Methylene tetrahydrofolate reductase gene mutation; A1298C and its relation to coronary artery disease

Thesis

Submitted for partial fulfillment of M.D degree in
Clinical and Chemical Pathology

By

Rasha Tharwat Mohamed Ali

M.B, B.Ch (Clinical and Chemical Pathology)
Ain Shams University

Supervised by

Prof. Dr. Iman Mohamed Amin Omar

Professor of clinical and chemical pathology
Faculty of Medicine-Ain Shams University

Dr. Amal Abd Al Hameed Mohamed

Assistant professor of clinical and chemical pathology
Faculty of Medicine-Ain Shams University

Dr. Mona Ahmed Ismail

Assistant professor of clinical and chemical pathology
Faculty of Medicine-Ain Shams University

Faculty of Medicine
Ain shams University
2010

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Acknowledgment

First and for most, thanks to Allah "The Most Merciful".

I would like to express my most gratitude to ***Prof. Dr. Iman Mohamed Amin Omar****, Professor of Clinical Pathology, Faculty of Medicine, Ain Sham University, for giving me the privilege of working under her meticulous supervision, and for her constant support, enthusiastic guidance and encouragement.*

My sincere thanks and appreciation are also to ***Dr. Amal Abd-Al Hameed Mohamed****, Assistant Professor of Clinical Pathology, Ain Shams University, for great help and big support and continuous encouragement.*

I also owe my deep thanks and gratitude to ***Dr. Mona Ahmed Ismail****, Assistant Professor of clinical Pathology, Ain Shams University, for her great support, patience and fruitful comments.*

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List of Acronyms

ACE: Angiotensin Converting Enzyme
APC: Activated Protein C
Apo: Apolipoprotein
aPTT: activated Partial Thromboplastin Time
AT: Antithrombin
ATIII: Antithrombin III
CAD: Coronary Artery Disease
CBC: Complete Blood Count
CBS: Cystathionine ß-Synthase
COC: Combined Oral Contraceptive pills
CRP: C- Reactive Protein
DNA: Deoxyribonucleic Acid
EBCT: Electron-Beam Computed Tomography
ECG : Electocardiogrm
EPCR: Endothelial Cell PC Receptor
FRET: Fluorescence Resonance Energy Transfer
FH: Familial Hypercholesterolemia
FV : Factor V
FVa: activated Factor V
FVLeiden : Factor V Leiden
FVIIIa: activated Factor VIII
FXa: activated Factor X
HDL: High Density Lipoproteins
HRT: Hormone Replacement Therapy
Hcy: Homocysteine
hs-CRP : high sensitivity C- Reactive Protein
Hs: Highly significant
K2-EDTA: Potassium Ethylene Diamine Tetra-Acetic acid
LC: Light Cycler
LDL: Low Density Lipoproteins

List of Acronyms (continued)

LDLR : Low Density Lipoproteins Receptor
Lp (a): Lipoprotein-a
MGPs: Magnetic Glass Particles
MTHFR: Methylyne Tetrahydrofolate Reductase
MI: Myocardial Infarction
NADPH: Nicotinamide Adenine Dinucleotide Phosphate Hydrogen
Ns: None sigificant
NTDs: Neural Tube Defects
PAI-1: Plasminogen Activator Inhibitor-1
P C: Protein C
PCR : Polymerase Chain Reaction
P S: Protein S
PLP: Pyridoxal 5' Phosphate
PT: Prothrombin Time
RNA: Ribonucleic Acid
S: Significant
SAH: S-Adenosyl Homocysteine
SAM: S-Adenosyl Methionine
sICAM-1: soluble Intercellular Adhesion Molecule type 1
Taq : Thermostable thermophilus aquaticus
TF : Tissue Factor
TFPI: tissue factor pathway inhibitor
tHcy: total Homocystein
TM: Thrombomodulin
Tm: melting Temprature
VO2 max :maximal oxygen consumption
VTE: Venous Thromboembolism
Vwf: Von Willebrand factor

INTRODUCTION

Thrombophilia is tendency to develop thrombosis as a consequence of predisposing factors that may be genetically determined, acquired, or both ***(Tripodi and Mannucci, 2001).*** Thrombotic disease can be classified into venous and arterial thrombosis. Both types of thrombosis are considered as distinct disease states that are characterized by different pathogenic mechanisms and underlying risk factors ***(Segers et al., 2007).***

An example of arterial thrombophilia is coronary artery disease (CAD). It is considered to be a leading cause of death world-wide, and most cases have a complex, multifactorial etiology that includes a substantial heritable component. Therefore attention have been brought to identification of new genes involved in CAD that may give information about pathogenesis and provide new therapeutic targets ***(Farrall et al., 2006).***

5, 10-Methylene tetrahydrofolate reductase (MTHFR) is a key enzyme of the homocysteine/methionine metabolic pathway. It catalyzes the conversion of 5,10-methylene tetrahydrofolate to 5-methyl tetrahydrofolate, the major methyl donor for the homocysteine remethylation pathway to methionine. Two common polymorphisms in the MTHFR gene, C677T and A1298C, have been described.

The C677T variant lies in exon 4 at the folate binding site of the MTHFR gene and results in the substitution of an alanine by a valine residue. This substitution causes reduced activity and thermolability of MTHFR and results in lower levels of 5-methyl tetrahydrofolate, an accumulation of 5,10-methylene tetrahydrofolate and increased plasma homocysteine levels. The A1298C variant results in substitution of glutamic by alanine residue. A1298C variant lies in exon 7 and results in a decrease in MTHFR enzymatic activity that is more pronounced in homozygotes (*C/C*) than heterozygotes (*A/C*), although it does not result in a thermolabile protein. Furthermore, unlike the MTHFR C677T variant, the A1298C variant allele is not associated with lower folate plasma level. However, compound heterozygotes for the A1298*C*and C677T genotypes tend to have increased plasma homocysteine levels and decreased plasma folate levels similar to C677T homozygotes ***(Cicek et al., 2004).***

Elevated levels of plasma homocysteine, an independent risk factor and a strong predictor of mortality in patients with coronary artery disease (CAD), can result from nutritional deficiencies or genetic errors, including methylenetetrahydrofolate reductase (MTHFR) C677T alone or in combination with A1298C polymorphism ***(Freitas et al., 2008).***

On the other hand though studies have shown that CAD patients have significantly increased prevalence with the 1298C MTHFR allele yet

hyperhomocysteinemia is not associated with the cardiovascular risk of the A1298C mutation. It is rather suggested that the 1298C allele of MTHFR presents a moderate genetic association with early onset CAD, independently from hyperhomocysteinemia ***(Szczeklik et al., 2001)***

AIM OF WORK

To study the role of MTHFR mutation; A1298C as genetic risk factor in patients with coronary artery disease and its relation to other standard risk factors.

THROMBOPHILIA

The widely accepted definition of thrombophilia is tendency to develop thrombosis as a consequence of predisposing factors that may be genetically determined, acquired, or both. This includes situations that are apparently not directly linked to the haemostatic system (e.g., hyperhomocysteinemia) ***(Tripodi and Mannucci, 2001).***

PATHOGENESIS

As early as in the mid 1800s, the German pioneer in the field of haemostasis, **Rudolf Virchow**, postulated that three major causes contribute to thrombin formation (Virchow's triad):

- Changes in the blood composition.
- Changes in the vessel wall.
- Changes in the blood flow.

It has been suggested that arterial thrombosis is dominated by vessel wall changes (atherosclerosis), the known risk factors for venous thrombosis can be divided into those that are attributable to changes in the blood flow (stasis) or to changed blood composition ***(Virchow, 1856).***

It has now become widely accepted that thrombosis is a multifactorial disease that may occur as the result of the interplay between two or more genetic, environmental (acquired) or behavioral risk factors. These factors together are capable of synergistically passing a certain anticoagulant threshold, thereby tipping the natural haemostatic balance between pro- and anticoagulant forces. When the threshold is passed, the natural anticoagulant systems are insufficient to balance the procoagulant factors, resulting in the development of a thrombotic event ***(Segers et al., 2007).***

Anticoagulant Mechanisms That Regulate Haemostasis

The formation of a stable plug by platelet aggregation and fibrin polymerization following vascular injury is a very rapid and important process that prevents extensive blood loss.

Two major systems can be discriminated that provide efficient control of the activity of pro- and anticoagulant pathways. A first system comprises circulating inhibitors (e.g. tissue factor pathway inhibitor (TFPI), antithrombin, α2 macroglobulin, antitrypsin) which can directly neutralize activated coagulation factors. A second important negative regulatory pathway of the coagulation cascade is the protein C (PC) pathway ***(Morrissey, 2001, Ye and Goldsmith, 2001, and Dahlbäck and Villoutreix, 2005).***

A key event in the initiation of the PC pathway is the formation of the thrombin-thrombomodulin complex. Thrombomodulin (TM) is a transmembrane protein present on intact endothelium, primarily that of the smaller vessels, that acts as a thrombin receptor by binding to thrombin exosite I and provides a binding platform for the substrate protein C. This thrombin exosite I plays an important role in the thrombin-mediated recognition and activation of factor V, factor VIII and fibrinogen (Figure 1) ***(Fuentes-Prior et al., 2000).***

As a consequence, the procoagulant properties of thrombin molecules, that escape a site of ongoing coagulation and migrate into the microvasculature, are lost upon binding to TM. In addition, TM-binding causes conformational changes near the active site of thrombin which alter the substrate specificity of thrombin such that thrombin becomes an anticoagulant protein that can efficiently activate PC in a process that is enhanced by the endothelial cell PC receptor (EPCR) ***(Fuentes-Prior et al., 2000, and Koeppe et al., 2005).***