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

Thesis

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BY

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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

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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 Treatment Panel (ATP) III includes abdominal 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.

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).***

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.