

G2691A and C2491T mutations of factor V gene and pre-disposition to myocardial infarction in Morocco

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Abstract. Coagulation factor Leiden mutation has been described as a common genetic risk factor for venous thrombosis; however, this mutation was reported to be practically absent in an African population. Recently, a novel non-sense mutation in the gene encoding factor V has been associated with the risk of occurrence of cardio-cerebrovascular diseases such as stroke and venous thrombosis. The aim of the present study was to investigate whether the factor V Leiden (FVL) and C2491T non-sense mutations are associated with the risk of developing myocardial infarction. Genotyping of FVL and C2491T FV was performed using the polymerase chain reaction restriction fragment length polymorphism method on a sample of 100 patients with myocardial infarction as well as 211 controls. In the study population, the frequency of the FVL mutation was practically zero. However, with regard to the C2491T mutation, the TT genotype was associated with an increased risk of myocardial infarction [odds ratio (OR)=3.16, 95% confidence interval (CI): 1.29-7.71, P=0.03]. A significant association between the C2491T FV mutation and the risk of myocardial infarction was identified using recessive (OR=2.74, 95% CI: 1.14-6.58, P=0.04), dominant (OR=1.85, 95% CI: 1.13-3.04, P=0.02) and additive (OR=1.88, 95% CI: 1.25-2.80, P=0.004) models. Furthermore, a positive correlation was found between the presence of the C2491T FV mutation and hypertension (P=0.02), which is associated with myocardial infarction. In conclusion, the results of the present

study suggested that the C2491T non-sense mutation of the FV gene may be a risk factor for myocardial infarction in a Moroccan population.

Introduction

Venous thrombosis is a major risk factor of myocardial infarction (MI) (1). Several studies have been performed to determine risk factors for thrombosis, among which coagulation factors were of major interest (2). The factor V Leiden (FVL) mutation is one of the most common causes of these thrombotic disorders, which, together with resistance to activated protein C, has long been considered a pre-disposing factor (3). Numerous studies have attempted to determine the association between the FVL mutation and thromboembolic diseases; which, however, has remained controversial (4-6). Certain studies have shown that the FVL mutation was associated with an increased risk of MI (7), while a meta-analysis only showed a modest association and a Danish study showed no association (8,9). Moreover, it has also been shown that the distribution of the frequency of FVL gene varies from one population to another, i.e., its frequency is higher in Europeans (10) than in African populations, particularly in Moroccans, in whom it is almost absent (11-13). Other mutations in the FV gene have been identified, including the C2491T variant, which was detected in a patient of Moroccan origin. It is a non-sense mutation in exon 13, where a C-to-T substitution at nucleotide 2,491 changed a glutamine codon at residue 773 to a stop codon (14) and thereby encodes a protein consisting of a FV heavy chain and a B domain comprising only 63 amino acids. Thus, this FV variant lacks ~90% of the B domain and the complete light chain (14). Recently, a case control study by our group showed that the C2491T FV mutation was associated with an increased risk of cerebral ischemia and that this risk was doubled in subjects carrying two morbid alleles (15). Furthermore, Hamzi *et al* (16) assessed the frequency of the C2491T FV mutation in a healthy Moroccan population [72.7% CC (wild-type), 23.7% CT (heterozygous mutated type) and 3.6% TT (homozygous mutated type); 84.54% C allele and 15.46% T allele). To the best of our knowledge, the present study was the first to assess the association between the C2491T FV non-sense mutation and MI. The objective of the present study was to assess the potential contribution of the

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Abbreviations: CI, confidence interval; HWE, Hardy-Weinberg equilibrium; LGPM, genetic and molecular pathology laboratory; MI, myocardial infarction; OR, odds ratio

Key words: factor V Leiden, C2491T, non-sense, mutation, myocardial infarction

FVL and C2491T mutations of the coagulation FV gene to the general risk of MI in a Moroccan patient population.

Materials and methods

Study population. The equation published by Dawson-Dawson-Saunders and Trapp was used to calculate the sample size with 80% power (17). The study population consisted of 311 subjects, including 100 patients who presented with MI ≤ 3 years ago, recruited at the Cardiology Service of CHU Ibn Rochd (Casablanca, Morocco) and 211 healthy control subjects recruited from volunteer blood donors with no history of MI. The following clinical data were collected from the medical records of each patient: Age, gender, status of smoking, hypertension, diabetes, dyslipidemia, obesity, similar familial cases and valvulopathy as well as left ventricular ejection fraction and coronary angiography results. A 5-ml blood sample was taken from each patient. The present study was approved by the Ethics Committee of Hassan II University (Casablanca, Morocco). Written informed consent was obtained from all participants prior to blood sampling and genetic analysis.

Genotyping analysis. DNA was extracted from the peripheral blood using standard methods described by Miller *et al* (18). The quality and quantity of the DNA were determined using a NanoVue Plus spectrophotometer (Biochrom Ltd., Cambridge, UK).

Genotyping of the G1691A FV and C2491T FV mutations was performed by polymerase chain reaction (PCR) restriction fragment length polymorphism amplification of each of the target alleles from genomic DNA, followed by restriction digestion with the corresponding enzymes *HindIII* and *HphI*, respectively, as previously described by Huber *et al* (19) and van Wijk *et al* (14). For FVL, PCR amplification and digestion

gave a fragment of 241 bp for the GG wild-type, three fragments of 241, 209 and 32 bp for the GA heterozygous type and two fragments of 209 and 32 bp for the AA mutated homozygous type. For the non-sense mutation C2491T FVC, the digested PCR amplification products were two fragments of 458 and 147 bp for the CC wild-type, three fragments of 605, 458 and 147 bp for the CT heterozygous type and one fragment of 605 bp for the TT mutated homozygous type (14).

Statistical analysis. Statistical analysis was performed using SPSS software version 19.0 (International Business Machines, Armonk, NY, USA). A Hardy-Weinberg equilibrium (HWE) was calculated for FVC and FVL polymorphisms for non-parametric data from cases and controls separately. Clinical parameters of patients with different genotypes were subjected to the χ^2 test. $P < 0.05$ was considered to indicate a statistically significant difference. To test the association between genotypes and the prevalence of MI, odds ratios (ORs) were calculated as a measure of the relative risk for MI and were presented with a 95% confidence interval (CI). To avoid the error type I, Bonferroni's correction was made to better interpret the results regarding the genetic association (20).

Results

A total of 311 subjects, including 100 MI patients and 211 controls, were enrolled in the present study. The mean age in the patient and control groups was 56.60 ± 2.17 and 54 ± 2 years, respectively. Genotyping analysis revealed that all subjects were void of the FVL mutation.

Table I shows the distribution of allelic and genotypic frequencies of C2491T FV in MI patients and controls. The distribution of the C2491T FV mutation was within the HWE in the cases and controls. The genotype frequencies of C2491T FV in MI patients were 57.0% CC (wild-type),

Table I. Distribution of the genotypes CC, CT and TT of the C2491T FV gene as well as the alleles C and T in patients with myocardial infarction (n=100) and healthy controls (n=211).

C2491T FV genotype/allele	Cases, n (%)	Controls, n (%)	OR (95% CI)	P-value	Corrected P-value ^a
CC	57 (57.0)	150 (71.1)	1		
CT	31 (31.0)	51 (24.2)	1.59 (0.93-2.75)	0.09	0.27
TT	12 (12.0)	10 (4.7)	3.16 (1.29-7.71)	0.01	0.03 ^b
CC+CT	88 (88.0)	201 (95.3)	1		
TT	12 (12.0)	10 (4.7)	2.74 (1.14-6.58)	0.02	0.04 ^b
CC	57 (57.0)	150 (71.1)	1		
CT+TT	43 (43.0)	61 (28.9)	1.85 (1.13-3.04)	0.01	0.02 ^b
Allele C ^d	145 (73.0)	351 (83.2)	1		
Allele T ^e	55 (27.0)	71 (16.8)	1.88 (1.25-2.80)	0.002 ^c	0.004 ^b
HWE P-value	0.08015				

^aBonferroni's correction was applied; ^bCorrected $P < 0.05$; ^c $P < 0.01$, MI vs. control. ^dNumbers include C allele in the associated genetic models; ^eNumbers include T allele in the associated genetic models. Genetic models: TT vs. CC+CT, recessive model for C2491T FV; CT+TT vs. CC, dominant model for C2491T FV; T vs. C allele, additive model for C2491T FV. CC/allele C was used as a reference (set as 1). OR, odds ratio; CI, confidence interval; FV, factor V; CC, wild-type homozygote C2491T FV; CT, heterozygote C2491T FV; TT, mutant homozygote C2491T FV; HWE, Hardy-Weinberg equilibrium.

Table II. Association of C2491T polymorphism frequencies with clinical and radiographic parameters in MI patients (n=100).

Parameters	n	CC, n (%)	CT, n (%)	TT, n (%)	P-value
Age (years)					0.18
<45	28	19 (67.9)	7 (25.0)	2 (7.1)	
≥45	72	35 (48.6)	24 (33.3)	13 (18.1)	
Gender					0.73
Female	30	18 (60.0)	8 (26.7)	4 (13.3)	
Male	70	36 (51.4)	23 (32.9)	11 (15.7)	
Hypertension					0.02 ^a
Yes	44	18 (40.9)	15 (34.1)	11 (25.0)	
No	56	36 (64.3)	16 (28.6)	4 (7.1)	
Diabetes					0.07
Yes	39	16 (41.0)	14 (35.9)	9 (23.1)	
No	61	38 (62.3)	17 (27.9)	6 (9.8)	
Smoking					0.08
Yes	45	21 (46.7)	19 (42.2)	5 (11.1)	
No	55	33 (60.0)	12 (21.8)	10 (18.2)	
Obesity					0.32
Yes	22	9 (40.9)	8 (36.4)	5 (22.7)	
No	78	45 (57.7)	23 (29.5)	10 (12.8)	
Dyslipidemia					0.23
Yes	27	11 (40.7)	10 (37.0)	6 (22.2)	
No	73	43 (58.9)	21 (28.8)	9 (12.3)	
Family history of MI					0.37
Yes	3	1 (33.3)	2 (66.7)	-	
No	97	53 (54.6)	29 (29.9)	15 (15.5)	
LVEF					0.76
Normal	44	21 (47.7)	16 (36.4)	7 (15.9)	
Moderately abnormal	41	23 (56.1)	12 (29.3)	6 (14.6)	
Severely abnormal	15	10 (66.7)	3 (20.0)	2 (13.3)	
Valvulopathy					0.56
Yes	28	14 (50.0)	8 (28.6)	6 (21.4)	
No	72	40 (55.6)	23 (31.9)	9 (12.5)	
Stenosis					0.13
Yes	66	32 (48.5)	21 (31.8)	13 (19.7)	
No	34	22 (64.7)	10 (29.4)	2 (5.9)	

^aP<0.05. LVEF, left ventricular ejection fraction; MI, myocardial infarction.

31.0% CT (heterozygous mutated type) and 12.0% TT (homozygous mutated type), and those in the control subjects were 71.1% CC, 24.2% CT and 4.7% TT. The allele frequencies were 73.0% C and 27.0% T in the MI patients vs. 83.2% C and 16.8% T in the controls (Table I). Statistical analysis revealed that the TT-mutated homozygous type of C2491T FV was significantly associated with an increased risk of MI (OR=3.16, 95% CI: 1.29-7.71, P=0.01) (Table I). Using three models of genotypic combination, a significant association with the risk of IM was determined with the recessive model (TT vs. CC+CT; OR=2.74, 95% CI: 1.14-6.58, P=0.02), the dominant model (CT+TT vs. CC; OR=1.85, 95% CI: 1.13-3.04, P=0.01)

and the additive model (T vs. C; OR=1.88, 95% CI: 1.25-2.80, P=0.002) (Table I).

Table II shows the association of C2491T polymorphism frequencies with clinical and radiographic characteristics. In the present study, MI patients aged ≥45 years (72%) were overrepresented compared to younger patients (age, <45 years; 28%), and there was a male predominance (70%). No positive correlation was observed between C2491T FV mutation and patient age, gender, diabetes, smoking status, obesity, dyslipidemia, family history of MI, valvulopathy and stenosis. However, the TT homozygous C2491T FV mutation was significantly associated with hypertension (P=0.02) (Table II).

Discussion

MI is a multifactorial condition associated with several risk factors, comprising constitutional, acquired and environmental factors (21-23). Constitutional or genetic factors may have a major role in the formation of occlusive thrombus (24) and the clinical progression of the rupture of atherosclerotic plaques. The association of MI with several molecular variants of coagulation factor genes, including FV and FII, have been studied; however, it has remained contradictory (7). In the present study, the association of mutations of the two FV genes FVL and C2491T FV with MI was explored. Previous studies by our group have revealed the total absence of the G2691A FVL mutation in a healthy population (12) and in stroke subjects (11), which was confirmed by the results of the present study. However, a previous meta-analysis showed that the G2691A FVL mutation was moderately associated with the risk of coronary disease (7).

In the present study, MI patients and healthy controls were assessed for the presence of the C2491T non-sense mutation in exon 13 of FV. The T-allelic frequency in the cases (27.0%) was significantly higher than in the controls (16.8%; $P < 0.05$). A significant association of the mutated TT genotype and the risk of MI was identified using three models of genotypic combination (recessive, dominant and additive). The risk for MI was shown to be increased if the patient is a carrier of the TT genotype. This result confirmed the role of the C2491T non-sense mutation in the FV gene with blood coagulation in the Moroccan population. The C2491T FV mutation in a Moroccan patient was first discovered by van Wijk *et al* (14), and Hamzi *et al* (16) assessed the frequency of this mutation in the general Moroccan population. As the present study was the first to assess the association between the C2491T FV mutation and MI, no direct comparison with the literature is possible. However, a recent study by Diakite *et al* (15) revealed a strong association between the T allele and TT genotype of the C2491T FV non- with the risk of ischemic stroke. Thus, the present study confirmed the role of this non-sense mutation in the pathogenesis of thromboembolic diseases. The non-sense mutation in exon 13 of the FV gene can induce thromboembolic diseases due to a possible decay of the FV mRNA as well as a lack of ~90% of the B domain and the complete light chain of the FV protein, leading to thrombophilia (14).

In conclusion, the present study demonstrated that non-sense mutation of C2497T FV was significantly associated with an increased risk of MI in a Moroccan population. Consistently, a significant correlation of the TT-type homozygote C2497T mutation with hypertension was identified. Evaluation of the C2491T non-sense mutation of the FV gene may aid in preventing/reducing the occurrence of thromboembolic diseases.

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