

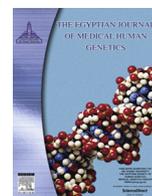
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Original article

Contribution of coagulation factor VII R353Q polymorphism to the risk of thrombotic disorders development (venous and arterial): A case-control study

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ABSTRACT

Background: Elevated factor VII (FVII) level is a risk factor for thromboembolic disorders. It was reported that the FVII R353Q polymorphism is associated with variation in plasma FVII levels, where Q allele carriers were more associated with lower levels of FVII than R allele carriers. However, the association between coagulation FVII R353 Q polymorphisms and the risk of thrombosis is uncertain.

Aim of the study: Is to investigate the contribution of factor VII R353Q gene polymorphism to the risk of thrombotic disorders development (venous and arterial) in a group of Egyptian patients.

Subjects and methods: This study was conducted on 310 subjects: 110 acute myocardial infarction (AMI) patients, 108 deep venous thrombosis (DVT) patients and 92 healthy controls. FVII R353Q genotypes were assessed using restriction fragment length polymorphism analysis.

Results: There were no statistically significant differences in the frequency of FVII R353Q polymorphism between each of the AMI and DVT patients and the control group ($P = 0.9, 0.1$). However the Q allele showed a significantly higher frequency in the AMI group (15.4%) vs. controls (8.7%) (OR: 1.92; 95% CI: 0.98–3.7). Bivariate analysis demonstrated no significant association between FVII R353Q genotypes and different studied risk factors, neither in arterial nor venous thrombosis.

Conclusion: FVII R353Q polymorphism did not contribute to an increased risk of thrombosis (arterial and venous); also carrying the Q allele (of R353Q) did not confer protection against acute thrombotic events.

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1. Introduction

Acute myocardial infarction (AMI), stroke, deep vein thrombosis (DVT) of the lower limbs and pulmonary embolism (PE) are the most common causes of arterial and venous thrombotic disorders, which causes high morbidity and mortality [1]. Genetic factors contribute significantly to the development of these disorders. The advent of molecular genetics, allowed the elucidation of the

role of mutations in certain genes and their impact on haemostatic disorders such as arterial and venous thrombosis [2].

Factor VII (FVII) is a Vitamin K-dependent inactive protease, which is converted to its active form upon contact with the tissue factor, and then it activates factor X and factor IX [2]. The activity of FVII has been associated with increased thrombotic risk [3,4]. However, circulating FVII levels may not accurately reflect their concentrations and activities, because they can be affected by diet, exercise, and drug therapy; therefore, genetic risk studies may be more reliable predictors of both circulating and local effects [5].

The FVII gene is located on chromosome 13; with five polymorphic sites [2] that can directly influence the FVII levels and also modulate its response to environmental stimuli [6]. Thus, they could represent interesting candidates for risk prediction of thrombotic events [1]. FVII R353Q is a common single

Abbreviations: OR, odds ratio; Arg, arginine; Gln, glutamine.

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base-change polymorphism in the FVII gene, that results in an Arg (R) to Gln (Q) substitution at position 353, which lies within the serine loop, close to the active serine 344 site [5,7]. It has been associated with variations in plasma FVII levels [7], where a 20% reduction in FVII levels [8] and coagulant activity were observed in carriers of the 353Q genotype compared with carriers of the 353R genotype [9]. However, the association between the R353Q FVII polymorphism and coronary heart disease is uncertain, with the protective role of the Q allele against AMI in several studies [10,11] is counterbalanced by numerous reports showing no such role in other populations [12–14], including the Framingham Heart study [15]. In addition, few studies have been unable to ascertain the role of factor VII genotype in venous thrombosis [16–18] and its implication on the response to antiplatelet therapy, where those at higher risk of venous thrombosis could gain more benefit from prolonged antiplatelet therapy [1]. Furthermore, the association in Arab was limited to a previous study in the Tunisian population [19]. The aim of this study was to investigate the contribution of factor VII R353Q gene polymorphism to the risk of thrombotic disorders development (venous and arterial) in a group of Egyptian AMI and DVT patients.

2. Subjects and methods

2.1. Study design and setting

A case-control study was conducted from April 2013 to May 2015 in Cardiac Intensive Care Unit, Cardiology Department, Specialized Medical Hospital, Department of Vascular Surgery and Department of Clinical pathology, Mansoura University.

2.2. Study groups

This study was conducted on 218 patients, divided into two groups. The first was the AMI group, comprised of 110 patients, diagnosed by elevated Troponin T and cardiac enzymes and/or electrocardiographic changes, 90 (81.8%) patients of whom had elevated ST segment. The second was the DVT group, comprised of 108 patients, diagnosed by Duplex examination and CT chest with contrast when pulmonary embolism was suspected; 15 (13.8%) patients of whom had recurrent DVT, 6 (5.5%) had DVT with PE, 3 had (2.77%) portal venous thrombosis and 1 case (0.92%) had upper limb DVT. In addition, 92 age and sex matched healthy unrelated volunteers were included as a control group. They were clinically proven to be free from AMI and DVT. This work was carried out in accordance with The Code of Ethics of the World Medical Association (Declaration of Helsinki) for experiments in humans. Institutional Review Board of Mansoura Faculty of Medicine (IRB.MFM) approved the study protocol and the informed written consent form (Code R/16.01.109).

All participants were subjected to full clinical assessment and routine laboratory testing. Risk factors, including hypertension, smoking and obesity were assessed in the AMI and DVT groups as well as the control group. Hyperlipidemia as a risk factor for arterial thrombosis was assessed in AMI group in comparison to the control group.

2.3. Blood collection and DNA analysis

Fasting morning venous blood samples from all participants were withdrawn and divided into 2.5 ml plain tubes for the estimation of serum total cholesterol (TC), triglycerides (TG) and high density lipoproteins (HDL-C) by enzymatic colorimetric methods using Hitachi 912 auto-analyser, Roche Diagnostics GmbH [20], while low density lipoprotein (LDL-C) was calculated by Friede-

wald equation [21] (for controls and AMI group). In addition, 2.5 ml were withdrawn into EDTA-containing tubes for genetic analysis.

Genomic DNA was extracted from peripheral blood leukocytes using the G-spin TM extraction kit (INTRON, Biotechnology, Inc). The FVII R353Q polymorphism was detected as previously described by Green et al. [7] using restriction fragment length polymorphism (RFLP). In short, the genomic region encompassing the polymorphic site in exon 8 of the FVII gene was PCR amplified, and then the 312 bp was digested with Msp1 restriction enzyme (Thermo Scientific, Fast Digest) and resolved on a 2% agarose gel electrophoresis to reveal the digested bands: Q (272 + 40 bp), R (205 + 67 + 40 bp) (Fig. 1). Fragment sizes were estimated using 50 bp DNA ladder.

2.4. Statistical analysis

Data entry and statistical analyses were performed using SPSS (statistical package of social sciences) version 16.0 (SPSS Inc., Chicago, IL, USA). Data were expressed as mean \pm SD (continuous variables), or as percentages of total (categorical variables). Student's *t* test was used to compare continuous variables in the studied groups. Categorical variables were compared using the Chi square test or Fisher exact test. Stat calc (Epi info program) was used for inter-group comparisons in different genotypes. Testing for deviation of genotype frequencies from the Hardy–Weinberg equilibrium (HWE) was calculated by applying Hardy–Weinberg model to current data (<http://www.oege.org/software/hwe-mr-calc.shtml>). A *P* value < 0.05 was considered statistically significant.

3. Results

AMI and DVT groups were comparable to the control group with regard to age ($P = 0.07$; 0.1) and gender ($P = 0.08$; 0.8). Statistically significant traditional cardiovascular risk factors, including higher BMI ($p = 0.03$), cigarette smoker ($P = 0.001$), elevated total serum cholesterol, triglyceride levels, low density lipoprotein and decreased high density lipoprotein ($P < 0.001$) were noted in AMI vs. the control group. Meanwhile, there was a higher smoking frequency in DVT patients compared to controls with a statistically significant difference ($P = 0.03$) (Table 1).

The distribution of RR genotype (common homozygous) was lower in AMI (76.4%) and DVT (73.14%) compared to the healthy control group (82.6%). However, RQ/QQ distribution was higher in AMI (23.6%) and DVT (26.82%) compared with controls (17.4%) with no statistically significant differences ($P > 0.05$). Meanwhile, there was higher frequency of the Q allele in the AMI group (15.4%) compared with controls (8.7%) with a statistically significant difference ($P = 0.04$) (OR: 1.92; 95%CI: 0.98–3.7). However, Q allele showed no statistically significant difference between DVT (14.9%) and the control group (8.7%) (Table 2).

The difference between the observed frequency of FVII R353Q genotypes and those expected in the general population in DVT and control groups according to HWE was not statistically significant ($P > 0.05$). However, the observed frequency in AMI group was statistically significant different from those expected in general population, which was called violation from HWE ($P < 0.05$) (Table 2).

Comparing lipid profile between RR and Q allele containing genotypes revealed no statistically significant differences with regard to TC, LDL-C and TG. However, significantly higher HDL-C in RR in comparison to carriers of Q allele containing genotypes ($P = 0.01$) (Table 3). Also, there was no statistically significant difference in the distribution R353Q gene among the clinical variants of venous thrombosis ($P = 0.7$) (Table 4).

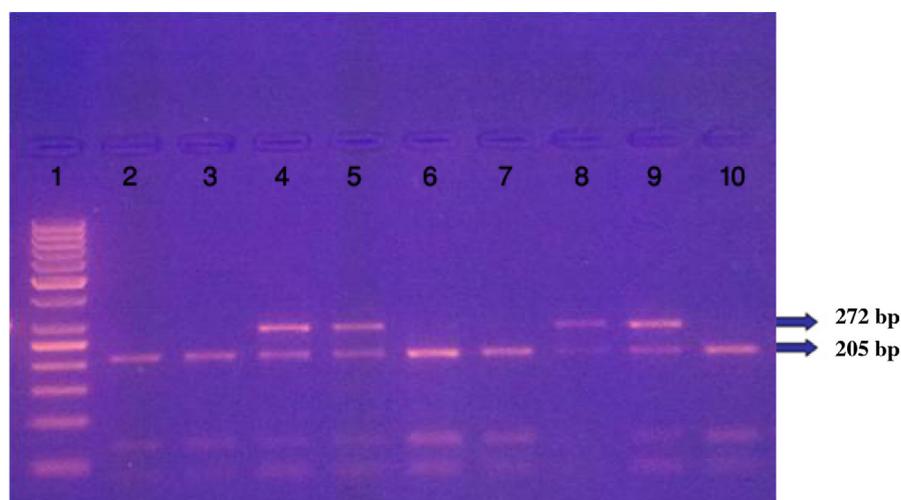


Fig. 1. RFLP detection of FVII R353Q gene polymorphism. Msp1 digested polymerase chain reaction products from individuals of different genotypes separated by agarose gel electrophoresis. The two alternative fragments of 272 bp (Q allele) or 205 bp (R allele) are shown; lane 1 showed molecular size markers 50 bp. Lanes 2, 3, 6, 7 and 10 showed homozygous RR genotype (205, 67, 40 bp), whereas lanes 4, 5, 8, and 9 showed heterozygous RQ genotype (272, 205, 67, 40 bp).

Table 1
Baseline characteristics of the studied groups.

	Studied groups			P value	
	Controls n = 92	AMI n = 110	DVT n = 108	P ¹	P ²
Age (years)	40.5 ± 10.6	43.15 ± 10.7	38.2 ± 9.7	0.07	0.1
Sex	72 (78.2%)	97 (88.2%)	67 (62%)	0.08	0.8
Male no (%)					
Smoking status	29 (31.5%)	79 (71.8%)	50 (46.2%)	0.001*	0.03*
Smoker no (%)					
BMI (kg/m ²)	25.7 ± 4.1	27.2 ± 4.7	26.7 ± 2.9	0.03*	0.2
Mean ± SD					
Cholesterol (mg/dl)	149.8 ± 29.15	181.7 ± 31.5	–	<0.001*	–
Mean ± SD					
TG (mg/dl)	94.15 ± 28.18	160.5 ± 66.0	–	<0.001*	–
Mean ± SD					
LDL-C (mg/dl)	79.5 ± 24.1	102.6 ± 27.7	–	<0.001*	–
Mean ± SD					
HDL-C (mg/dl)	54.7 ± 11.9	38.7 ± 10.7	–	<0.001*	–
Mean ± SD					

P¹: AMI vs. controls. P²: DVT vs. controls. BMI: body mass index.

LDL-C: low density lipoprotein. HDL-C: high density lipoprotein. TGs: triglycerides.

Table 2
Genotype and allelotype frequencies of FVII R353Q polymorphism in the studied groups.

FVII R353Q		Studied groups			P value	
		Controls (n = 92)	AMI (n = 110)	DVT (n = 108)	P ¹	P ²
		n (%)	n (%)	n (%)	OR (95% CI)	
Gene type	RR (r)	76 (82.6%)	84 (76.4%)	79 (73.14%)	0.9	0.1
	#RQ/QQ	16/0 (17.4%)	18 (16.4%)/8(7.2%)	28 (25.9%)/1 (0.92%)	1.02 (0.46–2.28)	1.74 (0.83–3.67)
Alleles	R(r)	168 (91.3)	186 (84.6)	186 (86.1)	0.04*	0.1
	Q	16 (8.7)	34 (15.4)	30 (14.9)	1.92	1.69
					(0.98–3.7)	(0.86–3.4)
HWE	$\chi^2 = 0.83$		$\chi^2 = 15.3$	$\chi^2 = 0.76$		
	p > 0.05		p < 0.001*	p > 0.05		

r: reference common homozygous group.

P¹: comparison between AMI & controls. P²: comparison between DVT& controls

#RQ/QQ are combined in analysis.

HWE: Hardy–Weinberg equilibrium for gene polymorphism.

Furthermore, bivariate analysis demonstrated no statistically significant association between FVII R353Q genotypes and differ-

ent studied risk factors neither in arterial nor venous thrombosis (data not shown).

Table 3

The lipid profile in AMI group according to different genotypes of FVII R353Q polymorphism.

	RR (N = 84) mean ± SD	RQ/QQ (N = 26) mean ± SD	P
Cholesterol (mg/dl)	184.3 ± 32.3	175.6 ± 24.5	0.2
LDL-C (mg/dl)	105.5 ± 28.4	94.8 ± 22.16	0.09
HDL-C (mg/dl)	40.3 ± 10.2	34.04 ± 11.34	0.01*
TGs (mg/dl)	162.4 ± 70.6	157.3 ± 45.7	0.7

LDL-C: low density lipoprotein. HDL-C: high density lipoprotein. TGs: triglycerides.

4. Discussion

Many studies have shown that increased factor VII activity is a risk factor for coronary heart disease (CHD) [22–24]. It is now well documented that certain polymorphisms in the factor VII gene are strongly associated with variations in levels of FVII [7,10,12,25]. The R353Q polymorphism has been associated with a decrease in plasma factor VII levels and could confer protection for AMI patients [26]. The current study revealed that the FVII R353Q polymorphism was not significantly different between AMI patients and controls and it was not significantly associated with a risk of AMI. However, the Q allele showed significantly higher frequency in AMI patients vs. controls (OR: 1.92; 95% CI: 0.98–3.7). This is in harmony with many reports who had found that the R353Q polymorphism was not associated with coronary heart disease [13–16,27]. However, it was in disagreement with other studies, which documented a protective nature of the 353Q allele [10,11,28,29]. These discrepancies could be attributable to differences in ethnicity, as a lower frequency of Q allele in controls was reported previously in Asian vs. Caucasian populations (8.8 vs. 14.2%) [30]. Differences in sample size could be another cause [19]. Furthermore, the very high frequency of 353Q allele in Gujarathi Indians, whose population is at higher risk for ischemic heart disease, shows its insignificant role in this disease [31].

The current study observed violation of HWE among the AMI group; however, cases do not need to be in HWE. In fact screening with HWE of data sets of affected individuals has been proposed as a relatively efficient method for detecting gene–disease associations [32].

The current study revealed non-significant differences in the lipid profile as a risk factor of AMI in bearers of different FVII genotypes with exception of significant higher mean values of HDL-C in RR bearers in comparison to carriers of Q allele containing genotypes. This was in partial agreement with Cheraghi et al. [33] and Criado-García et al. [34], but in contrast with Ben-Hadj-Khalifa et al. [19] who demonstrated negative correlation between R353Q and serum cholesterol.

In accordance with Corral [16] and Austin [35], the current study revealed that the FVII R353Q polymorphism was not signif-

icantly associated with DVT, with no significant difference in the distribution of R353Q genotype among the different types of venous thrombosis. Furthermore, Koster et al. [36] reported no difference in Q allele frequency between patients and controls in a study of 199 patients with venous thrombosis. Also, a previous study found no significant association of FVII R353Q polymorphism with Budd Chiari syndrome in Indian subjects [37]. However, FVII R353Q polymorphism was strongly associated with idiopathic venous thrombosis in a cohort of healthy white US women [18].

The present study demonstrated no significant association between FVII R353Q genotypes and different studied risk factors either in arterial or venous thrombosis. This was in agreement to Ben-Hadj-Khalifa et al. [19] who revealed non-significant association between Q allele-containing genotypes (R/Q and Q/Q) and coronary artery disease (CAD). Meanwhile, Iacoviello et al. [10] and Niccoli et al. [38] showed decreased risk of MI in smokers subjects carrying the Q allele.

The contribution of the FVII genetic variants to CAD and venous thrombotic risk, through influencing FVII production [10,11] is inconclusive due to ethnicity differences [19,30,6]. To the best of our knowledge, this is the first study that examined the contribution of FVII R353Q polymorphisms to arterial and venous thrombotic risk in a group of Egyptian patients except for an earlier study (with small sample size) that also didn't find an association between FVII R353Q polymorphism and the risk of cerebral venous thrombosis [39].

5. Limitations of the study

A convenient sample size was enrolled in the current study due to cost limitations. Therefore, a larger sample size is needed to confirm, or rule out the association between the R353Q polymorphism and thrombotic disorders. Factor VII levels were not measured because substantial numbers of recruited patients in the current study were receiving concomitant anticoagulant therapy.

6. Conclusion

FVII R353Q polymorphism is not associated with an increased risk of arterial and venous thrombosis, and carrying the Q allele (of R353Q) did not confer protection against acute thrombotic events.

Conflict of interest

We declare that we have no conflict of interests. This research received no specific grant from any funding agency in the public, commercial, or not-for-profit sectors.

Table 4

FVII R353Q polymorphism in relation to different clinical types of DVT patients.

Clinical type	Total n = 108	FVII R353Q		P OR (95%CI)
		RR n = 79	RQ/QQ n = 29	
		n %	n %	
Simple (r)	83 (76.87%)	60 (75.9)	23 (79.3)	0.7 1.21 (0.39–3.9)
Recurrent	15 (13.88%)	12 (15.1)	3 (10.3)	
DVT with PE	6(5.55%)	4 (5.1)	2 (6.9)	
PVT	3(2.77%)	2 (2.53)	1 (3.5)	
Upper limb DVT	1(0.93%)	1 (1.26)	0 (0)	

(r): reference group.

PE: pulmonary embolism. PVT: portal venous thrombosis. DVT: Deep vein thrombosis.

OR: odds ratio.

Authors' contributions

HA: Developed the study design, determined FVII polymorphism, participated in data interpretation and wrote the draft of the manuscript. RME: Developed the study design, determined FVII polymorphism, participated in data interpretation, wrote the draft and final version of the manuscript. NKA: Determined FVII polymorphism, participated in data analysis and interpretation. HE: Recruited and diagnosed DVT cases and interpretation of the results. SS & AH: Recruited and diagnosed AMI cases and interpretation of the results. E.K: Performed statistical analysis, interpreted the results and revised final draft. All authors read and approved the final manuscript.

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