of literature metabolic syndrome

Metabolic syndrome

Introduction:

Metabolic syndrome (MetS) is a cluster of the most dangerous risk factors for type 2 diabetes mellitus and cardiovascular disease (CVD), including abdominal obesity, hypertension, hyperglycemia and dyslipidemia(*Church et al.*,2009).

One in approximately every 4 or 5 adults has developed MetS depending on the environmental conditions and daily lifestyle habits of the country. The incidence of this syndrome has been estimated to increase with age for individuals over 50 years of age. MetS affects 33.7% of Iranian, 17% of population in Palestine(*Saberi et al.*, 2011), 7.4% of Egyptian adolescents(*Aboul Ella et al.*, 2010), 27% of the population in India, nearly 30% in Europe (*Cameron et al.*, 2004), and more than 40% in the US (*Ford et al.*, 2004).

MetS has been accepted worldwide as a clinical marker for earlier detection of cardiovascular disease and type 2 diabetes (*Alberti et al.*, 2008). People with MetS are estimated to have twice the risk of developing cardiovascular disease compared to healthy individuals and a five-fold increased risk of type 2 diabetes (*Grundy et al.*, 2005). However, the underlying pathophysiological processes leading to its development are unclear and there is confusion over its conceptual definitions and criteria, allowing the medical controversy over MetS to continue. An increase in total body fat and preferential upper body accumulation of fat is independently related to insulin resistance (IR). Obese women with a greater proportion of upper body fat tend to be more insulin-resistant, hyperinsulinemic, glucose-intolerant and dyslipidemic than obese women with a greater proportion of lower body fat. Therefore, the distribution of body fat is an important correlate of MetS(*Gupta et al.*, 2010).

Review of literature metabolic syndrome

History

The term "metabolic syndrome" dates back to at least the late 1950s, but came into common usage in the late 1970s to describe various associations of risk factors with diabetes that had been noted as early as the 1920s (*Gupta et al.*,2010).

- The Marseilles physician Dr. Jean Vague, in 1947, observed that upper body obesity appeared to predispose to diabetes, atherosclerosis, gout and calculi.
- In 1977, Haller used the term "metabolic syndrome" for associations of obesity, diabetes mellitus, hyperlipoproteinemia, hyperuricemia, and hepatic steatosis when describing the additive effects of risk factors on atherosclerosis.
- In 1977 and 1978, Gerald B. Phillips developed the concept that risk factors for myocardial infarction concur to form a "constellation of abnormalities" (i.e., glucose intolerance, hyperinsulinemia, hypercholesterolemia, hypertriglyceridemia, and hypertension) that is associated not only with heart disease but also with aging, obesity and other clinical states. He suggested there must be an underlying linking factor, the identification of which could lead to the prevention of cardiovascular disease; he hypothesized that this factor was sex hormones.
- In 1988, Gerald M. Reaven proposed insulin resistance as the underlying factor and named the constellation of abnormalities Syndrome X. Reaven did not include abdominal obesity, which has also been hypothesized as the underlying factor, as part of the condition.