*American Journal of Biomedical Research, 2016, Vol. 4, No. 3, 74-79*
Available online at http://pubs.sciepub.com/ajbr/4/3/3

DOI:10.12691/ajbr-4-3-3

# Methylenetetrahydrofolate Reductase C677T and Platelet Glycoprotein IIb/IIIa Genes Polymorphism in Myocardial Infarction Egyptian Patients in Ismailia City

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**Abstract** **Background**: Hyperhomocysteinemia and platelet glycoprotein GpIIIa polymorphism had been identified as risk factors for coronary atherosclerosis. The methylenetetrahydrofolate reductase *MTHFR* C677T variant has been shown to influence homocysteine metabolism, the interaction of plasma tHcy with other conventional risk factors remain uncertain in the clinical setting of acute myocardial infarction (AMI). The present study aimed to examine whether the *MTHFR* and platelet glycoprotein *IIIa* polymorphisms were associated with increased risk of (MI) in Egyptian patients. **Subjects and Method:** 150 newly diagnosed MI patients and 50 healthy matched subjects were recruited into this study, genotyping of the *MTHFR* C677T and *GpIIIa* 1565 $A^1/A^2$ polymorphisms were carried out by polymerase chain reaction−restriction fragment length polymorphism (PCR-RFLP) technique, plasma tHcy, and folic acid levels were estimated. **Results**: Fasting plasma total Hcy levels were significantly higher in MI patients than controls ($P$ <0.05), folate levels were significantly lower in MI patients than controls ($P$ <0.05), no significant differences were observed in the *MTHFR* C677T and *GpIIIa* genotypes frequencies between MI patients and controls ($P$ > 0.05). The frequency of the *MTHFR* C allele was 80.6 % and 76 % in MI patients and controls respectively and did not differ significantly between the two groups (P > 0.05). The frequency of risk allele, GpIIIa, $PIA^2$ was significantly higher in MI patients compared to controls (p<0.05), plasma tHcy level was significantly higher and folate level was significantly lower in MI patients carrying *MTHFR* CC and *GPIIIa* $PIA^2A^2$genotypes. **Conclusions**: In this population, the both risk alleles of *MTHFR* and *GpIIb/IIIa* polymorphisms had no major effect on the MI incidence, they were associated with higher homocysteine levels. A gene-environment interaction might increase the risk indirectly by elevating tHcy, especially when folate intake was low, our findings might support that *MTHFR* and *GpIIb/IIIa* polymorphisms as risk factors for MI.

***Keywords:*** *Myocardial infarction (MI), homocysteine (tHcy), folate, methylenetetrahydrofolate reductase (MTHFR), Glycoprotein (GP) IIb/IIIa*

**Cite This Article:** Kefah H Ali, Nagwan A Sabek, Loaa A Tag Eldeen, Emad F Ismail, and Gamela Nasr, "Methylenetetrahydrofolate Reductase C677T and Platelet Glycoprotein IIb/IIIa Genes Polymorphism in Myocardial Infarction Egyptian Patients in Ismailia City." *American Journal of Biomedical Research*, vol. 4, no. 3 (2016): 74-79. doi: 10.12691/ajbr-4-3-3.

## 1. Introduction

Coronary heart disease deaths in Egypt reached 107,232 or 23.14% of total deaths ranks 23th in the world [1]. Hypertension, raised blood glucose, hypercholesterolemia, raised fasting triglycerides, current tobacco smoking, low physical activity and obesity are perceived as customary danger elements that are thought to illustrate most coronary illness [2]. About 15% to 20% of those with coronary heart disease (CHD) had no recognized danger elements and miss to distinguish other danger elements that foresee a segment of CHD occasions and may enhance essential preventive efforts [3].

Many studies have demonstrated a positive correlation between plasma Homocysteine level and cardiovascular disorders [4], Hyperhomocysteinemia (Hhcy) is associated with endothelial dysfunction and accelerated atherosclerosis [5] leading to the general conclusion that Hcy is a pro-thrombotic factor [6].

Methylenetetrahydrofolate reductase (MTHFR) is the main regulatory enzyme for folate/ homocysteine metabolism. *MTHFR* converts 5,10-methylene-tetrahydrofolate (THF) into 5-methyl-THF, the dominant circulating form of folate. The 5-methyl-THF product donates a methyl group to homocysteine in the generation of *S*-adenosylmethionine [7]. A polymorphism in MTHFR gene C677T (rs1801133) results in a transformation of the amino acid alanine to valine at position 226 in the protein which was associated with a 50 % reduction of MTHFR enzyme activity, increase in plasma Hcy concentration and a decline in plasma folic acid concentration [8].

Platelet aggregation, as well, is an essential step in formation of the thrombus and in subsequent blood vessel wall remodeling [9]. The GPIIb/IIIa integrin, a platelet–

specific glycoprotein, exists as a heterodimer of two subunits: the αIIb chain and the β3 chain, functioning as a receptor for ligands such as fibrinogen, von Willebrand factor, and vitronectin [10], and is critical for the binding of platelets to the extracellular matrix of the blood vessel wall and to each other, thus facilitating platelet aggregation [11].

The *GPIIIa* gene located on chromosome 17, q21 to 22 with GpIIb to GpIIIa, contains a common polymorphism at position 1565 in exon 2, where the base can be either thymidine or cytosine, resulting in either proline or leucine at position 33 in the protein [12]. The two forms of *GPIIIa*, termed $PI^{A1}$ and $PI^{A2,}$ respectively, differ antigenically, resulting in an extra-cellular positioned conformational change of the 3-subunit. Therefore, it has been considered biologically plausible to suggest an impact of Leu33Pro on platelet aggregation and consequently on risk of ischemic cardiovascular disease [13].

Rupture of an atheromatous plaque, subsequent platelet aggregation, and thrombus formation are key events in the development of MI and sudden death in patients with CHD. The atherogenicity, thrombogenic potential and the damaging effect on lining arteries may be affected by these two polymorphisms; *MTHFR C677T* and platelet *GPIIIa* ($PIA^1$ and $PIA^2$). The study aimed to evaluate whether the *MTHFR* and *GpIIb/IIIa* variants were associated with risk of MI and to clarify their effect on the tHcy and folate plasma levels in MI Egyptians' patients.

## 2. Material and Methods

This cross-sectional study included 150 (MI) patients from the cardiovascular intensive care unit at Suez Canal university hospital and 50 healthy controls matched for age and sex. MI diagnosis was based on characteristic symptoms of stable angina pectoris according to the guidelines of the European Society of Cardiology ( location, character, and duration of pain and the relation of pain to exercise [14]. MI was confirmed by chest pain associated with specific ischemic ECG changes, high serum troponin and increased creatinine phosphokinase (CPK-MB) activity. All patients were undergone coronary angiography to determine the level of atherosclerotic lesion progression. The control subjects were selected from consecutive individuals who were judged to be free of coronary heart disease by history, clinical examination and electrocardiography. The study was approved by the Ethics Committee at the faculty of medicine, Suez Canal University Hospital and written informed consents were obtained from all participants. and conducted according to the guidelines of the Declaration of Helsinki (1964).

### 2.1. Extraction of Genomic DNA

Genomic DNA was extracted from whole blood using Gene JET™ Genomic DNA Purification Kit (Fermentas, CA, USA). DNA concentration was determined by Nano-Drop (Nano-Drop Technologies, Inc. Wilmington, USA). Genetic polymorphism was analyzed by (PCR-RFLP) method.

### 2.2. Genotype Determination

Detection of *MTHFR* gene C677T (rs1801133) polymorphism were carried out in 50 µL PCR reaction mixture containing ≈0.5 µg genomic DNA, 25µl Dream Taq™ Green PCR Master Mix (Fermentas, CA, USA), 50 pM from each of the following primers: forward (5′ GCA CTT GAA GAG AAG GTG TC-3′) and reverse (5′-AGG ACG GTG CGG TGA GAG TG-3′. DNA was amplified during 35 cycles with an initial denaturation for 5min at 94°C and a final extension of 5 min. at 72°C. The cycle program consisted of denaturation at 94°C for 30 seconds, annealing at 48°C for 30 seconds, and extension at 72°C for 30 seconds. PCR products were incubated for 5 min at 37°C. with 2 units of the Fast Digest *TaqI* restriction endonuclease, (#FD0674 Fermentas) and the digested products were size-fractionated on ethidium bromide-stained 2% agarose gels. The T allele generates a single band of 203 bp, and the C allele generates two bands 173 and 30 bp [15]. Figure 1.

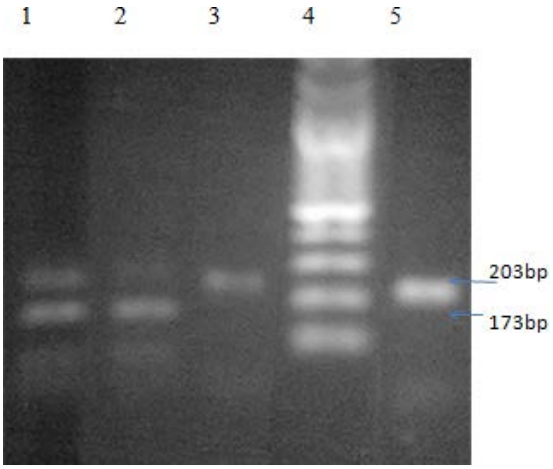


**Figure 1.** The PCR products of *MTHFR DNA* after enzyme digestion with*Taq1*. Lane 1 (CT heterozygote) ; Lane 2: (CC homozygote) Lane 3, 5 (TT homozygote); Lane 4: 100bp DNA marker.

For *GPIIIa* genotyping, PCR was carried out using forward primer 5'- TGG ACT TCT CTT TGG GCT CCT GAC TTA C -3' and reverse primer 5'-CGA TGG ATT CTG GGG CAC AGT TAT C- 3'. The samples were amplified for 35 cycles of denaturation at 95°C for 2min, annealing at 63°C for 60 seconds, and extension at 72°C for 2min. The cycles were preceded by initial denaturation at 95°C for 5 min. and followed by a final extension step at 72°C for 5 minutes. the PCR products were treated with *MspI* (#FD0544 Fermentas) for 5 min at 37°C, and visualized on 4% ethidium bromide-stained agarose gels, the 109 bp amplification product was cleaved into fragments of 66 bp and 43 bp. in $PI^{A2}$ allele [16]. Figure 2.

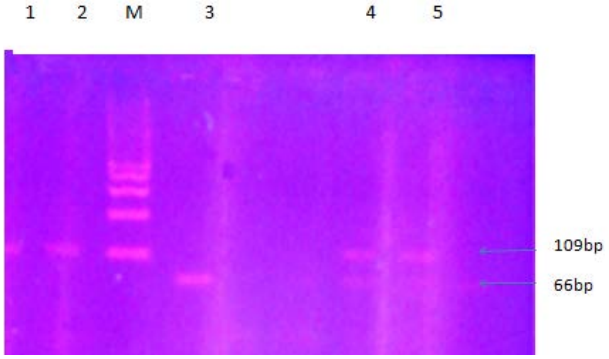


**Figure 2.** The PCR products of *GPIIIa DNA* after enzyme digestion with MspI. Lane M – 100bp Molecular DNA marker. Lane: 1-2 $PI^{A1A1}$ genotype, Lane3: $PI^{A2A2}$ genotype, lane 4,5 $PI^{A1A2}$ genotype

## 2.3. Laboratory Analysis

Plasma homocysteine and folate levels were assayed by Immulite 1000, using Homocysteine (PILKHO-10, 2006-12-29) and Folic Acid (LKFO1) respectively [17]. Fasting and 2 hours postprandial glucose levels were determined using the method of Trinder [18] on Roche Diagnostics Hitachi 912® system (Roche Diagnostics, Indianapolis, IN). Serum total cholesterol, serum triglycerides, HDL-cholesterol were estimated as previously described Alian et al.,[19] Siedel et al. [20]; Lopes-Virella et al.[21] LDL-cholesterol was calculated by the Friedewald Formula: LDL cholesterol = Total cholesterol – HDL cholesterol– Triglycerides /5). The formula is generally agreed to be inaccurate when triglycerides are greater than 400 mg/dl Marniemi et al. [22].

## 2.4. Statistical Analysis

Statistical analysis was carried out using the (SPSS version 17). values were expressed as mean ± SD. Differences between non-continuous variables, genotype distribution, allele frequency, and Hardy–Weinberg equilibrium were tested by chi-square analysis. The odds ratio (OR) for MI and their 95% confidence interval (CI) associated with each mutated allele was also calculated and a p-value < 0.05 was considered to be statistically significant.

# 3. Results

Clinical characteristics and biochemical parameters of the study subjects were shown in Table 1. In the present study MI patients were found to had significant higher tHcy level (14.0 ± 4.0 µmol/l vs. 12.0 ± 2.0 µmol/l, $p$ <0.05) and lower significant folate levels than controls (9.6 ± 3.7 vs. 10.3 ± 3.6 ng/ml. p< 0.05). Although no significant difference was found between patients and controls regarding age, gender and BMI, patients were found to had significantly higher prevalence of hypertension, hyperlipidemia, diabetes mellitus and smoking than controls.

**Table 1. Clinical characteristics and biochemical parameters of the study subjects**

| parameters | patients (N=150) | Controls (N=50) | p-value |
|---|---|---|---|
| Sex (M/F) (n) | 118/32 | 30/20 | 0.98 |
| Age (years) | 54.1 ±10.3 | 44.8±9.6 | 0.97 |
| BMI (kg/m$^2$) | 29.8±4.8 | 29.0± 4.4 | 0.44 |
| Smoking n (%) | 98 (65.3%) | 5 (5%) | 0.001* |
| Hypertension n (%) | 90 (60%) | 11(11%) | 0.002* |
| DM n (%) | 62(41.3%) | 4(4%) | 0.003* |
| SBP(mmHg) | 126.0 ±19.5 | 111.2± 9.7 | 0.001* |
| DBP (mmHg) | 77.6 ±11.6 | 77.0± 8.0 | 0.981 |
| FBG (mg/dl) | 141.0 ± 56.4 | 89.0 ±9.8 | 0.001* |
| Cholesterol (mg/dl) | 213.2 ± 51.7 | 164.4 ± 35.2 | 0.04* |
| TGs (mg/dl) | 167.4 ± 63.4 | 92.2 ± 40.4 | 0.001* |
| LDL-C (mg/dl) | 138.6± 48.5 | 127.0 ±25.6 | 0.261 |
| HDL-C (mg/dl) | 39.1 ± 13.2 | 49.0 ± 12.0 | 0.001* |
| Folate (ng/ml) | 9.6 ±3.7 | 10.3 ± 3.6 | 0.021* |
| tHcy (µmol/l) | 14.0 ± 4.0 | 12.0 ± 2.0 | 0.041* |

BMI=body mass index; SBP=systolic blood pressure; DBP=diastolic blood pressure; TG=triglyceride; LDL-C=Low Density Lipoprotein cholesterol; High Density Lipoprotein cholesterol= HDL-C; Homocystein = tHcy Diabetes Mellitus=DM. *P< 0.05 statistically significant.

## 3.1. *GPIIIa* and *MTHFR* Genotypes and Allele Frequencies in MI Patients and Controls

Both groups were assessed in this study, they were found to be in Hardy–Weinberg equilibrium. Table 2 showed the genotypes and allele frequencies of *GPIIIa* gene polymorphism in studied groups. The frequency of the mutant $PI^{A2}$ allele was significantly higher in MI patients than the controls (p < 0.05) also, the frequency of *GPIIIa* $PI^{A2A2}$ genotype was more frequent in MI patients than controls although the difference was not significant. No significant differences were found regarding *MTHFR* genotype or allele frequencies between the studied groups Table 3.

**Table 2. Genotypes and allele frequencies of GPIIIa gene polymorphism in the studied groups**

| Variables | Patients *n* (%) (N=150) | Controls *n* (%) (N=50) | p-value | OR | 95% CI |
|---|---|---|---|---|---|
| Genotype | | | | | |
| $PI^{A1A1}$ | 60 (40) | 26 (52) | 0.35 | 0.65 | (0.262to 1.614) ($A^1A^1$vs.other genotypes) |
| $PI^{A1A2}$ | 44 (29) | 14 (28) | 0.89 | 1.06 | (0.3908 to2.9150) ($A^1A^2$vs.othergenotypes) |
| $PI^{A2A2}$ | 46 (31) | 10 (20) | 0.36 | 1.66 | (0.5533 to 4.9824) ($A^2A^2$vs.other genotypes) |
| Allele | | | | | |
| $PI^{A1}$ | 164 (56) | 66 (66) | | | |
| $PI^{A2}$ | 136 (45) | 34 (34) | 0.04* | 0.621 | (0.387 to 0.990) |

*P< 0.05 statistically significant.

**Table 3. Genotypes and allele frequencies of *MTHFR C677T* gene polymorphism in studied groups**

| MTHFR | Patients (N=150) (%) | Controls (N=50) (%) | p- value | OR | 95% CI |
|---|---|---|---|---|---|
| genotype | | | | | |
| CC | 102 (68) | 32 (64 ) | 0.710 | 1.953 | (0.462 to 3.090) (CC vs. other genotypes) |
| CT | 38 (25.3) | 12 (24 ) | 0.89 | 1.07 | (0.374 to3.086) (CT vs. other genotypes) |
| TT | 10 (6.7) | 6 (12) | 0.40 | 0.523 | (0.115 to2.369) (TT vs. other genotypes) |
| Allele | | | | | |
| C allele | 242(80.7) | 76 (76) | 0.317 | 1.317 | 0.767 to 2.263 |
| T allele | 58(19.3 ) | 24 (24 ) | | | |

*P< 0.05 statistically significant.

### 3.2. Clinical and Functional Characteristics of MI Patients in Relation to *MTHFR and GPIIIa PI* Genotypes

Plasma tHcy level was significantly higher and folate level was significantly lower in MI patients carrying *MTHFR* CC and GPIIIa $PI^{A2A2}$ genotypes ($p < 0.05$). Except for systolic blood pressure which was statistically elevated in MI patients having *MTHFR* CC and GPIIIa $PI^{A1A1}$ genotypes, No significant differences were found regarding other measured parameters. (Table 4 - Table 5).

**Table 4. Characteristics and biochemical parameters of MI patients in relation to *MTHFR* gene genotypes**

| Parameters | Carrier of (CC) (N = 102 ) | Carrier of (CT+TT) (N = 48) | P- value |
|---|---|---|---|
| Age ( years) | 52.10 ± 9.5 | 55.21±10.1 | 0.44 |
| BMI (kg/m$^2$) | 30.23± 5.31 | 27.90±3.52 | 0.39 |
| SBP(mmHg) | 128.95±20.24 | 110±8.94 | 0.03* |
| DBP(mmHg) | 78.94±11.96 | 78.33 ± 9.30 | 0.91 |
| FBG (mg/dl) | 141.1±62.33 | 139.33 ± 52.4 | 0.42 |
| Cholesterol (mg/dl) | 205.16± 50.4 | 219.6± 52.7 | 0.79 |
| TG(mg/dl) | 165.7± 64.1 | 186.5 ± 63.6 | 0.94 |
| LDL-C (mg/dl) | 129.2± 48.0 | 145.10 ± 48.0 | 0.86 |
| HDL-C (mg/dl) | 39.4± 14.2 | 38.8± 12.4 | 0.47 |
| Folate (ng/ml) | 10.4± 3.3 | 11.9 ±4.0 | 0.031* |
| tHCY (µmol/l) | 13.1 ±5.3 | 12.05±2.16 | 0.022* |

Values are mean (SD); BMI=body mass index; SBP=systolic blood pressure; DBP=diastolic blood pressure; TG=triglyceride; LDL-C=LDL cholesterol; HDL-C=HDL cholesterol, tHcy= Homocysteine.
*P< 0.05 statistically significant.

**Table 5. Characteristics and biochemical parameters of MI patients in relation to *GPIIIa PI* gene polymorphisms**

| Parameters | Carrier of $PI^{A1A1}$ (N=60) | Carrier of $PI^{A2A2}$ (N= 46) | p-value |
|---|---|---|---|
| Age ( years) | 52.10 ± 9.0 | 53.9±13.0 | 0.11 |
| BMI (kg/m$^2$) | 30.2± 5.3 | 28.0±3.5 | 0.39 |
| SBP(mmHg) | 129.0±20.0 | 110.0 ±8.9 | 0.03* |
| DBP(mmHg) | 78.9±11.6 | 78.3 ± 9.0 | 0.91 |
| FBG (mg/dl) | 141.8±62.0 | 130.0 ±42.0 | 0.11 |
| Cholesterol (mg/dl) | 205.2± 50.4 | 213.6 ± 48.7 | 0.86 |
| TG(mg/dl) | 165.4±64.0 | 178.0 ±74.0 | 0.13 |
| HDL-C (mg/dl) | 39.4± 14.2 | 35.8 ± 13.0 | 0.72 |
| LDL-C (mg/dl) | 129.2±48.0 | 139.5 ±39.1 | 0.18 |
| Folate (ng/ml) | 11.6±6.5 | 10.4±3.8 | 0.02* |
| tHCY (µmol/l ) | 12.1 ±5.3 | 14.5±4.5 | 0.05* |

Values are mean (SD); BMI=body mass index; SBP=systolic blood pressure; DBP=diastolic blood pressure; TG=triglyceride; LDL-C=LDL cholesterol; HDL-C=HDL cholesterol, tHcy = Homocystein
*P< 0.05 statistically significant.

## 4. Discussion

Hyperhomocysteinemia (Hhcy) was known of being associated with increased thrombotic tendency, which had been considered as a risk factor for coronary vascular disease (CVD), and atherosclerosis [23], MI patients had significant elevation in plasma tHcy compared to controls ($p<0.05$) with a mean value of 14.1 µmol/L, which is considered as mild Hhcy which was associated with activation of coagulation systems in patients with premature atherosclerotic arterial disease and elevated factor VIIa, and with thrombin generation in patients presenting with an acute coronary syndrome [24].

Mild hyperhomocysteinemia is not associated only with coronary artery disease (CAD), but also with acute MI and multi-vessel CAD, and might explain the pro-thrombotic effect of homocysteine in acute coronary syndromes. Many factors may cause hyperhomocysteinemia; including malnutrition, vitamins B12, B6, folic acid deficiencies, chronic renal failure, hypothyroidism, cancer, drugs, and gene mutations [25]. Therefore, the present study aimed to assess whether the *MTHFR* and *GpIIIa* polymorphisms were associated with increased risk of MI and to clarify their effect on the tHcy and folate blood levels in MI Egyptian patients.

Epidemiologic studies present convincing evidence that hyperhomocysteinemia is associated with increased risk of various diseases, such as those of a cardiovascular nature. Most of these studies suggested that a significant association is observed for total plasma homocysteine (tHcy) levels above 12 to 15 µmol/L, experimental data suggested that homocysteine (Hcy) could impair normal cellular and physiologic functions [26].

Several studies have suggested that mild to moderate elevations of plasma homocysteine are associated with increased risk of vascular disease. Homocysteine levels tend to be higher in individuals homozygous for the thermolabile variant of *MTHFR* gene, due to a C-to-T transition at position 677, four studies found an increased risk of atherothrombotic stroke in carriers of the TT genotype of the 677C>T variant in *MTHFR*, but 7 other studies did not find any association [27].

Our finding showed a significantly higher plasma homocysteine level was associated with a significantly lower folate level in MI patients than the controls, similar findings were observed in the National Heart Lung and Blood Institute (NHLBI) Family Heart Study by Jacques et al. [28]. Our findings showed that the *MTHFR* genotypes frequency were similar among patients and controls which was agree with Jing et al. [27] found that the frequency of the three genotypes of *MTHFR* were in similar distribution among both MI patients and controls, also our findings were in agree with Pandey et al. [25], who found no significant association between T allele and CAD in Indian patients (C vs. T; OR= 1.24, 95% CI: 0.84-1.85, (p= 0.25).

In the present study, we observed high tHcy and low folate levels in MI patients who are carrying *MTHFR* CC genotype compared to patients carrying (CT+TT) genotypes ($p<0.05$). Our results were inconsistent with the results of other studies Liag et al. [29], Andreassi et al. [30], According to these studies, the homozygous TT genotype among the *MTHFR* mutations had reportedly caused a marked increase in plasma homocysteine concentrations. This difference between our finding and theirs might be due to many factors, hence, the overall effect of this mutation on homocysteine concentration in plasma depends on study design, inclusion criteria, ethnic background, age and vitamin intake of the population.

The association of elevated tHcy and lower folate levels suggests that the *MTHFR* gene mutation may increase plasma tHcy levels when folate intake is low. These findings further support the hypothesis that folate may stabilize the MTHFR enzyme and be consistent with the observation that, oral supplementation of folic acid normalizes hyperhomocysteinemia due to thermolabile *MTHFR* polymorphisms Jacques et al. [28].

The frequency of *MTHFR* polymorphic allele occurrence displayed a variation among different ethnic groups Qyang et al.[31]. The highest prevalence is reported in the Japanese population (59.8%) Wu [32] and, it decreases toward western parts of Asia and Europe; 43.3% in China Zheng et al. [33].

In this study, the relation between *GP IIIa* $Pl^{A}$ polymorphism and increased risk of MI was tested. A significantly higher prevalence of $Pl^{A2}$ allele in MI patients than controls matched for sex and age was found ($p< 0.05$). This result is matched with Weiss et al.[34], where, the prevalence of $Pl^{A2}$ found to be 2.1 fold higher in MI or unstable angina cases compared to the controls, previous studies elucidated that the $Pl^{A2}$ variant was associated with higher activation of GP IIb/IIIa receptor after adenosine di-phosphate stimulation, Angiolillo et al. [35].

Our finding demonstrated that higher non-significant differences in relative risk factors in MI patients carrying $Pl^{A2/A2}$ genotype compared to the carrier of $Pl^{A1/A1}$ genotype, for FBG, triglyceride, cholesterol, HDL-C, and LDL, also our finding demonstrated that MI patients carry GPIIIa $Pl^{A2A2}$ genotype had significantly elevated plasma tHcy level than the controls ($p<0.05$). Our results agree with Duan et al.[36] who failed to confirm the association between particular type of GPIIIa Pl polymorphism and cardiovascular risk.

$H_2S$ produced by platelet, harvested from hyperhomocysteinemic patients, activates arachidonic acid cascade by phosphorylating phospholipase A2. This cascade of events primes the platelet to be more responsive to endogenous stimuli contributes to the increased thrombotic events associated with hyperhomocysteinemia, Roberta et al. [37].

The results of such studies are of great importance because better understanding of the relationship between genotype and nutrition influencing the plasma total homocysteine level, and cardiovascular health may improve the cardiovascular diagnostic tests (ie, measurement of biologic markers). Furthermore, formulation of the appropriate supplementation scheme for lowering plasma tHcy should be based not only on current nutrient recommendations but also on an understanding of gene polymorphism and gene-nutrient interactions.

**In conclusion**: In this population, while the risk alleles of *MTHFR* and *GPIIIa* polymorphisms had no major effect on the MI incidence, they associated with higher plasma homocysteine and low folate levels and might play important role in increasing the susceptibility to the risk of MI.

## Acknowledgements

The authors are indebted to the participants for their utmost cooperation.

## Funding

The study was supported by the researchers

## Conflict of Interest

The authors declare that they have no competing interests.

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