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Association of *factor V Leiden R506Q*, *FXIIIVal34Leu*, and *MTHFR C677T* polymorphisms with acute myocardial infarction

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Abstract

Background: Acute myocardial infarction (AMI) is a leading cause of death and morbidity around the world. Although the association between thrombophilia and AMI is well-established, controversial data are present on the association between thrombophilic polymorphisms and AMI. The aim of this study was to investigate the association of three thrombophilic polymorphisms including *factor V Leiden* (*FVL*), *MTHFRC677T* (methylenetetrahydrofolate reductase), and Coagulation *factor XIIIVal34Leu* with AMI in East of Iran.

Result: There were no statistically significant differences between the patients and control groups in terms of the distributions of allelic and genotypic frequencies of *FVL* and *FXIIIVal34Leu* polymorphisms (*P*-value > 0.05). Subjects who carried *CT* genotype of *MTHFR C677T* polymorphism were at a 2.03-fold higher risk for AMI (*P*-value: 0.02, OR 1.76, 95% CI 1.07–2.75). Furthermore, patients with *MTHFR* 677*CT* (*P*-value < 0.001, $\beta = -0.90$, 95% CI − 1.33, − 047) or *677CC* (*P*-value < 0.001, $\beta = -1.04$, 95% CI − 1.47, − 0.61) genotypes showed significantly Lower creatinine levels compared with patients having the *MTHFR* 677TT. No association was observed between the other remaining polymorphisms and AMI (*P*-value > 0.05).

Conclusion: Our findings showed that *MTHFRC677T* polymorphism could contribute to AMI susceptibility and increase creatinine levels in east Iran population. This was the first study to examine the association of these three polymorphisms with AMI in east Iran.

Keywords: Acute myocardial infarction, Factor V Leiden, Methylenetetrahydrofolate reductase, Factor XIII, PCR

Background

AMI is one of the leading causes of death in both developed and developing countries. According to data from the World Health Organization, 17.9 million people die from cardiovascular diseases worldwide each year, accounting for 31% of all deaths. The cause of 85% of these deaths is either AMI or stroke [1, 2]. AMI occurs when plaque that has built up in the walls of coronary arteries erodes or ruptures, resulting in a transient, partial, or complete occlusion of the arteries [3]. Although the exact association of risk factors for AMI has not been established, a growing number of studies have shown that age, race, ethnicity, alcohol use, blood pressure abnormalities, diabetes, obesity, and an unhealthy lifestyle increase the risk of AMI. A genetic predisposition is now recognized as a significant risk factor for atherosclerosis, leading to coronary artery disease, myocardial ischemia, and AMI [4]. Several mutations such as *factor V Leiden* (*FVL*) [5], *MTHFRC677T* [6], and *FXIIIVal34Leu* [7]

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polymorphisms have been identified as inherent risk factors for thrombosis.

The *R506Q* polymorphism increases *FVL* resistance to degradation by activating protein C and accentuating thromboembolic risk [8, 9]. Coagulation *factorXIIIVal34Leu* polymorphism influences the balance between thrombus formation and dissolution [5]. This polymorphism can play a protective role against myocardial infarction, but the results of studies on this topic have been contradictory [10, 11]. The *MTHFRC677T* polymorphism is associated with a reduction in the catalytic activity of the enzyme, which in turn could lead to total homocysteine accumulation and endothelial dysfunction, both of which are two well-known risk factors for coronary artery disease [12, 13]. Despite numerous studies examining the relationship between these polymorphisms and the susceptibility to AMI, the results of these studies have been contradictory. Thus, further studies are needed to provide a clear picture of this association. Moreover, to the best of our knowledge, no study has yet been conducted on this very topic in Iran. Therefore, we conducted this study to investigate the association of the *FVL*, *FXIIIVal34Leu*, and *MTHFRC677T* polymorphisms with susceptibility to AMI.

Methods

Study population

In this case–control study, we collected 300 participants including 150 AMI patients and 150 ethnically matched healthy volunteers from the Iranian population. The AMI patients included in this study were patients with AMI admitted to Razi Hospital of Birjand, Iran in 2019–2021 who did not receive any intervention medication. All cases were selected based on the criteria of the world health organization [14]. Inclusion criteria for AMI patients were age between 18 and 65 years and positive angiography with 50% or more stenosis of at least one coronary artery. Angiography was carried out and evaluated by experienced cardiologists who were blinded to patients' genotype. Clinical electrocardiography (ECG) and cardiac enzyme findings were used to make the diagnosis of AMI. Non-inclusion criteria for all participants including patients and healthy controls were: history of previous angiography, heart failure, history of a coagulation disease, deep vein thrombosis, hepatic dysfunction, renal dysfunction, advanced cancer, history of thyroid disease and consumption of related medication, organ inflammation, pregnancy, and lactation.

Concentrations of total cholesterol (Chol), high density lipoprotein (HDL), low density lipoprotein (LDL), triglycerides (TG), and fasting blood sugar (FBS), were measured using an automatic analyzer (BT-4500, Biotecnica, Italy). After informed consent was obtained, venous blood sample was collected from all participants. The study protocol was evaluated and approved by the Ethics Committee of Birjand University of Medical Sciences (Ref. ID: IR.BUMS.REC.1399.264).

DNA isolation and SNP selection and genotyping

Blood samples were stored at − 20 °C until use. Genomic DNA for PCR was extracted from peripheral blood by DNA extraction kit. Genotype determination was performed using tetra-primer amplification refractory mutation system-polymerase chain reaction (T-ARMS-PCR) assay. Primer sequences and fragment sizes are shown in Table 1. All primers used in this research were designed by a web primer design program (Primer1) [15]. Amplification was done in Eppendorf thermal cycler (Germany). Following amplification, PCR products were separated by 2% agarose gel electrophoresis using a 100 bp ladder.

Table 1 Primers used in the T-ARMS-PCR for genotyping of *FVL* G1691A, *MTHFRC677T*, and *FXIIIval34leu* gene polymorphisms

SNP	Primer sequence	Product size (bp)
FVL (rs6025)	Forward inner primer (A allele): GAGCAGATCCCTGGACAGTCA	Common: 242
	Reverse inner primer (G allele): ACTTCAAGGACAAAATACCTGTATTCATC	
	Forward outer primer (5′—3′): GAACATCTTAGAGTTTGATGAACCCAC	G Allele: 175
	Reverse outer primer (5′—3′): CCCATTATTTAGCCAGGAGACCTAA	A Allele: 117
MTHFR (*rs1801133*)	Forward inner primer (T allele): TTGAAGGAGAAGGTGTCTGCGGGCGT	Common: 407
	Reverse inner primer (C allele): CAAAGAAAAGCTGCGTGATGATGAAATAGG	
	Forward outer primer (5′—3′): CCCAGCCACTCACTGTTTTAGTTCAGGC	C Allele: 273
	Reverse outer primer (5′—3′): GGTGAGAGTGGGGTGGAGGGAGCTTAT	T Allele: 190
FXIII (*rs5985*)	Forward inner primer (T allele): CTGCCCACAGTGGAGCTTCAGGACT	Common: 414
	Reverse inner primer (G allele): TGACGCCCCGGGGCACTAC	
	Forward outer primer (5′—3′): CGGCAAAATGTGTTGCTCAAGTGCT	G Allele: 268
	Reverse outer primer (5′—3′): TAAAACCAGAGATTGGCAGGGGGCT	T Allele: 190

SNP, single nucleotide polymorphism; bp, base pair; FVL, Factor V Leiden; *MTHFR*, Methylenetetrahydrofolate reductase

Bands of PCR products were visualized under UV transilluminator. Sequencing was performed to confirm the genotypes initially identified by the T-ARMS-PCR. The final T-ARMS-PCR was conducted with a total volume of 20 µl containing 50 ng of template DNA, 1 µL of each inner primer, 0.25 µL of each outer primer (each primer has a concentration of 10 µM), and 10 µL of 1X PCR Master mix (amplicon, Denmark). PCR amplification conditions are shown in Table 2.

Statistical analysis

The data were analysed using SPSS version 16.0. The mean and standard deviation (SD) of normally distributed data were calculated, and the Student's *t* test was used to compare groups. To compare genotype distribution and allele frequency between groups, the χ^2-test was used. The allele and genotype frequencies in various patient subgroups were analysed using Fisher's exact test. The Chi-square was used to assess the Hardy–Weinberg equilibrium in the control group. The association between polymorphisms and risk of AMI was assessed by logistic regression. Data that were not normally distributed were expressed as medians, and the Mann–Whitney U test was used to compare patients. Multiple groups were compared using Kruskal–Wallis tests. The χ^2-test was used to compare patients' categorical data, which were expressed as percentages. Odds Ratio (OR) along with 95% Confidence Interval (CI) were estimated in order to assess the risk of the association between AMI and the studied gene polymorphisms and Linear regression was used for estimate of gene polymorphisms on clinical characteristics. Statistical significance was defined as $P<0.05$.

Results

Study group characteristics

This study enlisted the participation of 300 Iranian subjects comprising 150 individuals with primary MI (men = 77; women = 73) and 150 healthy individuals (male = 75; female = 75). The demographic and clinical characteristics of AMI patients and the control group are shown in Table 3. The mean age of the AMI patients and the controls was 57.94 ± 7.72 and 47.06 ± 8.70, respectively ($P<0.001$). In addition, 50% of the control group ($n=75$) and 51.3% of the case group ($n=77$) were male and there was no significant difference in the distribution of sex between the two groups ($P=0.53$). Unlike HDL which was lower in AMI patients, total and LDL

Table 2 PCR procedure for T-ARMS-PCR genotyping of *FVL* G1691A, *MTHFR* C677T, and FXIIIval34leu polymorphisms

	FVLG1691A		***MTHFRC677T***		***FXIIIval34leu***	
Initial denaturation	95 °C—5 min		94 °C—5 min		95 °C—5 min	
Denaturation	95 °C—30 s	25 cycle	94 °C—1 min	30 cycle	95 °C—30 s	33 cycle
Annealing	57.1 °C—25 s		65 °C—45 s		69.1 °C—25 s	
Extension	72 °C—30 s		72 °C—45 s		72 °C—30 s	
Final extension	72 °C—10 min		72 °C—5 min		72 °C—10 min	

Table 3 Baseline characteristics of the individuals included in the study

Variables	Cases (Mean ± SD)	Controls (Mean ± SD)	*P*-value
Age	51.78 ± 8.18	49.30 ± 9.04	< 0.03
Gender (male/female)	(77/73) Ratio 1.05	(75/75) Ratio 1	0.53
BMI (kg/m^2)	24.8 ± 3.6	24.0 ± 2.9	< 0.01
FBS (mg/dl)	124.5 ± 48.7	87.5 ± 14.2	0.00
HDL (mg/dl)	42.3 ± 8.6	46.1 ± 8.0	0.00
LDL (mg/dl)	120.0 ± 63.4	87.0 ± 26.3	0.00
Total Cholesterol (mg/dl)	176.5 ± 51.4	152.4 ± 33.7	0.00
Triglycerides (mg/dl)	116.0 ± 66.0	113.6 ± 35.9	0.69
Creatinine (mg/dl)	1.1 ± 0.5	0.9 ± 0.7	0.00
Diabetes, %	17.0	0.0	0.00
Dyslipidemia, %	6.1	0.0	0.02
Smoker, %	11.3	2.0	0.02

Values are mean ± SD or *n* (%), BMI, body mass index; FBS, fasting blood sugar; HDL, high density lipoprotein; LDL, low density lipoprotein; Statistical significance $P<0.05$

cholesterol, creatinine, and FBS concentrations were higher in these patients compared with the controls ($P<0.001$). Diabetes, dyslipidemia, and smoking were all more common in the AMI group than in the control group ($P<0.001$).

Genotyping data

All the studied polymorphisms in the control group followed Hardy–Weinberg equilibrium (HWE) ($P>0.05$). Table 4 summarizes the genotypic and allelic frequencies of the *FVL*, *MTHFRC677T* and *FXIIIVal34leu* polymorphisms. There were no statistically significant differences between the patients and control groups in the distribution of allelic and genotypic frequencies of *FVL*, and *FXIIIVal34leu* polymorphisms ($P>0.05$). However, there was a significant association between *CT* genotype of *MTHFRC677T* polymorphism and AMI (OR 1.76, 95% CI 1.07–2.75, $P=0.02$).

Association of genotypes with clinical characteristics

No association was observed between clinical characteristics and *FVL* and *FXIIIval34leu* polymorphisms. The association between *MTHFRC677T* polymorphism and clinical characteristics of AMI patients is shown in Table 5. According to our findings, patients with the *TT* genotype of *MTHFR* polymorphism had higher creatinine levels (2.07 ± 0.19 mg/dl; $P<0.001$).

Discussion

Although there was no significant association between *FVL* and *FXIIIVal34Leu* polymorphisms and AMI. A meaningful relationship was found between *CT* genotype of *MTHFR* polymorphism and AMI. Furthermore, higher creatinine levels were observed in patients with the *MTHFR677TT* genotype.

Factor V is a blood protein that aids in the conversion of prothrombin to thrombin by acting as a cofactor. After clot formation, activated protein C, a natural anticoagulant, cleaves and inactivates *FVa*. The *FVL* polymorphism makes factor V resistant to APC inactivation so it will remain active for a longer period and facilitate increased thrombin production, resulting in an increased risk of thrombosis. We found no association between *FVL* and occurrence of AMI, which is in line with Msalati et al. who reported that the *FVL* mutation is not significantly associated with MI [16]. Similarly, *FVL* has not been linked to myocardial infarction or stroke in prospective cohort studies [17–19]. In addition, Mahmoodi et al. found no association between *FVL* and increased risk of subsequent atherothrombotic events and mortality in high-risk participants with coronary heart disease

Table 4 Genotypic and allelic frequencies of *FactorVG1691A*, *MTHFRC677T* and *Factor XIII V34L* polymorphisms in AMI patients and control group

Polymorphisms	Genotype	Control, *n* (%)	Case, *n* (%)	*P*	OR	95% CI	Control *P*′ HWE
FVLG1691A	GG	149 (99.3)	144 (96)	1			0.96
	GA	1 (0.7)	5 (3.3)	0.13	5.17	0.59–44.8	
	AA	0 (0.0)	1 (0.7)	0.99	1.6	0.00	
Allele							
G		299 (99.7)	293 (97.7)	1			
A		1 (0.3)	7 (2.3)	0.068	0.14	0.017–1.145	
MTHFRC677T	CC	88 (58.7)	69 (46)	1			0.66
	CT	55 (36.7)	74 (49.3)	**0.02***	1.76	1.07–2.75	
	TT	7 (4.7)	7 (4.7)	0.66	1.27	0.43–3.81	
Allele							
C		231 (77)	212 (70.7)	1			
T		69 (23)	88 (29.3)	0.08	1.39	0.96–2.00	
Factor XIIIV34L	GG	102 (68)	103 (68.7)	1			0.85
	GT	43 (28.7)	46 (30.7)	0.82	1.059	0.64–1.74	
	TT	5 (3.3)	1 (0.7)	0.13	0.19	0.02–1.68	
Allele							
G		247 (82.3)	252 (84)	1			
T		53 (17.7)	48 (16)	0.58	0.88	0.57–1.36	

AMI, acute myocardial infarction; OR, odds ratio; CI, confidence interval; HWE, Hardy–Weinberg equilibrium; The OR reference of genotypes and alleles is 1 (GG genotype and G allele for the FVL G1691A polymorphism, CC and C for MTHFR C677T, and GG and G for Factor XIIIV34L)

*Statistically significant *P*-value < 0.05

Table 5 The associations between clinical characteristics and genotypes for *MTHFR* C677T polymorphism in AMI patients

	CC	CT	TT	*P* value
MTHFR C677T				
Sex				
Male, No (%)	36 (46.8)	36 (46.8)	5 (6.5)	0.57
Female, No (%)	33 (45.2)	38 (52.1)	2 (2.7)	
Diabetes,				
Yes, no (%)	11 (44)	11 (44)	3 (12)	0.11
No, no (%)	58 (47.5)	61 (50)	3 (2.5)	
Dyslipidemia, No (%)				
Yes	4 (44.4)	5 (55.6)	0 (0.0)	1.00
No	65 (47.1)	67 (48.6)	6 (4.3)	
Hypertension				
Yes, no (%)	5 (31.3)	9 (56.3)	2 (12.5)	0.10
No, no (%)	64 (48.9)	63 (48.1)	4 (3.1)	
Smoker				
Yes, no (%)	6 (35.3)	10 (58.8)	1 (5.9)	0.77
No, no (%)	63 (47.4)	64 (48.1)	6 (4.5)	
FBS (mg/dl)	96 (31.0)	99 (30.0)	100 (37.5)	0.56
HDL (mg/dl)	44 (11.0)	44 (9.0)	47 (13.5)	0.77
LDL (mg/dl)	87 (41.0)	98 (46.0)	95 (63.5)	0.55
Total Cholesterol (mg/dl)	152 (54.0)	172 (50.0)	170 (68.5)	0.25
Triglycerides (mg/dl)	110 (64.0)	116 (71.5)	99 (68.0)	0.42
Creatinine (mg/dl)	0.9 (0.36)	1.0 (0.40)	1.3 (1.46)	CC-CT $P^a = 0.10$ CC-TT $P^a = 0.00$ CT-TT $P^a = 0.00$

Values are mean ± SD or *n* (%), FBS fasting blood sugar, HDL high density lipoprotein, LDL low density lipoprotein, Statistical significance $P < 0.05$, a. Kruskal Wallis Test

[20]. In contrast to these studies, some previous studies reported the association of *FVL* with MI [5, 21]. Therefore, the association between *FVL* and AMI is still a controversial topic. It is worthy of note that in the present study as in the study by Ezzat et al. [21], one myocardial infarction patient had the mutated homozygous genotype AA (0.7%).

Mechanical stabilization of fibrin clots and the protection of newly formed fibrin from fibrinolysis are two main hemostatic functions of coagulation *FXIII* [22]. The *FXIIIVal34Leu* polymorphism has been reported to increase the activation rate of this coagulation factor by thrombin that affects the clot structure [23]. According to research findings, individuals with the 34Leu allele have clots with thinner fibrin fibers [24]. The effect of this polymorphism may vary depending on the plasma levels of fibrinogen and thrombin in different populations [25]. In our study, there were no differences between AMI patients and healthy groups in the prevalence of the *FXIII* genotypes, which is consistent with some previously published research [26, 27]. In contrast, some studies have shown a protective role of this polymorphism against thrombosis [28]. By the same token, a meta-analysis of the published data revealed that the *Leu34* allele provides moderate but significant protection [29]. Ethnicity has been reported as a major driver behind *Val34Leu* frequencies around the world [30, 31].

One of the interesting findings from our study was the association of *CT* genotype in *MTHFRC677T* polymorphism with the risk of AMI. As indicated by the results of previously published studies, the common *C677T* (*rs1801133*) single nucleotide polymorphism is associated with lower *MTHFR* enzyme activity and plasma homocysteine concentration [32]. For instance, Kang et al. reported that the mean total plasma homocysteine level in patients with *MTHFRC677T* polymorphism was significantly higher than the normal value [33]. It has also been proved that hyperhomocysteinemia is associated with endothelial dysfunction [34]. Although the exact mechanism of homocysteine toxicity is unknown, it is believed that homocysteine causes atherosclerosis by negatively affecting the vascular endothelium [35]. Unfortunately, the present study did not measure plasma homocysteine levels. Most importantly, however, we found that patients with the *MTHFR677TT* genotype have higher levels of creatinine. Since homocysteine is crucial for the formation of creatine and creatinine, the

increase in serum creatinine can be a reflection of high levels of serum homocysteine [36].

Given the differences in sample sizes, ethnicity, and geographical conditions, the frequency of *FVL*, *MTHFRC677T*, and *FXIIIVal34Leu*polymorphisms in this population differs from that of other populations.

The main study limitation is small sample size of study population, which prevented further examination of the studied gene polymorphisms in relation to the clinical characteristics of the patients. We believe that more research involving a larger number of people will yield more conclusive results. Furthermore, the two study groups in the present study were not age-matched due to the time constraints and our limitation in patient selection. Finally, AMI patients had more cardiovascular risk factors (Table 3), which naturally made them more at risk of developing AMI.

Conclusion

To the best of our knowledge, this was the first study to examine the association of these three mutations with AMI in east Iran. These results may imply the effects of *MTHFR C677T* polymorphism and higher mean serum creatinine levels in the pathogenesis of AMI. Therefore, screening of these parameters might be used for clinical risk assessment.

Abbreviations

BMI: Body mass index; Chol: Cholesterol; FBS: Fasting blood sugar; FVL: *Factor V Leiden*; HDL: High-density lipoprotein; LDL: Low-density lipoprotein; MT: Methylenetetrahydrofolate reductase; T-ARMS-PCR: Tetra primer-amplification refractory mutation system-polymerase chain reaction; TG: Triglyceride; Val-34Leu: Valine-34-Leucine.

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Author contributions

AG performed the experiments, and wrote the manuscript. AR and NM performed the experiments. GAS and KD collected the studied samples, critical revision of the manuscript. FS analysed the data. NA designed the study, physical examination of the patients, revised the manuscript. SMS designed the study, revised the manuscript, and supervised the project. All authors read and approved the final manuscript.

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Availability of data and materials

The datasets used and/or analysed during the current study are available from the corresponding author on reasonable request.

Declarations

Ethics approval and consent to participate

The study protocol was evaluated and approved by the Ethics committee of Birjand University of medical sciences (Ref. ID: IR.BUMS.REC.1399.264).

Consent for publication

After informed consent was obtained, venous blood sample was collected from all participants.

Competing interests

The authors declare that they have no competing interests.

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