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Association of *C677T MTHFR* and *G20210A FII* prothrombin polymorphisms with susceptibility to myocardial infarction

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Abstract. Myocardial infarction (MI) is a common complex pathology, localized in the main leading causes of mortality worldwide. It is the result of the interaction of genetic and environmental factors. The aim of the present study was to investigate the potential association of C677T 5,10-methylenetetrahydrofolate reductase (*MTHFR*) (rs1801133) and *G20210A* factor II prothrombin (*FII*) (rs1799963) polymorphisms with the susceptibility of MI. Following extraction by the standard salting-out procedure, DNA samples of 100 MI patients and 182 apparently healthy controls were genotyped by polymerase chain reaction-restriction fragment length polymorphism using *Hinf*I and *Hind*III restriction enzymes, respectively. The results show a significant association of the *G20210T FII* polymorphism with the MI risk. The frequencies of the heterozygote genotype GA, homozygous mutated AA and the *G20210A* allele was higher among patients compared to controls (GA: 59 vs. 5.5%, P<0.001; AA: 10 vs. 0%, P=0.003; and 20210A: 39.5 vs. 2.7%, P<0.003), suggesting that this polymorphism may be a potential genetic marker for MI. No significant association was observed between the *C677T MTHFR* and MI occurrence, and there was more heterozygote CT in the patient group compared to the controls. As a multifactorial disease, the development of MI may be the result of numerous factors that influence synergistically its occurrence. Thus, further studies are merited to try to better assess these associations (gene-gene and gene-environment interactions).

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Abbreviations: MI, myocardial infarction; *MTHFR*, 5,10-methylenetetrahydrofolate reductase; *FII*, factor II prothrombin; CI, confidence interval; OR, odds ratio

Key words: myocardial infarction, susceptibility, association, Moroccan population, *C677T MTHFR*, *G20210A FII*

Introduction

According to the World Health Organization, every year ~50 million people succumb due to ischemic heart diseases, particularly myocardial infarction (MI), which is a worldwide leading cause of fatality (1). In general, MI is a result of myocardium necrosis, which occurs when the latter does not receive enough oxygen, due to a sudden occlusive thrombosis of the coronary artery that irrigates this section of myocardium (2).

Several studies have identified that genetic in addition to known risk factors (including age, gender, arterial hypertension, smoking, diabetes and dyslipidemia) strongly increases the risk of MI occurrence (3). Among the numerous genes that were previously found to be in association with MI susceptibility, 5,10-methylenetetrahydrofolate reductase (*MTHFR*) and factor II prothrombin (*FII*) genes are the most widely reported.

The *MTHFR* gene is located on chromosome 1 at the 'Ip36.3' position; the corresponding cDNA sequence comprises 11 exons spanning 2.2 kb (4). The main product of the *MTHFR* gene is a protein of 77 kDa with catalytic activity, composed of 656 amino acids (5). It is involved in folate metabolism by catalyzing the irreversible conversion of 5,10-methylenetetrahydrofolate (5,10-CH_2-FH_4) into 5-methyltetrahydrofolate (5-CH_3-FH_4), which is the major circulating form of folic acid and the cosubstrate for remethylation of homocysteine to methionine (6,7).

In its 5-methyl form, folate participates in single carbon transfers that occur during nucleotide synthesis; S-adenosylmethionine formation; remethylation of homocysteine to methionine; and methylation of DNA, proteins, neurotransmitters and phospholipids (8).

The *C677T* allele of the *MTHFR* gene is the conversion of cytosine (C) to thymine (T) at position 677, which results in a conversion of alanine to valine at the binding site of the flavin adenine dinucleotide, the cofactor of *MTHFR* enzyme (9). This allele is commonly known as 'labile'; it facilitates the separation of the enzyme from its cofactor, reducing the activity of the encoded enzyme at ≥37˚C (10,11). Thus, if the folate intake is insufficient, the activity of the homozygote decreases

by 50-60% at 37˚C, and by 65% at 46˚C; heterozygotes are in the intermediate range, and homozygotes tend to have slightly increased plasmatic homocysteine levels (10).

Prothrombin is a precursor of thrombin. It has an essential role in fibrin formation and coagulation procedure. Numerous studies have demonstrated that a single base-pair change in the prothrombin gene (*G20210A*) is associated with increased plasma prothrombin levels (12-15); this has generated significant interest and it has been suggested that this polymorphism may lead to increased risks of arterial and venous thrombosis (16).

Located on chromosome 11 (11p11), in a 3'-untranslated region of the prothrombin gene, the G20210A variant replaces a single base of guanine (G) with adenine (A) at position 20210, where the pre-mRNA receives the poly A-tail (17). Patients with one copy of this polymorphism have ~5-fold greater risk of blood clot formation compared to patients without. The risk becomes 50-fold higher among subjects with two copies of the *20210A* allele. In such conditions, they become prone to earlier or severe arterial and venous thrombosis, particularly with the addition of a family history of such events (12,18,19).

Although mutation in the prothrombin gene has been frequently studied, data regarding this mutation to the risks of MI are limited and their interpretation has been controversial (13-15).

In the present study, the main aim was to investigate the association of the *C677T* and *G20210A* variants of *MTHFR* and *FII* prothrombin genes with MI among Moroccan patients.

Materials and methods

Patients. In the present study, 100 Moroccan MI patients were recruited from the Department of Cardiology (Ibn Rochd University Hospital Center, Casablanca, Morocco), in addition to 184 DNA samples of apparently healthy subjects from the DNA bank of the general population available in the Laboratory of Genetics and Molecular Pathology (Medical School, University Hassan II, Casablanca, Morocco). Written consent was obtained from all participants, and blood samples were collected into 4cc EDTA tubes and were stored at -20˚C or treated immediately. For the patients, genomic DNA was extracted from peripheral blood leukocytes using the salting-out procedure (20), and DNA was quantified using spectrophotometry. The study has been performed in accordance with the Declaration of Helsinki and has been approved by the ethics committee of The School of Medicine, Casablanca, Morocco.

Polymorphism analysis. *C677T MTHFR* and *G20210A FII* polymorphisms were analyzed using polymerase chain reaction and restriction fragment length polymorphism techniques, as respectively described by Frosst *et al* (21) and Danneberg *et al* (22). Following cleavage of amplified fragments using *Hinf*I and *Hind*III restriction enzymes, respectively, DNA bands were stained with ethidium bromide and visualized under ultraviolet light.

Statistical analysis. Statistical analyses were performed using SPSS software 21.0 (IBM Corp., Armonk, NY, USA). χ^2 test was used to determine statistical significance of association/non-association between genotypes and clinical parameters. Hardy-Weinberg Equilibrium (HWE) test was performed in the cases and controls groups for the two polymorphisms analyzed. Odds ratio (OR) was calculated to estimate the association between genotypes and risk of MI, using a confidence interval (CI) of 95%, and P<0.05 was considered to indicate a statistically significant difference.

Table I. HWE for the *C677T MTHFR* and *G20210A FII* distributions among the cases and controls.

	HWE cases		HWE controls	
Genotypes	χ^2	P-value (P>0.05)	χ^2	P-value (P>0.05)
C677T MTHFR	1.65	0.43[a]	0.68	0.457[a]
G20210A FII	5.5	0.06[a]	0.16	1[a]

[a]Statistically significant difference. HWE, Hardy-Weinberg Equilibrium; *MTHFR*, 5,10-methylenetetrahydrofolate reductase; FII, factor II prothrombin.

Results

Patient characteristics. The study cohort consisted of 100 MI patients and 182 apparently healthy controls. The distributions of *C677T MTHFR* and *G20210A FII* polymorphisms were in HWE in the case and control groups (Table I). The average age of patients was 58.60±2.17 years, and 66% of them were >50 years of age with a male predominance (70 males vs. 30 females).

Correlation analysis for the polymorphisms. Among the seven risk factors analyzed in the study, *C677T MTHFR* was positively correlated with obesity (P=0.02). No correlation was observed between *G20210A FII* with any one of these risk factors (Table II).

For the *C677T MTHFR* polymorphism, the genotypic and allelic frequencies were 38, 52 and 10%, respectively, for the CC, CT and TT genotypes, and 64 and 36%, respectively, for the 677C and 677T alleles among the cases. In the control group, the frequencies were 52.2, 41.8 and 6%, respectively, for the CC, CT and TT genotypes, and 73.1 and 26.9%, respectively, for the 677C and 677T alleles (Table III).

For the *G20210A FII* variant, the genotypic and allelic frequencies among the cases were 31, 59 and 10%, respectively, for the GG, GA and AA genotypes, and 60.5 and 39.5%, respectively, for the *20210G* and *20210A* alleles. Among the healthy controls, 94.5% were homozygous for the 20210G allele. The 20210A allele was absent in the homozygous form (0% AA), and only 5.5% carried one copy of it (GA). Allelic frequencies were 97.3% for the 20210G allele vs. 2.7% for the 20210A allele (Table III).

No statistically significant association was observed between the *C677T MTHFR* polymorphism and MI risk [CT: OR=1.71 (95% CI, 1.02-2.84), P=0.61; TT: OR=2.27 (95% CI, 0.88-5.82), P=0.64; and 677T: OR=1.35 (95% CI, 0.89-2.03), P=0.75], suggesting that even patients carrying at least one copy of the 677T allele are not protected and this variant may not be a genetic risk factor for MI susceptibility in Morocco.

Table II. Distribution of *C677T MTHFR* and *G20210A FII* polymorphisms according to risk factors among MI patients.

		MTHFR, n (%)				*FII*, n (%)			
Risk factors	Patients, n (n=100)	CC	CT	TT	P-value	GG	GA	AA	P-value
Age, years									
≤50	34	13 (38.23)	19 (55.88)	2 (5.89)	0.36	13 (38.23)	18 (52.94)	3 (8.83)	0.53
>50	66	25 (37.87)	33 (50.00)	8 (12.13)		18 (27.27)	41 (62.12)	7 (10.61)	
Gender									
Male	70	30 (42.85)	34 (48.57)	6 (8.58)	0.33	22 (31.42)	44 (62.85)	4 (5.73)	0.1
Female	30	8 (26.67)	18 (60.00)	4 (13.33)		9 (30.00)	15 (50.00)	6 (20.00)	
HTA									
Presence	44	16 (36.37)	25 (56.81)	3 (6.82)	0.46	11 (25.00)	28 (63.63)	5 (11.37)	0.5
Absence	56	22 (39.28)	27 (48.21)	7 (12.51)		20 (35.71)	31 (55.35)	5 (8.94)	
Diabetes									
Presence	39	10 (25.64)	25 (64.10)	4 (10.26)	0.12	8 (20.51)	27 (69.23)	4 (10.26)	0.17
Absence	61	28 (45.90)	27 (44.26)	6 (9.84)		23 (37.70)	32 (52.45)	6 (9.85)	
Smoking									
Presence	45	19 (42.22)	22 (48.88)	4 (8.89)	0.49	13 (28.89)	29 (64.44)	3 (6.66)	0.47
Absence	55	19 (34.54)	30 (54.54)	6 (10.92)		18 (32.72)	30 (54.54)	7 (12.74)	
Obesity									
Presence	22	8 (36.36)	8 (36.36)	6 (27.28)	0.02[a]	7 (31.81)	12 (54.54)	3 (13.65)	0.79
Absence	78	30 (38.46)	44 (56.41)	4 (5.13)		24 (30.76)	47 (60.25)	7 (8.99)	
Dyslipidemia									
Presence	27	8 (29.63)	16 (59.26)	3 (11.11)	0.59	9 (33.33)	15 (55.55)	3 (11.12)	0.9
Absence	73	30 (41.09)	36 (49.31)	7 (9.59)		22 (30.13)	44 (60.27)	7 (9.59)	

[a]P<0.05. *MTHFR*, 5,10-methylenetetrahydrofolate reductase; *FII*, factor II prothrombin; HTA, arterial hypertension.

Table III. *C677T MTHFR* and *G20210A FII* polymorphism frequencies and genetic distribution among the cases and controls.

Genes	Genotypes	Cases, n (%) (n=100)	Controls, n (%) (n=182)	OR (95% CI)	P-value (P<0.05)
MTHFR	CC	38 (38.0)	95 (52.2)	Ref	
	CT	52 (52.0)	76 (41.8)	1.71 (1.02-2.84)	0.61
	TT	10 (10.0)	11 (6.0)	2.27 (0.88-5.82)	0.64
	C	128 (64.0)	171 (73.1)	Ref	
	T	72 (36.0)	87 (26.9)	1.35 (0.89-2.03)	0.75
FII	GG	31 (31.0)	172 (94.5)	Ref	
	GA	59 (59.0)	10 (5.5)	32.73 (15.11-69.71)	<0.001[a]
	AA	10 (10.0)	0 (0.0)	115 (1.75-7332)	0.003[a]
	G	121 (60.5)	182 (97.3)	Ref	
	A	79 (39.5)	0 (2.7)	238.83 (4.48-12581.7)	<0.001[a]

[a]P<0.05. *MTHFR*, 5,10-methylenetetrahydrofolate reductase; *FII*, factor II prothrombin; OR, odds ratio; CI, confidence interval.

By contrast, the *G20210A FII* polymorphism was highly associated with MI risk among patients carrying even a single or double copy of the *20210A* allele [P-value (GA) <0.001 and P-value (AA)=0.003)] (Table III). These findings suggest that this variant may be a potential genetic marker for MI in Moroccan population.

Discussion

With a complex pathology, numerous factors are involved in the occurrence of MI. In interaction with environmental factors, the genetic background has an essential role in MI susceptibility. A number of studies began, over the few past

years, focusing on the implication of hemostatic markers in MI development, and have suggested that numerous genes affecting coagulation proteins are prothrombotic risk factors (23,24), among which are the FII (*G20210A* variant) and *MTHFR* (C677T variant) genes.

Several studies have investigated the association of prothrombotic genetic markers, including *FII* and *MTHFR* genes with MI risk, but have provided divergent and inconclusive results (5,25,26).

To the best of our knowledge, this is the first study to explore the potential association of the *G20210A FII* and *C677T MTHFR* polymorphisms with MI risk in the Moroccan population.

In the present study sample, the majority of patients were >50 years of age (66%) and were predominantly male (70 vs. 30% who were female). This was in agreement with the Croatian study by Jukic *et al* (27) regarding the implication of the ABO blood group genotypes and the prothrombotic mutations of factor V Leiden, prothrombin *G20210A FII* and *MTHFR C677T* on MI occurrence. The study noted that 75.3% of the patients recruited were >55 years of age, with a male predominance (63.2 male vs. 36.8% female). In addition, the same study reported that males had a 1.5-fold greater risk of developing MI compared to females (P<0.05), and that older age patients (>55 years) had >20-fold greater risk (OR=21.1; 95% CI, 12.64-35.23).

Hyperhomocysteinemia is known to be an important risk factor for cardiovascular diseases, such as MI. The C677T polymorphism of *MTHFR* gene has been associated with decreased *MTHFR* enzyme efficiency and its thermolability (28). Previous studies have reported that individuals carrying a double copy of the 677T allele had significantly increased plasma levels of homocysteine, and suggested that this variant may be a potential genetic risk factor for cardiovascular diseases (29-31).

According to the present data, the *C677T MTHFR* variant was significantly associated to obesity (P=0.02) (Table II). The CT genotype was the most frequent among patients (52%) compared to the other genetic profiles (38% CC and 10% TT). In healthy controls, the CC genotype was the most frequent (52.2%) vs. 41.8 and 6% for the CT and TT genetic profiles, respectively. Similar findings were reported by Spiroski *et al* (32), which found that the CT genotype was the most frequent genotype among patients (51.3%) vs. 35.5% CC and 13.2% TT. According to this study, the T allele frequency was higher in patients compared to controls, which consists with the present findings (T allele frequency in patients group was 36% compared to 26.9% among healthy controls).

Other studies reported that the higher frequency of homozygous mutated genotype TT (18-19%) was found in the Italian population (29,33,34). It was 16.7% in Greece, 6.2% in Germany and 6% in Croatia (35,36). Xuan *et al* (37) reported that the 677T allele frequency was 28.99% in Caucasian children vs. 42.28% in Asian pediatric patients, and 31.76% in the Caucasian maternal vs. 41.51% in the Asian maternal population.

Regarding the present results, no statistically significant association was observed between the *C677T MTHFR* polymorphism and MI risk in Morocco. Numerous studies have investigated this association among different populations and have noted similar findings (38-43). Other studies suggest a significant association of *C677T MTHFR* with MI risk (30,37,44,45).

The *G20210A* polymorphism of the *FII* prothrombin gene is correlated with higher plasma levels of prothrombin among subjects carrying this variant compared to normal subjects, making them prone to blood clots and thrombotic events (13,19,46,47). Regarding the biology, high plasma concentrations of prothrombin associated with the presence of the *G20210A FII* polymorphism may confer an elevated risk for cardiovascular disease (48).

The present results show that no genetic profile was the most frequent in the patient group compared to the controls, and no statistically significant association of the *G20210A FII* polymorphism with any one of the risk factors analyzed was observed. There was more heterozygote genotype (GA) among patients compared to the healthy controls (59 and 5.5%, respectively). Consistent results were reported by Ercan *et al* (48) among the Turkish population, and even the heterozygote exhibited a significant association of the *G20210A FII* polymorphism with coronary artery disease risk.

According to the present data, the homozygous mutated genotype (AA) frequency was 10% among patients and 0% among controls. The *20210A* allele frequency was higher in the patient group (39.5%) compared to the control group (2.7%). The study performed by Ercan *et al* (48) reported similar results among healthy controls, signaling that the homozygous mutated profile of *G20210A FI*I polymorphism was absent in the control group. The study also reported that this genotype was not detected among patients. Other similar findings were reported by Doggen *et al* (14), signaling that the *20210A* allele was present in only 2% of healthy controls, and suggesting that the *G20210A FII* polymorphism is associated with the risk of venous thromboembolism (OR=2.8). In 2009, Gohil *et al* (49) reported that this variant was correlated the venous thromboembolism risk (OR=3.2), and that the risk associated with the homozygous genetic profile of the polymorphism was 6.7-fold higher.

The present results show that the *G20210A* polymorphism of the *FII* prothrombin gene was significantly associated with susceptibility to MI. The results of the previous studies regarding this association remain inconsistent (14,15,20,48,50-55).

The divergences in results regarding the association of *C677T MTHFR* and *G20210A FII* variants with MI risk may be explained by numerous factors, such as differences in genetic background among the studied populations; differences in the selection of patients and controls; studies sample sizes and ethnicity.

In conclusion, to the best of our knowledge this is the first study to evaluate the association of *C677T MTHFR* and *G20210A FII* prothrombin polymorphisms with MI in Morocco. According to the data, no significant association was observed between the *C677T MTHFR* variant and MI risk. The heterozygote genetic profile (CT) was more frequent among patients compared to the controls. By contrast, the *G20210A* FII variant was significantly correlated to MI risk, with a predominance of the heterozygote genotype (GA) and the high frequency of the *20210A* allele among patients compared to the controls, thus suggesting that this polymorphism may be a potential genetic marker for MI in

Morocco. As a multifactorial disease, the development of MI may be synergistically influenced by numerous factors at different intensities. Thus, further investigations are required to assess these associations in greater detail (gene-gene and gene-environment interactions).

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