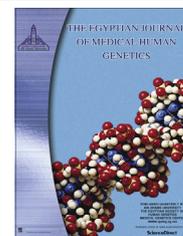




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ORIGINAL ARTICLE

## Prevalence of thrombophilic gene polymorphisms (FVL G1691A and MTHFR C677T) in patients with myocardial infarction

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### KEYWORDS

Thrombophilia;  
MI;  
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**Abstract** *Background:* Inherited thrombophilia may be caused by mutations, polymorphisms in a variety of genes mainly involved in haemostatic pathways.

Aim of the study, was to find the prevalence of thrombophilic gene factor V Leiden (FVL) and methylene tetrahydrofolate reductase (MTHFR) gene polymorphism in patients with myocardial infarction (MI), aiming at early diagnostic methods and guiding preventive procedures.

*Subjects and methods:* This study was carried on 30 patients who survived their first MI as compared to 15 healthy volunteers. Patients and controls were subjected to history, physical examination. Factor VL G1691A and MTHFR C677T genotypes were determined by RT PCR.

*Results:* The prevalence of heterozygous FVL GA genotype was significantly higher among MI patients as compared to the control group. The prevalence of mutant homozygous AA was significantly higher in MI patients as compared to control. The low risk cases had a higher frequency of GA genotype as compared to high risk cases.

As regards MTHFR C677T gene polymorphism, the prevalence of heterozygous MTHFR C677T CT genotype showed significant increase in MI patients compared with the control group. The prevalence of mutant homozygous TT genotype was significantly higher in MI patients as compared to the control group. The low risk cases had a higher frequency of heterozygous MTHFR C677T CT genotype than high risk cases.

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*To conclude:* The prevalence of heterozygous (FVL G1691A) and MTHFR C677T gene polymorphisms was significantly increased in MI patients compared with the control group and these gene polymorphisms are probably risk factors for myocardial infarction among Egyptian cases especially if integrated with other environmental and genetic risk factors. We recommended screening high risk patients for this polymorphism and the use of specific thromboprophylaxis to prevent recurrent thrombotic disease.

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## 1. Introduction

Myocardial infarction (MI) is one of the leading causes of death worldwide. MI has a high prevalence especially in the elderly [1]. However, the age of MI onset has decreased because of environmental risk factors and genetic polymorphisms [2].

Myocardial infarction reflects cell death caused by myocardial ischemia. Myocardial infarction may occur with typical symptoms, or even without symptoms, being detected only by ECG, cardiac biomarker elevations, or cardiac imaging [3]. Myocardial infarction is influenced by genetic and environmental factors. Family history of coronary artery disease, obesity, inflammation, hypertension, dyslipidemia, diabetes, and smoking, all are factors that constitute a major risk factor for MI [4].

Many susceptible gene candidates have been confirmed to be highly associated with the onset and prognosis of MI. Even some well-known etiologic risk factors probably have genetic backgrounds [5].

Thrombophilia refers to a series of acquired and inherited conditions that confer a tendency to thrombus formation. Inherited thrombophilia may be caused by mutations and polymorphisms in a variety of genes mainly involved in haemostatic pathways. These genes are included in coagulation, fibrinolysis, and platelet glycoprotein receptor function and homocysteine metabolism. Thrombophilic genes are well recognized as common risk factors for venous thromboembolic disorders. However, the relationship between inherited thrombophilias and arterial disease is still unresolved [6,7].

The exact relationship between thrombophilia and MI is not well established. In the last years the attention has been focused on polymorphisms influencing some biological functions [8]. Among the candidate markers for inherited cardiovascular disease (CVD) risk, are variations in the genes for blood coagulation factors V (FV) and methylene tetrahydrofolate reductase (MTHFR) [9]. Thrombophilia may contribute to the development of MI in a specific group of young patients [10].

FV Leiden mutation (FVL) is caused by single point mutation of guanine to adenine transition at nucleotide 1691 in the factor V gene. This mutation leads to a substitution of amino acid arginine by glutamine at position 506. This results in failure of activated protein C (APC) to recognize a major cleavage site on factor V, which led to increased thrombin generation [11]. Carriers of FVL develop hypercoagulability. Most homozygotes for FVL were reported to get at least one venous thromboembolic (VTE) event in their life time [12].

Methylene tetrahydrofolate reductase (MTHFR) is a key enzyme in homocysteine (Hcy) metabolism. MTHFR is responsible for the circulating form of folate, 5-methyl-tetrahydrofolate, which provides methyl groups for the remethylation of homocysteine to methionine [13]. Hyperhomocysteinemia is the consequence of decreased activity of MTHFR and is associated

with a slight increase in the risk for thrombosis [14]. A common mutation (C677T) has been identified in the MTHFR gene. It results from the alteration of alanine to valine amino acid. Common C677T polymorphism in MTHFR has been associated with an increased risk for the development of cardiovascular disease, Alzheimer's disease, depression in adults, and neural tube defects in the fetus [15]. The population frequencies of homozygous MTHFR C677T vary in the United States from 44.6% in Caucasians, 42% in Hispanics and 25.6% African American [16].

The present work was designed to study the prevalence of the thrombophilic (Factor VL G1691A and MTHFR C677T) gene polymorphisms in patients with myocardial infarction, aiming at early diagnostic methods and guiding preventive procedures.

## 2. Subjects and methods

This cross sectional study was conducted at Internal Medicine Department at Al-Zahraa University Hospital and Sayed Galal University Hospital, Cairo, Egypt.

The study was carried on two groups. The patient group consisted of 30 patients who survived their first acute myocardial infarction based on clinical, electrocardiographic (ECG) and laboratory data (24 males, 6 females, and mean age  $45.5 \pm 4.8$  years).

A control group ( $n = 15$ ) consisted of healthy volunteers, (11 males, 4 females, mean age  $43.2 \pm 4.3$  years), Control subjects were free of clinical manifestations of coronary artery disease; none of them had history of venous thrombosis, hypertension or diabetes.

Cases were further classified into high risk group with two or more risk factors and low risk group with no or only one risk factor.

Important risk factors are previous cardiovascular disease, old age, tobacco smoking, high serum levels of low-density lipoprotein (LDL) cholesterol, triglycerides and low levels of high density lipoprotein (HDL) cholesterol, diabetes, high blood pressure, lack of physical activity, obesity, chronic kidney disease, and chronic high stress [17,18].

The exclusion criteria for all groups include: history of previous venous thrombosis, liver disease, or any collagen diseases, presence of any form of cancer, diabetes, history of acquired causes of thrombophilia, such as antiphospholipid antibody syndrome, and recent surgery or trauma.

The work was done after taking acceptance of all patients and controls to share in the study as well as acceptance of ethics committee of the university.

The works have been carried out in accordance with the code of Ethics of the World Medical Association (Declaration of Helsinki) for experiments involving humans.

All patients and controls were subjected to the following:

- 1- All participants gave a complete medical history that included cardiovascular risk factors, such as obesity, smoking habits, hypertension, diabetes, dyslipidemia, and family history of MI. Subjects were deemed dyslipidemic when their total cholesterol concentration was above 220 mg/dl or their triglyceride concentration was above 200 mg/dl, or if they were receiving lipid-lowering drugs. Smoking history was coded by grouping patients into non-smokers who had never smoked and those who were or had been smokers. A family history of MI was defined based on its presence in first-degree relatives.
- 2- Complete general examination including blood pressure measurement and measurement of body mass index (BMI), body mass index is defined as the individual's body weight divided by the square of his or her height in meter ( $\text{kg}/\text{m}^2$ ).
- 3- Electrocardiographic examination (ECG).
- 4- Routine laboratory investigations which include fasting blood sugar, liver function and kidney function tests, complete blood picture and lipid profiles.
- 5- Factor VL G1691A and MTHFR C677T genotypes were determined by RT PCR.

### 2.1. Laboratory investigations

Venous blood samples were obtained in the morning after an overnight fasting (10 h) from all patients and controls. Blood samples were divided into two portions as follows:

- First portion, 2 ml was put in EDTA tube for genetic analysis by RT (PCR)
- Second portion, 5 ml was put in a plain tube, left to clot then centrifuged at 1500 rpm for 10 min. Serum was separated and used for estimation of biochemical variables.

This included: glucose, creatinine, cholesterol, triglycerides, high density lipoprotein-cholesterol (HDL-c). These were measured using COBAS® INTEGRA 400 plus, Autoanalyzer using Kits supplied from Roche Diagnostics, Mannheim, Germany (as routine follow up).

### 2.2. DNA extraction and molecular genetic screening

Genomic DNA was extracted from whole EDTA blood by Roche Diagnostics high pure PCR template preparation Kit (Cat.-No. 11 796 828 001). The following loci of genes encoding for proteins involved in blood coagulation were analyzed: (1) factor V Leiden mutation and (2) MTHFR C677T.

Eluted DNA was stored in screw cap tubes at  $-80^\circ\text{C}$  and thawed once at time of use.

### 2.3. Detection of factor V Leiden gene polymorphism

FVL G1691 A, Kit Cat No (03 610 179 001) supplied by Roche Diagnostics GmbH, Mannheim, Germany, was used for detection of factor V Leiden mutation in the extracted DNA samples. The Kit is tested on the Light Cycler 2 Instrument (RT, PCR).

#### 2.3.1. Detection

1. A 222 bp fragment of the factor V gene is amplified from human genomic DNA using specific primers.
2. The resulting PCR fragments are analyzed with hybridization probes labeled with Light Cycler Red 640 (detected in channel 640n). The genotype is identified by running a melting curve with specific melting points ( $T_m$ ) The wild type factor V DNA exhibits a  $T_m$  of  $66.0^\circ\text{C}$  in channel 640. The mutant Factor V exhibits a  $T_m$  of  $57^\circ\text{C}$  in channel 640.

The specimen preparation was performed according to the workflow procedures described in the package insert.

The reagent include, 2  $\mu\text{l}$  of the light cycler factor V Leiden mutation detection mix, 2  $\mu\text{l}$  of the light cycler factor V reaction mix and 11  $\mu\text{l}$  of PCR grad water supplied with the kit. Mix gently and transfer to a Light Cycler Capillary (Light Cycler 2 Instrument). 5  $\mu\text{l}$  of sample or control DNA was added to each capillary or well for a final reaction volume of 20  $\mu\text{l}$ . (Negative control: template DNA was replaced by Water) (Positive control: contains provided control DNA along with the kit) then run was start.

#### 2.3.2. Light cycler programming

The protocol consists of four programme steps:

Denaturation, one cycle  $95^\circ\text{C}$  for 10 min; Cycling, 45 cycles of  $95^\circ\text{C}$  for 10 s,  $58^\circ\text{C}$  for 10 s and  $72^\circ\text{C}$  for 15 s; Melting curve analysis, one cycle of  $95^\circ\text{C}$  for 20 s,  $40^\circ\text{C}$  for 20 s then  $85^\circ\text{C}$  for 0 s; followed by a single Cooling cycle  $40^\circ\text{C}$  for 30 s.

#### 2.3.3. Specimen result

The assay result for the FV Leiden data in channel 640 "Tm Calling" Analysis mode (LightCycler 2 Instrument) should always exhibit a melting curve profile for the homozygous wild-type, homozygous mutant or heterozygous genotype. The negative control must show no signal (Fig. 1).

#### 2.3.4. Interpretation of data

Homozygous G/G variant, the wild type factor V DNA exhibits a  $T_m$  of  $66^\circ\text{C}$  in channel 640

Heterozygous G/A variant exhibits two peaks one at  $66^\circ\text{C}$  and another one at  $57^\circ\text{C}$  in channel 640

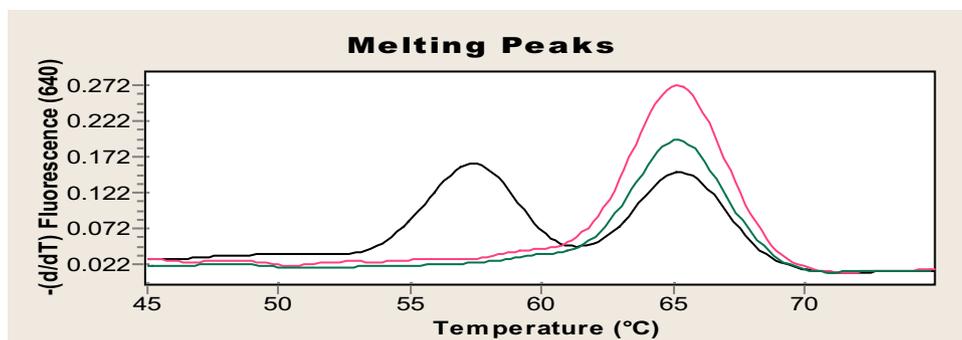
Homozygous A/A variant: The mutant factor V Leiden exhibits a  $T_m$  of  $57^\circ\text{C}$  in channel 640.

### 2.4. Detection of MTHFR C677T gene polymorphism

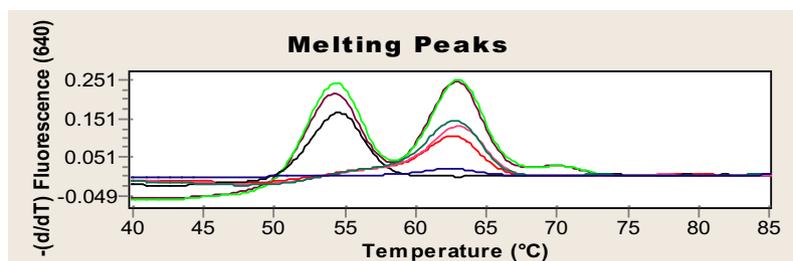
The Light Mix Kit (MTHFR C677T) Cat No (40-0095-16) supplied by Roche Diagnostics GmbH, Mannheim, Germany was used for detection of MTHFR C677T polymorphic loci in the extracted DNA samples. The Kit is tested on the Light Cycler 2 (RT PCR).

#### 2.4.1. Detection

- 1- A 233 bp fragment of the human MTHFR gene is amplified with specific primers. The resulting PCR fragments are analyzed with hybridization probes labeled with Light Cycler Red 640 (detected in channel 640n).



**Figure 1** Light cycler 2 instrument Roch master, sample data for factor V leiden at channel 640.



**Figure 2** Light cycler 2 instrument Roch master, sample data for MTHFR C677T at channel 640.

2- The genotype is identified by running a melting curve with specific melting points ( $T_m$ ). The wild type MTHFR C677 DNA exhibits a  $T_m$  of 63.0 °C in channel 640. The mutant MTHFR 677 exhibits a  $T_m$  of 54.5 °C in channel 640.

#### 2.4.2. Preparation of light cycler reaction mix

The reaction mix for each sample (single reaction mix) consists of the following: 7.4  $\mu$ L PCR grade water, (provided with the Roche Master kit), 1.6  $\mu$ L magnesium solution (Roche Fast-Start kit) + 4  $\mu$ L reagent mix (primers and probes) + 2  $\mu$ L Roch master (enzymes). Mix gently, and transfer 15  $\mu$ L each of the reaction mix to a LightCycler Capillary (LightCycler 2 Instrument). Add 5  $\mu$ L of sample or control DNA to each capillary or well for a final reaction volume of 20  $\mu$ L (Negative control: template DNA was replaced by Water) (Positive control: contains provided control DNA).

#### 2.4.3. Light cycler programming

The protocol consists of four programme steps:

Denaturation, one cycle 95 °C for 10 min; Cycling, 45 cycles of 95 °C for 5 s, 60 °C for 10 s and 72 °C for 15 s; Melting curve analysis, one cycle of 95 °C for 20 s, 40 °C for 20 s then 85 °C for 0 s; followed by a single Cooling cycle 40 °C for 30 s.

#### 2.4.4. Data analysis

View MTHFR C677T data in channel 640 “ $T_m$  Calling” Analysis mode (LightCycler 2 Instrument). The negative control must show no signal (Fig. 2).

#### 2.4.5. Interpretation of data

Homozygous C/C variant, the wild type MTHFR C677 DNA exhibits a  $T_m$  of 63 °C in channel 640.

Heterozygous C/T variant exhibits two peaks one at 54.5° and another one at 63 °C in channel 640.

Homozygous T/T variant: The mutant MTHFR 677T exhibits a  $T_m$  of 54.5 °C in channel 640.

#### 2.5. Statistical analysis

Data were analyzed using SPSS statistical package for Social Sciences version 13 for Windows. Quantitative variables were described using mean ( $\pm$ ) standard deviation and categorical data by using percentage. Comparison between groups was done using Chi square ( $\chi^2$ ) test for qualitative variables,  $t$ -test for normally distributed variables.  $P$  value < 0.05 was considered statistically significant.

### 3. Results

The baseline demographics and clinical characteristics of the included patients are shown in Table 1. The majority of the patients were men (80%). 40% of cases were below the age of 45 years. Obesity was observed in 30% of patients. There was history of smoking in 73.3% of patients. Hypertension was detected in 30% of patients. Family history of MI or ischemic heart disease (IHD) was detected in 40% of patients. Hypercholesterolemia was observed in 43.3% of patients.

Regarding risk factors, cases were classified into high risk group with two or more risk factors and low risk group with no or only one risk factor.

The classical risk factors, hypertension, obesity, smoking and family history were significantly higher in the MI patient group than in the control. The value of HDL-cholesterol was significantly lower in patients than in controls, although serum triglycerides, total cholesterol, LDL-cholesterol levels were found to be significantly higher in the patient group, (Table 2).

Parameter		Myocardial infarction ( <i>n</i> = 30)
Gender	Male	24 (80%)
	Female	6 (20%)
Age	< 45 years	12 (40%)
	> 45 years	18 (60%)
BMI (kg/m <sup>2</sup> ) <i>N</i> (%)	Mean ± SD	28.9 ± 3.1
	Obese	9 (30%)
	Non obese	21 (70%)
Smoking: <i>N</i> (%)	Smoker	22 (73.3%)
	Non smoker	8 (26.7%)
Blood pressure: <i>N</i> (%)	Normal	21 (70%)
	Hypertensive	9 (30%)
Family history of MI or IHD	Absent	18 (60%)
	Present	12 (40%)
Hypercholesterolemia No (%)		13 (43.3%)

The percentage distribution of the different genotypes for FV Leiden (G1691A) in the study population showed that the GG genotype variant (the wild type) was the most prevalent one, Fig. 3 followed by GA (heterozygous), Fig. 4 and then the (mutant homozygous) AA variant respectively, Fig. 5. There was significant increase in the heterozygous FVL GA genotype among MI patients (26.7%) compared with

the control group (6.7%). The prevalence of homozygous AA was significantly increased in MI patients (3.3%) as compared to the control group (0%). Additionally the allele frequency of the mutated A allele in MI patients (16.7%) showed significant increase when compared with the control group (3.3%), as shown on Tables 3 and 4.

As regards the MTHFR C677T gene polymorphism, The percentage distribution of the different genotypes in the study population showed that, the CC genotype variant (the wild type), Fig. 6 was the most prevalent one followed by CT (heterozygous), Fig. 7 and then the (mutant homozygous) TT variant respectively, Fig. 8. There was a significant increase in the heterozygous MTHFR:C677T CT genotype in MI patient group (53.3%) compared with the control group (13.3%). The prevalence of homozygous TT was significantly increased in MI patients (6.7%) as compared to the control group (0%). Additionally the allele frequency of the mutated T allele in MI patients (33.3%) showed significant increase when compared with the control group (6.7%), as shown in Tables 3 and 4.

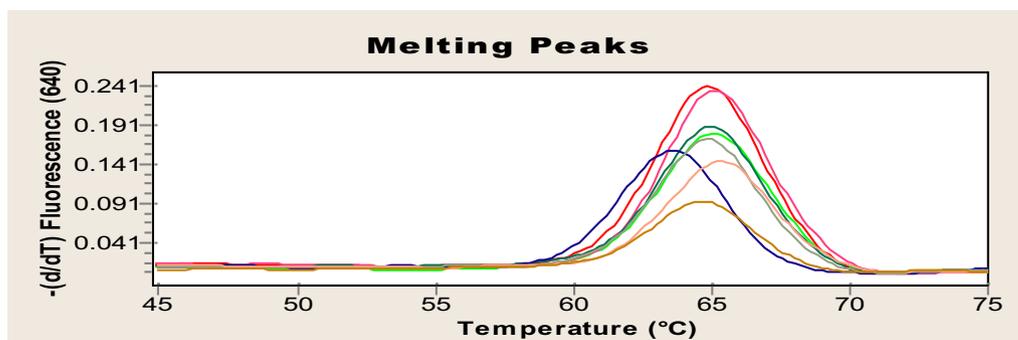
Table 5 represents distribution of FVL genotypes among low risk and high risk subjects and control group. Results showed that low risk cases had a higher frequency of GA genotype as compared to high risk cases and control (28.6%, 25%, and 6.7%) respectively. The prevalence of homozygous AA was also significantly increased in low risk cases (7.1%). None of the high risk cases or control group had a genotype AA.

Table 6 represents distribution of MTHFR C677T genotypes among low risk and high risk subjects and control group. Results showed that low risk cases had a higher

**Table 2** Comparison of clinical and biochemical data of the studied groups.

Parameter	Healthy control ( <i>n</i> = 15)	Myocardial infarction ( <i>n</i> = 30)	<i>P</i> value
Age (years)	43.2 ± 4.3 years	45.5 ± 4.8 years	> 0.05
BMI (kg/m <sup>2</sup> )	25.2 ± 2.5	28.9 ± 3.1	< 0.05
Systolic blood pressure (mmHg)	122 ± 8	145 ± 15	< 0.05
Diastolic blood pressure (mmHg)	78 ± 7	88 ± 10	< 0.05
Total cholesterol (mg/dl)	156 ± 18	195 ± 25	< 0.05
Triglycerides (mg/dl)	115 ± 20	155 ± 28	< 0.05
HDL-C (mg/dl)	44 ± 2.1	39 ± 3.1	< 0.05
LDL-C (mg/dl)	80 ± 13.5	105 ± 18.1	< 0.05
Smoking <i>N</i> (%)	4 (26.6%)	22 (73.3%)	< 0.05
Family history of MI	1 (6.6%)	12 (40%)	< 0.05

*P* < 0.05 is statistically significant, *P* > 0.05 is not statistically significant.



**Figure 3** Cases with wild type (homozygous Factor V) with GG genotype.

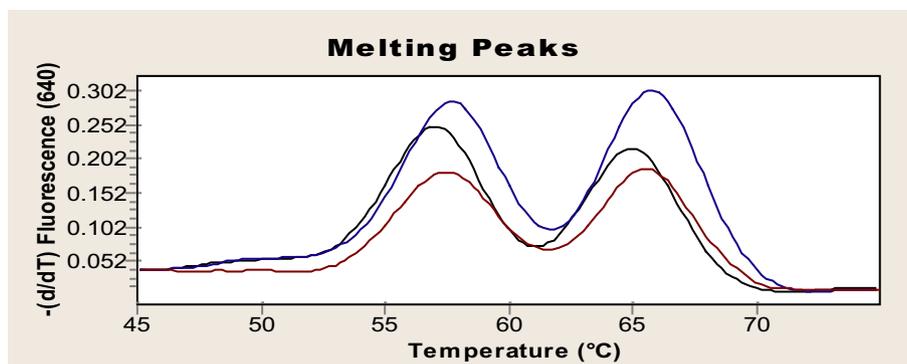


Figure 4 Cases with heterozygous factor V Leiden mutation with GA genotype.

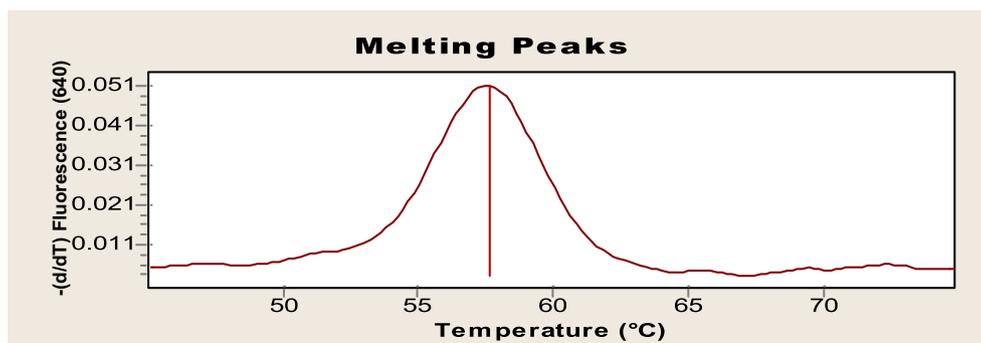


Figure 5 Case with mutant (homozygous Factor V Leiden mutation) with AA genotype.

Table 3 Genotype frequencies in patients and controls.

Mutation/polymorphism	Genotype	Patients $N = 30$	Controls $N = 15$	$P$ value
		$N\%$	$N\%$	
Factor V:G1691A (FV Leiden)	GG	21/30 (70%)	14/15 (93.3%)	< 0.05
	GA	8/30 (26.7%)	1/15 (6.7%)	< 0.05
	AA	1/30 (3.3%)	0/15 (0%)	< 0.05
MTHFR:C677T	CC	12/30 (40%)	13/15 (86.7%)	< 0.05
	CT	16/30 (53.3%)	2/15 (13.3%)	< 0.05
	TT	2/30 (6.7%)	0/15 (0%)	< 0.05

$P < 0.05$  is statistically significant.

Table 4 Allele frequency of the mutations/polymorphisms in patients and controls.

Mutation/polymorphism	Allele	Patients $N = 60$	Controls $N = 30$	$P$ value
		$N\%$	$N\%$	
Factor V:G1691A FV Leiden	G	50/60 (83.3%)	29/30 (96.7%)	< 0.05
	A	10/60 (16.7%)	1/30 (3.3%)	< 0.05
MTHFR:C677T	C	40/60 (66.7%)	28/30 (93.3%)	< 0.05
	T	20/60 (33.3%)	2/30 (6.7%)	< 0.05

$P < 0.05$  is statistically significant.

frequency of heterozygous MTHFR:C677T CT genotype as compared to high risk cases and control (64.3%, 43.75%, and 13.3%) respectively. The prevalence of homozygous TT

genotype was significantly increased in low risk cases (14.3%). None of the high risk case or the control group had genotype AA.

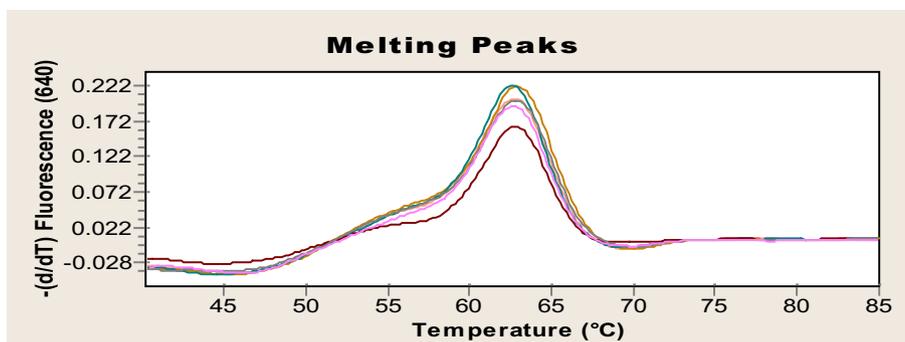


Figure 6 Cases with wild type (homozygous MTHFR C677T) with CC genotype.

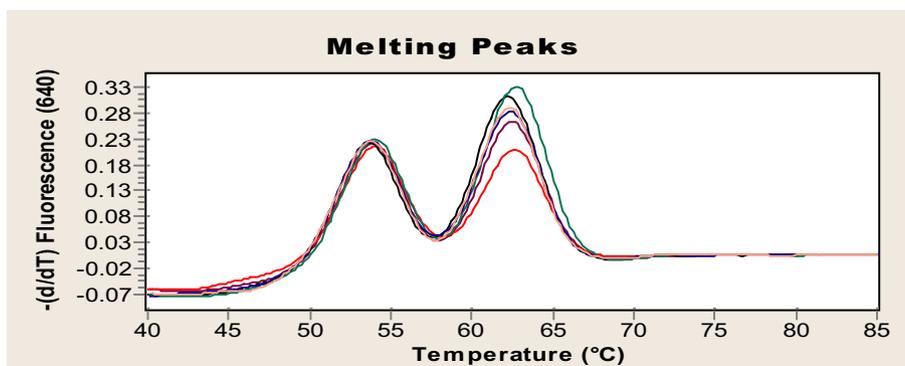


Figure 7 Cases with heterozygous form of MTHFR (C677T) with CT genotype.

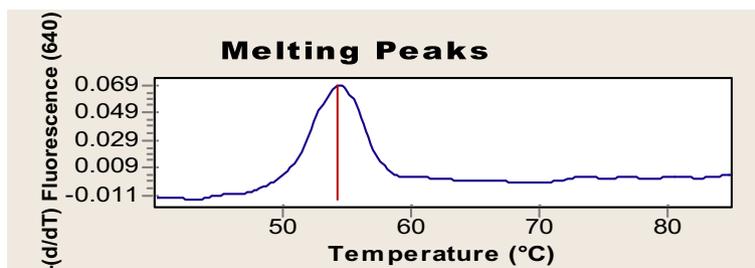


Figure 8 Case with mutant (homozygous form of MTHFR C677T) with T/T genotype.

**Table 5** Distribution of FVL genotypes among low risk and high risk subjects and control group.

Studied groups	Factor V Leiden genotype		
	GG N (%)	GA N (%)	AA N (%)
Control group (n = 15)	14/15 (93.3%)	1/15 (6.7%)	0/15 (0%)
Total cases (n = 30)	21/30 (70%)	8/30 (26.7%)	1/30 (3.3%)
High risk cases (n = 16)	12/16 (75%)	4/16 (25%)	0/16 (0%)
Low risk cases (n = 14)	9/14 (64.3%)	4/14 (28.6%)	1/14 (7.1%)

#### 4. Discussion

Myocardial infarction may be a minor event in a lifelong chronic disease, it may even go undetected, but it may also be a major catastrophic event leading to sudden death [3]. Accurate diagnosis of MI has life-saving implications and requires a careful assessment of both the patient's history and

the findings on physical examination, 12-lead ECG, and cardiac biomarker assays (cardiac-specific troponins T or I, or muscle and brain fraction of creatine kinase (CK-MB) [19].

Genetic factors play a role in the development of coronary artery disease. Among these genetic factors is the thrombophilic gene. The association between mutations/polymorphisms in thrombophilic gene and myocardial infarction as well as their

**Table 6** Distribution of MTHFR:C677T genotypes among low risk and high risk subjects and control group.

Studied groups	MTHFR:C677T genotype		
	CC N (%)	CT N (%)	TT N (%)
Control group (n = 15)	13/15 (86.7%)	2/15 (13.3%)	0/15 (0%)
Total cases (n = 30)	12/30 (40%)	16/30 (53.3%)	2/30 (6.7%)
High risk cases (n = 16)	9/16 (56.25%)	7/16 (43.75%)	0/16 (0%)
Low risk cases (n = 14)	3/14 (21.4%)	9/14 (64.3%)	2/14 (14.3%)

role as risk factors contributing to disease pathogenesis and progression needs clarification.

In this study, classical risk factors, hypertension, obesity, smoking and family history were significantly high in the MI patient group. The value of HDL-cholesterol was significantly lower in patients than in controls, although serum triglycerides, total cholesterol, LDL-cholesterol, levels were found to be significantly higher in the patient group. These results were in agreement with Segev et al. [10].

In our study BMI was higher among MI patients than control and obesity was observed in 30% of patients. This could be explained by the fact that obesity is a risk factor for ischemic heart disease. Several studies reported that elevations of both BMI and waist circumference are associated with increased incidence of cardiovascular events [20,21].

In the present study we reported high prevalence of cigarette smoking among patients groups (73.3%). These results were in agreement with the results obtained by Heuschmann et al. [22]. Rosendaal et al. demonstrated that interaction of the FVL polymorphism with environmental factors such as smoking increases the risk of MI [23].

In the present study we found that 30% of patients were hypertensive. These results were in agreement with the results obtained by Lewington et al. [24]. In addition, Turnbull reported that lowering high BP is associated with a 30% to 40% reduction in risk of stroke [25].

In this work, two potential thrombophilic mutations/polymorphisms (FVL) and MTHFR C677T were investigated in a group of patients with myocardial infarction and compared to the control group.

In this study, the percentage distribution of the different genotypes for FV Leiden (G1691A) in the study population showed that the wild type GG genotype variant was the most prevalent one, followed by heterozygous GA, and then the mutant homozygous AA variant respectively. There was significant increase in the heterozygous FVL GA genotype among MI patients (26.7%) compared with the control group (6.7%). One MI patient only had the mutant homozygous AA genotype (3.3%) as compared to the control group (0%). Additionally the allele frequency of the mutated A allele in MI patients (16.7%) showed significant increase when compared with the control group A allele frequency (3.3%).

Several studies have addressed the effect of factor V Leiden on coronary artery disease. In a large study, among 224 patients with coronary artery disease, the factor V mutation was found more often in the patients than in 196 controls [26]. Rosendaal et al. demonstrate an association between FV G1 691A polymorphisms and increased risk of MI in young women [23].

Consistent with our results, the result reported by Mannucci et al., on a large cohort of Italian patients who had MI,

1880 patients with MI and an equal number of controls, they demonstrated that the gain of function variant A allele of FV Leiden (G1691A) was associated with an increased risk of MI [27]. Satra et al. examined 523 patients using exercise-rest myocardial perfusion single photon emission computed tomography; they reported an association between FVL (heterozygous variant) and decrease myocardial perfusion, suggesting that the process of coronary artery disease and also patient prognosis may be adversely modified by FVL [28].

In a study by Emiroglu et al., among 220 patients, the prevalence of the heterozygous FVL mutation GA variants was associated with higher prevalence of totally occluded coronary arteries. Furthermore they demonstrated that the risk of left ventricular aneurysm formation was significantly higher in FVL heterozygote group compared to wild type GG variants [29].

As regards distributions of FVL genotypes among low risk and high risk subjects and control group, results showed that low risk cases had a higher frequency of GA genotype as compared to high risk cases and control (28.6%, 25%, and 6.7%) respectively. The prevalence of homozygous AA was significantly increased in low risk cases (7.1%). None of the high risk cases or control group had genotype AA.

These results were in agreement with Van de Water and colleagues, who reported that the frequency of factor V Leiden was significantly increased (14.6%) in patients aged < 50 years in the study compared with 3.6% in the control group [30]. Also Settin et al. reported that, factor FV Leiden (G1691A) mutation represents a significant risk factor for AMI. They concluded that frequency of FV Leiden (G1691A) mutation was high among patients with AMI. So families of affected subjects should be genotyped and counseled for proper prophylaxis [31].

FV Leiden (G1691A) mutation (FVL) an arginine to glutamine missense mutation in the Factor V (FV) gene at position 506 is the most prominent risk factor for venous thromboembolism [32]. Factor V Leiden is found in approximately 5% of Caucasians, and increases the risk of thrombosis five to eight-fold for heterozygous carriers and 50–80-fold for homozygous carriers [33]. Several mechanisms contribute to explanation of prothrombotic tendency that is present in carriers of FVL mutation. The amino acid substitution in FV resulting in a failure of APC to recognize a major cleavage site on factor V, lead to increased thrombin generation [11]. In case of FV Leiden mutation APC is not able to bind the mutant FV region around the most favored cleavage site at Arg 506 and consequently APC cannot regulate the activity of FVL via the proteolysis independent mechanism [34].

On the other side, several studies did not report any association between FVL mutation and coronary artery disease. The atherosclerosis, thrombosis, and vascular biology Italian study

group, investigated the association between FV Leiden (G1691A) mutation/polymorphisms and myocardial infarction, in a large sample of young patients with myocardial infarction chosen because, their disease is more likely to be related to a genetic predisposition to a prothrombotic state. They reported that FVL G1691A were not associated with an increased risk of myocardial infarction [35].

In cohort of Indian patients consisting of 51 patients aged below 50 years presenting with acute coronary syndromes, Himabindu et al. reported that factor V Leiden mutation is not a predisposing factor for acute coronary syndromes [36]. Also Yamada et al., found that FV G1691A was not associated with an increased risk of AMI [37].

As regards MTHFR C677T gene polymorphism, the percentage distribution of the different genotypes in the study population showed that the wild type CC genotype was the most prevalent one followed by heterozygous CT, and then the mutant homozygous TT variant respectively. There was a significant increase in the heterozygous MTHFR C677T CT genotype among MI patients (53.3%) compared with the control group (13.3%). The prevalence of homozygous MTHFR TT genotype was significantly increased in MI patients (6.7%) as compared to the control group (0%). Additionally the allele frequency of the mutated T allele in MI patients (33.3%) showed a significant increase when compared with the control group T allele frequency (6.7%).

Our results are consistent with previous studies that showed a significant increase in the frequency of the MTHFR C677T polymorphism among patients with CAD. Den Heijer et al. reported that the population frequencies of homozygous MTHFR C677T vary by ethnic group and the heterozygous MTHFR C677T mutation is more common than the homozygous variant [16].

Kluijtmans et al. reported that both the homozygous TT mutant variant and heterozygous CT genotype result in elevated homocysteine concentration concluded that the homozygous TT genotype is a modest but significant risk factor for CAD [38]. The finding in our study is consistent with a previous report by Gülec *et al.*, in which MTHFR C677T transition was found to be a risk factor for premature MI [39].

Almawi et al. in a sample from the Bahrain population studied a total of 96 patients with CAD and 404 healthy subjects and in the study done by Ghazouni et al. on 352 Tunisian patients with CAD, and 390 matched healthy subjects they demonstrated that significantly higher frequency of T allele and homozygous TT genotype was seen in patients vs. control subjects, and they concluded that MTHFR C677T mutation is associated with CAD and with elevated homocysteine levels [40,41].

In contrast to our results, Hsu et al. reported that the C677T mutation of the MTHFR gene is not associated with the risk of coronary artery disease or venous thrombosis among Chinese in Taiwan [42]. Yilmaz et al. and Caner et al. did not find any association between MTHFR C677T polymorphism and increased risk for CAD in Turkish patients [43,44].

As regards the distributions of MTHFR C677T genotypes among low risk and high risk subjects and control group. Our results showed that the low risk cases had a higher frequency of heterozygous MTHFR CT genotype as compared to high risk cases and control (64.3%, 43.75%, and 13.3%) respectively. The prevalence of homozygous TT genotype was significantly increased in low risk cases (14.3%). None

of the high risk cases neither the control group had genotype AA.

The present study is in agreement with a previous study by Alam et al. who demonstrated positive association of MTHFR C677T gene polymorphism and CAD and they recommended further study with larger sample size including assessment of vitamin status to better clarify the relationship between MTHFR genotypes and CAD [45]. Hendy et al. also reported that there is a strong relationship between MTHFR C677T gene polymorphism and hypertension, hypercholesterolemia and hyperhomocysteinemia [46].

MTHFR plays a central role in the metabolism of the amino acid homocysteine. Deficiency of MTHFR may be associated with an increase in plasma homocysteine, which in turn is associated with an increased risk of vascular disease [47]. A common polymorphism that may contribute to hyperhomocysteinemia has been reported in the *MTHFR* gene. The C677T (Ala-to-Val) transition, which produces thermolability and somewhat reduced enzyme activity in vitro, was first described by Kang et al. [48].

Hyperhomocysteinemia has been identified as a risk factor for atherosclerosis. Experimental evidence suggests that homocysteine facilitates the vascular oxidative process, thereby altering the coagulation system, and reduces the vasomotor regulation of the endothelium [49]. Outinen et al. reported that Hyperhomocysteinemia may contribute to both atherosclerotic and thrombotic processes by modulating vascular cell proliferation and promoting prothrombotic activity in the vascular wall [50].

The contradictory results for V Leiden (G 1691 A) and MTHFR (C677T) between different studies could be attributed to relatively small sample size, and ethnic heterogeneity.

## 5. Conclusion

This study observed that factor V Leiden (FVL G1691 A) and methylene tetrahydrofolate reductase (MTHFR C677T) gene polymorphisms are probably risk factors for MI among Egyptian cases particularly if integrated with other environmental and genetic risk factors. We recommended screening high risk patients for this polymorphism and the use of specific thromboprophylaxis to prevent recurrent thrombotic disease. Further studies in a large sample size are needed to confirm these results.

## Conflicts of interest

All authors declare no conflict of interest. There is no financial and personal relationship with other people or organizations that could inappropriately influence this work.

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