The J. of the Egyptian Society of Haematology and Research, Vol. 2, No. 1, March: 45-50, 2006

Factor FVII Arg^{353}Gln Polymorphism and its Relevance to Ischemic Complications Following Coronary Catheter Interventions

TAGHRID GAAFAR, M.D.*; HALA ALI, M.D.*; SHAHIRA EL SHAFIE, M.D.*; NAHLA LEHTA, M.D.* and MAGED AL-ABBADY, M.D.**

The Departments of Clinical Pathology, Faculty of Medicine, Cairo University and Cardiology**, National Heart Institute.*

ABSTRACT

Factor VII polymorphisms have been suggested in some studies to show an association with coronary artery disease (CAD) especially its fatal outcome myocardial infarction, and there is a known association between FVII levels and polymorphic variants in the gene. The aim of this study was to study the influence of FVII Arg^{353}Gln polymorphism on the plasma levels of both FVIIc and FVIIa and to assess its role as a risk predictor of complications following coronary catheter interventions. A total of 24 patients with CAD who had undergone percutaneous catheter intervention (PCI) were followed up for 30 days and assessed for occurrence of ischemic complications which included myocardial infarction (MI), death or need for target vessel revascularization. A control group of 20 age and sex matched subjects was also included. The FVII Arg^{353}Gln polymorphism was determined by PCR/REFLP assay. The frequencies of FVII genotypes did not show significant differences between the CAD group and the controls or between the males and the females. Carriers of the Gln^{353} allele had a significantly lower levels of total FVII activity (FVIIc, -37%, p<0.01) and of activated circulating FVII (FVIIa, -50%, p<0.01). In this study 5 (21%) patients suffered from major complications during the 30 day follow up after PCI. No patients with Gln^{353} allele had complications following PCI, while 26% of patients with Arg^{353}/Arg^{353} genotype had complications. In this study, we confirmed that Factor VIIa levels and VIIc levels, are influenced by factor VII gene codon 353 polymorphism and our preliminary results indicate that the Gln^{353} allele might be protective against the thrombotic complications following PCI, yet it did not reach statistical significance mostly due to the small number of cases. Further prospective studies are needed to assess this protective role of the Gln^{353} against thrombogenesis.

Key Words: *Factor VII polymorphism catheter intervention.*

INTRODUCTION

Despite recent advances in angiography technology, acute complications including death, delayed abrupt closure and periprocedural myocardial infarction (MI), continue to occur in 10-15% of patients undergoing percutaneous coronary intervention (PCI) **[1]**. Because numerous observational studies have now confirmed a close association between ischemic complications of PCI and late mortality, prevention of such complications remains a central goal of the practicing intervention cardiologist **[2]**.

In recent years, it has become increasingly clear that the coagulation cascade plays a critical role in the pathogenesis of such complications. All forms of catheter intervention based revascularization produce local endothelial injury, thus exposing underlying tissue factor, which binds to and activates circulating factor VII (FVII). The activated form of factor VII is then free to activate other clotting factors (including factors IX and X), thus leading to the generation of thrombin, platelet activation and ultimately to the formation of thrombus at the site of the catheter induced arterial injury **[3]**. It might be that the risk of subacute ischemic complications after catheter-induced endothelial injury should be influenced by circulating levels of FVII. In theory, higher levels of circulating FVII should increase the risk of an ischemic complication, while lower levels of circulating FVII would be protective

Elevated levels of plasma FVII may lead to a prothrombotic state and increase vascular events. The Northwick Park Heart Study showed that elevated FVII coagulant activity (FVIIc) levels in western countries are risk factors for

ischemic heart disease (IHD), particularly its fatal outcome **[4]**. Other studies **[5,6]** also indicated that plasma FVII levels were related to coronary heart disease.

Plasma levels of FVII are influenced by environmental and genetic factors. Several polymorphisms influencing FVII activity have been recently identified. The substitution of glutamine for arginine at position 353 in the catalytic domain (R353Q) and a 10-bp insertion in the promoter region (5'F7) may be responsible for one third of the variations in plasma FVII levels **[7]**. The -401 G/T polymorphism may account for 18% of the variations in FVII Ag and FVIIa levels **[8]**. The FVII gene is also characterized by a polymorphism involving a variable number of 37-bp repeats in intron 7(IVS7) **[9]**. The rare alleles of the above polymorphisms are generally associated with the decreased levels of FVII. In contrast, the rare -402A allele of the -402G/A polymorphism, which may account for 28% of the variation in plasma FVII levels, is associated with the increased plasma FVII levels **[8]**. Allelic frequencies among populations were different **[10]**.

It has been suggested that the Gln^{353} allele protects against myocardial infarction **[11]**. Thus, presence of the Gln^{353} allele may consequently also be protective in other situations in which thrombus formation is a fundamental pathophysiological mechanism, as it is for adverse events complicating coronary catheter interventions.

This study aimed at investigating the relationship between FVII Arg^{353}Gln polymorphism and the plasma level of FVII coagulant activity and activated FVIIa in patients with coronary artery disease (CAD). Also, the possible role of this polymorphism as a risk predictor of complications following coronary catheter interventions has been evaluated.

PATIENTS AND METHODS

This study was carried out on 24 patients suffering of coronary artery disease and were receiving coronary catheter intervention at the National Heart Institute. Coronary artery disease was defined on the basis of angiographic criteria as stenosis ≥50% in a major coronary artery or its major branches. Our study included 8 (33.3%) patients with stable angina, 10 (41.7%) patients with unstable angina and 6 (25%) patients with myocardial infarction. They were17 males and 7 females with a mean age of 50±8.5 years (range 38-68 years). Patients were followed up for one month after coronary catheter interventions and assessed for occurrence of complications as the need for target-vessel revascularization, periprocedural MI or death. We routinely performed post-procedure creatine kinase, MB fraction levels for all patients. The CK levels greater than twice normal were established as criteria for suspecting MI. In addition, a post-procedural electrocardiogram was carried out. In our study, 5 patients presented with complications following the intervention; 2 patients needed target vessel revascularization while 3 patients had periprocedural MI. A control group of 20 age and sex matched healthy subjects were also included in the study. All patients were subjected to the following:

1- Clinical examination and history taking including smoking, diabetes mellitus, hypertension, use of contraceptive pills for females and family history of coronary artery disease.

2- ECG to determine the type of ischemic heart disease.

3- Coronary angiography was performed according to standard techniques and type of lesion was assessed as follows:

Type A lesion: High success rate-low risk-discrete <10mm-concentric-smooth contour-little or no calcification-less than totally occlusive-absence of thrombus.

Type B lesion: Moderate success rate-60-85%-moderate risk-tubular 10-20mm-eccentric-irregular contour-moderate to heavy calcification-total occlusion <3 month-some thrombus are present.

Type C lesion: Low success rate <60%-high risk-diffuse >2cm-total occlusion >3 month-some thrombus are present.

4- Routine laboratory investigations: Included complete blood picture, erythrocyte sedimentation rate, liver function tests, kidney function tests, blood glucose level, cardiac enzymes and lipid profile.

5- Coagulation studies: Estimation of prothrombin time & concentration, INR, activated partial thromboplastin time and fibrinogen level.

6- Assay of factor VII level:

a- Total activity of Factor VII (FVIIc) was determined using a clotting assay with STA-Deficient VII supplied by Diagnostica-Stago, France (Cat.No.00743) **[12]**.

b- Activated Factor VII (FVIIa) was measured using Staclot VIIa-rTF, Diagnostica-Stago, France (Cat.No.00281) **[13]**.

7- Genetic analysis of factor VII $Arg^{353}Gln$ polymorphism by PCR/REFLP:

Genomic DNA was extracted from peripheral-blood lymphocytes by phenol chloroform method. Amplification conditions were an initial cycle at 93°C for 3min, then subsequent 35 cycles for 60 seconds at 93°C, 60 seconds at 55°C and 2min at 72°C, then a final elongation step at 72°C for 5min. (Perkin Elmer 9600, USA). Primers for the $Arg^{353}Gln$ polymorphisms were 5'-GGG AGA CTC CCC AAA TAT CAC-3' and 5'-ACG CAG CCT TGG CTT TCT CTC-3' **[14]**. Twenty microliters of the PCR amplification product (312bp) were digested with 10 units Msp1 restriction enzyme under the conditions described by the manufacturer (Promega, USA). Fragments of 206bp, 67bp and 39bp were detected in the presence of the Arg^{353} allele, and 273bp and 39bp bands indicated the Gln^{353} allele.

Statistical analysis:

Data management and statistical analysis of this work was performed using SPSS 11 computer system. Analysis included descriptive statistics with calculation of mean and SD and frequency distribution. Mean values were compared using student *t* test and *p*-value was calculated. Identifying relationships between different variables was performed using chi-square test.

RESULTS

General characteristics of patients in the study:

The prevalence of the potential risk factors in our studied patients did not differ between the two genotypes (Table 1) except for the type of lesion in coronary angiography. Patients having the Arg^{353}/Gln^{353} genotype were significantly associated with type A lesion ($p<0.01$), while Arg^{353}/Arg^{353} genotypes were significantly associated with types B and C lesions.

Prevalence of FVII polymorphism:

The Arg^{353}/Gln^{353} genotype was found in 5 (20.8%) patients and 4 (20%) normal controls, while the Arg^{353}/Arg^{353} genotype was found in 19 (79.2%) patients and in 16 (80%) control subjects (Table 2). The distribution of these two genotypes was not significantly different between patients and controls ($p>0.05$) nor between males and females. The allelelic frequencies of Arg^{353} and Gln^{353} were 89.6%, 10.4% and 90%, 10% in the CAD group and controls, respectively. No homozygous (Gln^{353}/Gln^{353}) cases were found in this study.

FVII polymorphism and FVII level:

Genotype-phenotype relationship analysis was performed with data from the entire study population. On comparing the plasma level of FVIIc and FVIIa between the two genotypes, it was evident that the plasma levels of factor VIIa and FVIIc were significantly influenced by FVII polymorphism (Table 3). The mean level of factor VIIa was 50 percent lower in patients with the Arg^{353}/Gln^{353} genotype than in those with the Arg^{353}/Arg^{353} genotype and FVIIc was 37 percent lower in patients with the Arg^{353}/Gln^{353} genotype than in those with the Arg^{353}/Arg^{353} genotype. The plasma FVIIa mean values were significantly higher in patients who showed complications after PCI versus the non-complicated group (Table 5).

FVII polymorphism and ischemic complications following PCI:

No patients with Arg^{353}/Gln^{353} genotype had complications following PCI, while 26% of patients with Arg^{353}/Arg^{353} genotype allele had complications. In this study the Gln^{353} allele appears to be protective against complications following PCI, yet it did not reach statistical significance mostly due to the small number of cases (Table 4).

Table (1): Comparison of baseline potential risk factors stratified by genotype.

	Arg^{353}/Arg^{353}	Arg^{353}/Gln^{353}	*p* value
N	19	5	
Age (Yrs)	49.6±8.4	52.2±9.6	NS
Male/Female (%)	14/5	3/2	NS
Smoking (%)	52.6	20.0	NS
Diabetes (%)	21.1	40	NS
Hypertension (%)	36.8	40	NS
Hypercholesterolemia (%)	78.9	100	NS
Acute MI (%)	26.3	20	NS
Lesion type A (%)	5.3	100	<0.01
Lesion type B (%)	68.4	0	<0.01
Lesion type C (%)	26.3	0	<0.01

Table (2): Distribution frequencies of FVII genotypes between patients and controls.

Genotypes	Patients (n=24)	Controls (n=20)	*p* value
Arg^{353}/Arg^{353}	19 (79.2%)	16 (80%)	NS
Arg^{353}/Gln^{353}	5 (20.8%)	4 (20%)	

Table (3): Comparison of FVIIc and FVIIa levels between the different genotypes.

	Genotype	Mean	S.D	*p* value
FVIIc (%)	Arg^{353}/Arg^{353} (n=35)	84.9	19.2	<0.01*
	Arg^{353}/Gln^{353} (n=9)	53.2	17.6	
FVIIa (mU/dl)	Arg^{353}/Arg^{353} (n=35)	43.29	16.39	<0.01*
	Arg^{353}/Gln^{353} (n=9)	21.44	8.63	

Table (4): Distribution frequencies of FVII genotypes in CAD patients with and without complicated PCI.

Genotypes	Complicated PCI (n=5)	Non-complicated PCI (n=19)	*p* value
Arg^{353}/Arg^{353}	5 (100%)	14 (73.7%)	NS
Arg^{353}/Gln^{353}	0 (0%)	5 (26.3%)	

Table (5): Comparison of FVIIc and FVIIa levels between patients with and without complicated PCI.

		Mean	S.D	*p* value
FVIIc (%)	Non-complicated PCI (n=19)	70.89	22.70	NS
	Complicated PCI (n=5)	81.40	15.78	
FVIIa (mU/dl)	Non-complicated PCI (n=19)	31.69	15.48	<0.01*
	Complicated PCI (n=5)	53.75	16.52	

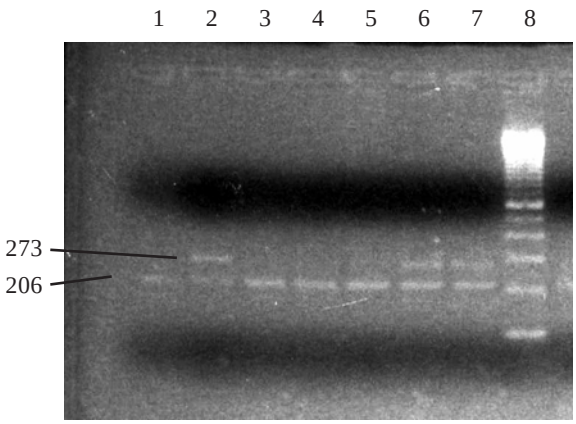

Fig. (1): Gel electrophoresis showing FVII Arg^{353}Gln polymorphism, lanes 2,6,7: heterozygous FVII Arg^{353}/Gln ^{353} cases, lanes 1,3,4,5: Homozygous FVII Arg^{353}/Arg^{353} cases, lane 8: Molecular weight marker.

DISCUSSION

During catheter interventions the protective endothelial lining of the arterial wall is disrupted, and such catheter-induced endothelial injury may trigger thrombogenesis. Increased FVII activity represents a risk factor for thrombotic events complicating coronary catheter interventions. Previous studies had shown an association between FVII levels and polymorphic variants in the gene. Several investigators found that Arg^{353}Gln polymorphism was protective against the development of myocardial infarction in patients with CAD **[15,16]**. The aim of this study was to investigate the relationship between FVII Arg^{353}Gln polymorphism and the plasma levels of both FVIIc and FVIIa, as well as the possible role of this polymorphism in the protection against complications following coronary catheter interventions.

In this study, the allelelic frequencies of Arg^{353} and Gln^{353} were 89.6%, 10.4% and 90%, 10% in the CAD group and controls, respectively. No homozygous (Gln^{353}/Gln^{353}) cases were found in this study. The frequency of the Arg^{353}/Arg^{353} and the Arg^{353}/Gln^{353} genotypes were 79.2% and 20.8% respectively in our CAD patients with no homozygous cases. These frequencies were similar to that reported by other investigators as Girelli et al. **[15]** who reported frequencies of 69.1%, 28.6% and 2.3% in the Arg^{353}/Arg^{353}, Arg^{353}/Gln^{353} and Gln^{353}/Gln^{353} respectively. The frequency of Gln^{353} allele in our controls was similar to those

reported in controls in other studies from northern Italy (for example, 10 percent of our controls carried the Gln^{353} allele, as compared with 16.5 percent of the healthy controls in the study by Ardissino et al. **[17]** and 16.2 percent in the study by Girelli et al. **[15]**.

It has been evidenced by our findings (Table 3) that the Gln^{353} mutation possesses functional importance, that consists in a FVIIc reduction of 37% in Gln^{353} carriers. An analogous phenomenon could be observed in FVIIa levels, with a more reduction of 50% in the Gln^{353} heterozygotes. These phenotypical findings accord well with the literature **[11,14,18,19]** and may explain the expected protective effect of the Gln^{353} allele. The $Arg^{353}Gln$ site was noted initially to associate with a 20% to 30% variance in factor VII levels in males and females and in different ethnic groups **[10]**. Many subsequent studies have confirmed that carriers of the allele coding for Gln^{353} have lower factor VII levels.

An interesting finding in this study is the significant association of FVII Arg^{353}/Gln^{353} genotypes with the atheromatous lesion type A, (Table 1) which is characterized by having no thrombus while FVII Arg^{353}/Arg^{353} genotypes were either type B or C which may have a thrombus.

In this study five (21%) patients suffered from major complications during the first 30 days after PCI, in form of myocardial infarction and need for target vessel revascularizton. When evaluating the role of FVII $Arg^{353}Gln$ genotype as a risk predictor of these ischemic complications, we found that the incidence of complications was 0% in Arg^{353}/Gln^{353} genotype and 26% in Arg^{353}/Arg^{353} genotype. Although all complicated cases were of the Arg^{353}/Arg^{353} genotype and none of them was of the Arg^{353}/Gln^{353} genotype, the difference was statistically insignificant. This might be due to the relatively small number of subjects studied in our work, yet there seems to be a trend towards a protective role for the Gln^{353} allele in prevention of complications following PCI. This relationship has been evaluated by Mrozikiewicz et al. **[14]** and they provided a clear evidence that the Gln^{353} allele of coagulation FVII is associated with a substantial risk reduction by two thirds following coronary catheter interventions.

In the present study, the mean plasma FVIIa level was significantly higher in the complicated group ($p<0.01$), however, FVIIc levels were higher in the complicated group when compared to the non complicated, yet it did not reach statistical significance. It is well accepted that precise genetic markers may provide a better measure of individual lifelong exposure to a putative risk factor than related plasma measurements, which may vary over time **[20]**. This may be particularly true of factor VIIc levels. Whereas genetic markers are probably the strongest determinants of these levels, **[21]** a number of well-known, transient, environmental influences, **[22,23]** may obscure the relation with thrombotic complications when a single measurement is made. By contrast to functional variations of FVIIc or FVIIa levels, the FVII genotype is a constant predictor of the lifelong tendency of individual FVII activity. Similar explanation was reported by Girelli et al. **[15]** who concluded that Gln^{353} allele was protective in their CAD patients against MI yet they found that the mean levels of factor VIIa did not differ significantly between those with a history of myocardial infarction and those without it.

We concluded that the polymorphism strongly influenced the plasma levels of both FVIIc and FVIIa. Also, our preliminary results indicate that absence of the Gln^{353} allele in patients who suffered ischemic complications following PCI might shed light on its protective role against the risk of such complications. Because the number of subjects included in this study was small, these results require further confirmation in larger scale prospective studies.

REFERENCES

1- The EPISTENT Investigators. Randomized placebo-controlled and balloon-angioplasty-controlled trial to assess safety of coronary stenting use of platelet glycoprotein IIb/IIIa blockade. Evaluation of Platelet IIb/IIIa Inhibitor for Stenting. Lancet. 1998, 352 (9122): 87-92.

2- Califf RM, Abdelmeguid AE, Kuntz RE. Myonecrosis after revascularization procedures. J Am Coll Cardiol. 1998, 31: 241-51.

3- The RESTORE investigators. Effects of platelet glycoprotein IIb/IIIa blockade with tirofiban on adverse cardiac events in patients with unstable angina or acute myocardial infarction undergoing coronary angioplasty. Randomized efficacy study of tirofiban for outcome and restenosis. Circulation. 1997, 96: 1445-53.

4- Meade TW, Mellows S, Brozovic M, et al. Haemostatic function and ischemic heart disease: Principal results of the Northwick Park Heart Study. Lancet. 1986, 2: 533-537.

5- Heinrich JL, Balleisen H, Schulte G, Assman G, van der Loo J. Fibrinogen and factor VII in the prediction of coronary risk: Results from the Procam study in healthy men. Arterioscler Thromb. 1994, 14: 54-59.

6- Junker R, Heinrich J, Schlte H, van der Loo J, Assman G. Coagulation factor VII and the risk of coronary heart disease in healthy men. Arterioscler Thromb Vasc Biol. 1997, 17: 1539.

7- Bernardi F, Matchetti G, Pinotti M, Arcieri P, Baroncini C, Papacchini M, Zepponi E, Ursicino N, Chiarotti F, Mariani G. Factor VII gene polymorphisms contribute about one third of the factor VII level variation in plasma. Arterioscler Thromb Vasc Biol. 1996, 16: 72-76.

8- Van't Hooft FM, Silveira A, Tornvall P, Iliadou E, Ehrenborg E, et al. Two common functional polymorphisms in the promoter region of the coagulation factor VII gene determinig plasma factor VII activity and mass concentrations. Blood. 1999, 93: 3432.

9- Pinotti M, Toso R, Girelli D, et al. Modulation of factor VII levels by intron 7 polymorphisms: Population and in vitro studies. Blood. 2000, 95 (11): 3423-8.

10- Lane A, Cruikshank JK, Mitchell J, Henderson A, Humpheries S, Green FR. Genetic and environmental determinants of factor VII coagulant activity in ethnic groups at different risk of coronary heart disease. Atherosclerosis. 1992, 94 (1): 43-50.

11- Iacoviello L, DiCastelnuovo A, de Knijff P, et al. Polymorphisms in the coagulation factor VII gene and the risk of myocardial infarction. N Engl J Med. 1998, 338: 79-85.

12- Miller GJ, Stirling Y, Esnouf MP, et al. Factor VII-deficient substrate plasmas depleted of protein C raise the sensitivity of the factor VII bioassay to activated factor VII: An international study. Thromb Haemost. 1994, 71: 38-48.

13- Morrissey JH, Macik BG, Neuenschwander PF, Comp PC. Quantitation of activated factor VII levels in plasma using a tissue factor mutant selectively deficient in promoting factor VII activation. Blood. 1993, 81: 734-44.

14- Mrozikiewicz PM, Casrobi I, Ziemer S, Laule M, Meisel C, Stangl V, Rutsch W, Wernecke K, Baumann G, Roots I, Stangl K. Reduced procedural risk for coronary catheter interventions in carriers of the coagulation factor VII-Gln^{353} gene. J Am Coll Cardiol. 2000, 36 (5): 1520-1525.

15- Girelli D, Russo C, Ferraresi P, Olivieri O, Pinotti M, Friso S, Manzato F, Mazucco A, Bernardi F, Corrocher R. Polymorphisms in the Factor VII Gene and the Risk of Myocardial Infarction in Patients with Coronary Artery Disease. N Engl J Med. 2000, 343: 774-780.

16- Xu G, Jin G, Fu G, Ma J, Shi Y, Tang O, Shan J. Polymorphism in the coagulation factor VII gene and the risk of myocardial infarction in patients undergoing coronary angiography. Chin Med J. 2003, 116 (8): 1194-7.

17- Ardissino D, Mannucci PM, Merlini PA, et al. Prothrombotic genetic risk factors in young survivors of myocardial infarction. Blood. 1999, 94: 46-51.

18- Ghaddar HM, Folsom AR, Aleksic N, et al.: Correlation of factor VIIa values with factor VII gene polymorphism, fasting and postprandial triglyceride levels, and subclinical carotid atherosclerosis. Circulation. 1998, 98: 2815-21.

19- Jeffery S, Poloniecki J, Leatham E, Bevan D, Ireson N, Talbot S, Cole D, Kaski JC. A protective contribution of the Q allele of the R353Q polymorphism of the Factor VII gene in individuals with chronic stable angina? Int J Cardiol. 2005, 100 (3): 359-9.

20- Ridker PM, Stampfer MJ. Assessment of genetic markers for coronary thrombosis: Promise and precaution. Lancet. 1999, 353: 687-688.

21- Bernardi F, Arcieri P, Bertina RM, et al. Contribution of factor VII genotype to activated FVII levels: Differences in genotype frequencies between northern and southern European populations. Arterioscler Thromb Vasc Biol. 1997, 17: 2548-2553.

22- Balleisen L, Bailey J, Epping P-H, Schulte H, van de Loo J. Epidemiological study on factor VII, factor VIII and fibrinogen in an industrial population. I. Baseline data on the relation to age, gender, bodyweight, smoking, alcohol, pill-using, and menopause. Thromb Haemost. 1985, 54: 475-479.

23- Marckmann P, Bladbjerg E-M, Jespersen J. Diet and blood coagulation factor VII - a key protein in arterial thrombosis. Eur J Clin Nutr. 1998, 52: 75-84.