Polymorphisms in the genes for coagulation factor II, V, VII in patients undergoing coronary angiography*

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Abstract: Objective: To determine whether polymorphisms in the genes for coagulation factor II, V, VII could predispose an individual to increase risk for coronary artery disease (CAD) and/or myocardial infarction (MI) in Chinese. Methods: We screened coagulation factor II(G20210A), V(G1691A), VII (R353Q and HVR4) genotype in 374 patients undergoing coronary angiography by polymerase chain reaction and restriction fragment length polymorphism (PCR-RFLP) assay. Results: The R353Q and HVR4 genotype of the factor VII distribution was in accordance with Hardy-Weinberg equilibrium. The frequencies of FVII genotype or allele did not show statistically significant differences between CAD group and controls or between male and female. The frequencies of the Q allele and (RQ + QQ) genotype were significantly higher among the CAD patients without myocardial infarction (MI) history than among those with MI history ($P < 0.05$). However, HVR4 polymorphism was not significantly different within groups. We only find one normal control of factorII (G20210A) mutation. No coagulation factor V(G1691A) mutation was found in the CAD patients and controls. Conclusion: The factor II(G20210A), V(G1691A) mutation is absent and may not be a major genetic factor for CAD and/or MI; the Q allele of the R353Q polymorphism of the factor VII gene may be a protective genetic factor against myocardial infarction in Chinese.

Key words: Coagulation factor, Polymorphism, Coronary angiography, Myocardial infarction
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INTRODUCTION

Coronary artery disease (CAD) continues to be the major cause of morbidity and mortality in our country. The activity of blood coagulation factors has been shown to be an important risk indicator for CAD. However, circulating levels of coagulation factors may not accurately reflect the true coagulation status, because they may be affected by diets, exercise and drug therapy. Then genetic risk studies may be reliable predictors of both circulating and local effect. We investigated the genetic variants of coagulation factor II(G20210A), V(G1691A), VII (R353Q and HVR4) as candidate genes for CAD in 374 patients undergoing coronary angiography. It is worth mentioning that in our study all of the patients have received coronary angiography, excluding those who had substantial coronary disease, although it was not clinically evident. Our main purpose is to determine whether these gene polymorphisms could predispose an individual to increase risk for CAD and/or MI in Chinese.

MATERIALS AND METHODS

Study population

A total of 374 patients receiving coronary angiography at the Zhejiang University Second Affiliated Hospital were divided into two groups: (1) CAD group: 234 cases (182 male and 52 female, aged 61.78 ± 9.38 years). (2) Controls (normal coronary arteriograms): 140 cases (102 male and 38 female, aged 62.51 ± 9.98).

According to the presence or absence of his-

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tory of MI, the CAD group was further divided into two groups: (1) MI subgroup: 102 cases (77 male and 25 female, aged 60.14 ± 8.78); (2) No MI subgroup: 132 cases(101 male and 31 female, aged 62.14 ± 9.41).

Selecting coronary angiography

Selecting coronary angiography was done using standard techniques. Most patients were catheterized because of angina, but other indications included previous MI, atypical chest pain and aortic regurgitation. Coronary artery disease was defined on the basis of angiographic criteria as stenosis $\geqslant 50\%$ in major coronary artery or major branch.

Gene polymorphisms

Genomic DNA was extracted from peripheral-blood lymphocytes by the standard phenol-chloroform method. The PCR amplification was performed in a GeneAmp System 2400 (Perkin-Elmer, USA) to 94 ℃ for 5 minutes, followed by 35 cycles of 1 minute at 94 ℃, 1 minute at 56 ℃, 1 minute at 72 ℃, and the final cycle was at 72 ℃ for 5 minutes in a 50 μl reaction containing 5 μl genomic DNA and 50 pmol of each primer, 200 μmol/L of each dNTP, 1.5 mmol/L $MgCl_2$, 1 unit of Taq polymerase, 10 × PCR buffer (Promega) (Table 1). Following amplification, a restriction digestion was performed to detect the factor II, V, VII sequence polymorphisms with the enzyme (Hind III [Promega], Mnl1 [MBI], and Msp1 [Promega]) under the conditions described by the manufacture. These digestion products and the PCR products were separated using electrophoresis through 2% agarose gel or 10% polyacrylamide gel and staining with ethidium bromide and visualized under UV light.

Table 1 Coagulation factor II, V, VII polymorphisms

Gene	Primers(5'-3')	Allele	Reference
II(G20210A)	(1) TCT AGA AAC AGT TGC CTG GC (2) ATA GCA CTG GGA GCA TTG AAGC	20210G(345bp) 20210A (322bp, 23bp)	Poort *et al*., 1996
V(G1691A)	(1) ACC CAC AGA AAA TGA TGC CCAG (2) TGC CCC ATT ATT TAG CCA GCA G	1691G (104bp, 82bp, 37bp) 1691A (141bp, 82bp)	Ridker *et al*., 1995
VII(R353Q)	(1) GGG ACA CTC CCC AAA TAT CAC (2) ACG CAG CCT TGG CTT TCT CTC	353R (206bp, 67 bp, 39bp) 353Q (273bp, 39bp)	Geen *et al*., 1991
VII(HVR4)	(1) AAT GTG ACT TCC ACA CCT CC (2) GAT GTC TGT CTG TCT GTG GA	H6 (443bp) H7 (480bp)	Marchetti *et al*., 1991

Statistical analysis

The frequencies of the alleles and genotypes were counted and compared by the Chi-square test with the values predicted by the assumption of Hardy-weinberg equilibrium. Chi-square analysis and Fisher exact test were used to compare the genotype distribution among each group. Odds ratios (OR) and their 95% confidence intervals (95%CI) were used to estimate the risk association to the genotypes for the presence or absence of a MI history. All statistical procedures were performed with SPSS 10.0 software package. P values below 0.05 were considered statistically significant.

RESULTS

General characteristics of the patients in the study

No significant differences were found in the prevalence of the CAD risk factors between CAD group and controls or between MI subgroup and no MI subgroup, including age, sex, smoking, hypertension and diabetes mellitus by T-test and Chi-square test ($P > 0.05$) (data not shown).

Coagulation Factor II, V polymorphisms and CAD

We found one normal control of the coagulation factor II G20210A gene mutation, the incidence was 0.27%. No coagulation factor V (G1691A) gene mutation was found in 374 patients(Fig.1 and Fig.2). The coagulation factor II, V polymorphisms were not associated with an increased risk for CAD and MI.

Coagulation Factor VII polymorphism and CAD

Three genotypes of R353Q (RR, RQ and QQ) and three genotypes of HVR4 (H7H7, H7H6 and H6H6) were found in this study (Fig.3 and Fig.4).

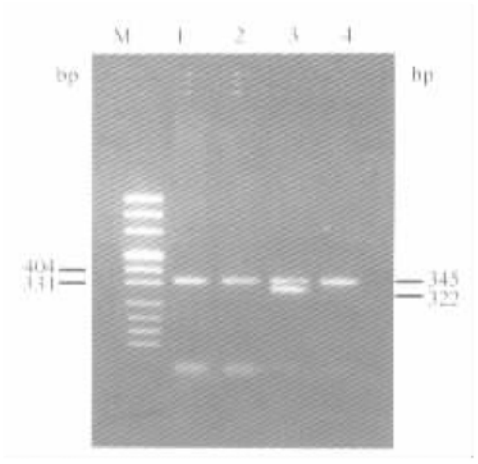

Fig.1 Results of factor II G20210A genotype
M: Marker; 1: PCR product; 2,4: GG genotype; 3: GA genotype

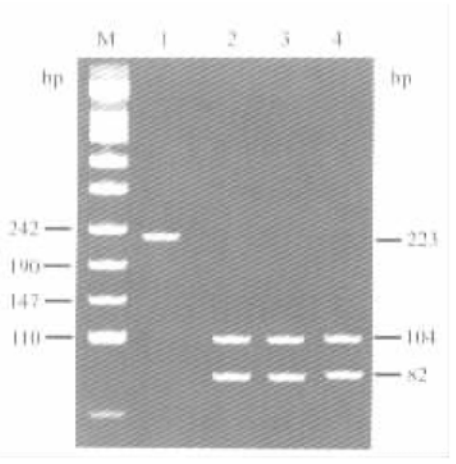

Fig.2 Results of factor VG1691A genotype
M: Marker; 1: PCR product; 2,3,4: GG genotype

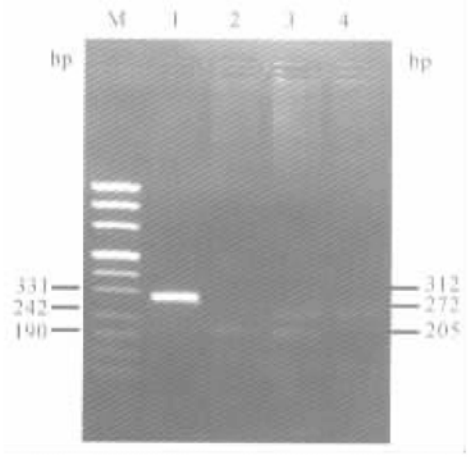

Fig.3 Results of FVII R353Q genotype
M: Marker; 1: PCR product; 2: RR genotype; 3: PQ genotype; 4: QQ genotype

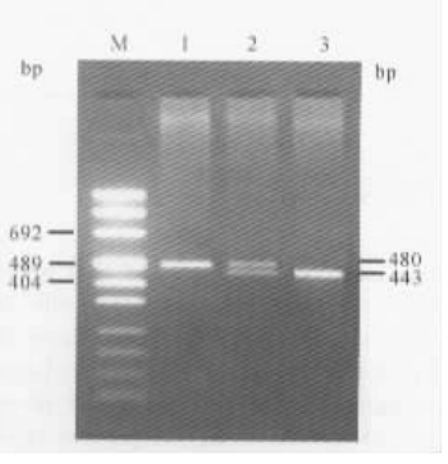

Fig.4 Results of VII HVR4 genotype
M: Marker; 1: H7H7 genotype; 2: H6H7 genotype; 3: H6H6 genotype

We did not identify the H5 allele in our study, which is a very rare allele in the general population. The genotype distribution for both male and female was in accordance with Hardy-weinberg equilibrium by Chi-square test ($P > 0.05$). The frequencies of FVII genotypes and alleles did not have statistically significant differences in the presence or absence of CAD ($P > 0.05$) (Table 2).

Coagulation Factor VII polymorphism and MI

Among 234 CAD patients, there were 102 patients with MI history and 132 patients without MI history. Due to the low number of subjects homozygous for the Q allele, we concentrated our analysis on the combined group (RQ + QQ). The frequencies of the Q allele and (RQ + QQ) genotypes were significantly higher among the CAD patients without MI history than among those with MI history ($P = 0.031$, odds ratio 0.37, 95% CI, 0.15 – 0.94 for the Q allele and $P = 0.037$, odds ratio 0.37, 95% CI, 0.14 – 0.97 for the RQ and QQ genotypes). This subgroup of the Q carriers had a 63% reduction of the risk of MI as compared with carriers of the R allele (Table 3).

There was one homozygous QQ individual (a 68-years-old, male) without MI history. This individual had two stenosis vessels and received one stent on the left anterior descending coronary artery. However, the polymorphism of HVR4 was not significantly different among those with and without MI history ($P > 0.05$) (Table 3).

Table 2 Distribution frequency of Factor VII genotype and allele by the presence and absence of CHD (%)

Group	N	R353Q genotype			Allele		HVR4 genotype			Allele	
		RR	RQ	QQ	R	Q	H6H6	H6H7	H7H7	H6	H7
CHD	234	89.6	10.0	0.4	94.6	5.6	53.2	34.2	12.6	70.6	29.4
Controls	87.2	12.3	0.5	93.2	6.8	35.2	48.3	16.5	59.3	40.7	

Table 3 Genotypes distribution of FVII polymorphisms in CHD with and without a past history of MI (%)

Genotype	MI group $n = 102$	No MI group $n = 132$	P	OR (95% CI)
R353Q (exon 8)				
RR	96(94.1)	113(85.6)	0.037	1.0
RQ + QQ	6(5.9)	18 + 1(14.4)		0.37 (0.14 – 0.97)
R	97.0	92.4	0.031	1.0
Q	3.0	7.6		0.37 (0.15 – 0.94)
HVR4(intron7) H6H6				
H6H7	53(52.0)	72(54.5)	0.926	1.0
H7H7	36(35.2)	44(33.3)		1.11 (0.63 – 1.96)
H6	13(12.8)	16(12.2)		1.10 (0.49 – 2.49)
H7	69.6	71.2	0.592	1.0
	30.4	28.8		1.12 (0.75 – 1.66)

DISCUSSION

It has long been established that atherosclerosis plays an essential etiological role in most cases of CAD; however, the importance of coagulation pathway as a pathogenic mechanism for the development of CAD and/or MI is now receiving more scrutiny. In the present study, we investigated the association of the coagulation factor II(G20210A), V(G1691A), VII (R353Q and HVR4) gene polymorphisms with CAD and/or MI in Chinese.

One genetic variation in the 3'-UT region of the coagulation factor II(FII) gene, a G to A transition at nucleotide position 20210, had been reported to influence the regulation of FII expression and have a 2.8-fold increased risk of venous thrombosis (Poort *et al*., 1996). FII is encoded by a 21-kb-long gene localized on chromosome 11, position 11p11-q12. We only found one normal control of the FII(G20210A) gene mutation in 374 subjects. The prevalence of the FII20210GA genotype in our Chinese (0.27%) was rather low compared with that in European countries such as Swiss(4.8%)(Redondo *et al*., 1999), Italy (4%) (Ferraresi *et al*., 1997), and Austria (2%) (Watzke *et al*., 1997). The results was in agreement with other reports (Ridker *et al*., 1999) indicating that the FII(G20210A) gene polymorphism should not be considered a risk factor for CAD and/or MI.

Bertina *et al*. recently described the G→A mutation at nucleotide 1691 of the gene coding for the coagulation factor V(FV), which leads to resistance of the FV to activated protein C (Bertina *et al*., 1994). Association of the FV (G1691A) gene mutation with venous thrombosis is well established in the literature, but the role of the mutation in arterial thrombotic events is controversial. Rosendssl *et al*. reported a relatively high prevalence of the FV(G1691A) gene mutation in young females smokers who had suffered from myocardial infarction (Rosendaal *et al*., 1997). However, no FV(G1691A) gene mutation was found in our study. This result indicated that the FVG1691A gene mutation might not be a risk factor for CAD in Chinese.

Coagulation factor VII (FVII) is a vitamin K-dependent protease that plays an important role in the extrinsic pathway of blood coagulation. Two common polymorphisms, a guanine-to-adenine substitution at 353 position in exon 8 resulting in substitution of arginine (R) by glutamine (Q) in the FVII protein (R353Q) and a variable number of 37-bp repeats in intron 7 (HVR4), had been extensively studied in the past years. Recent studies reported that the com-

bined RQ and RR genotypes of FVII R353Q were correlated to a reduced risk of CAD in 2,574 patients (OR = 0.78, 95% CI, 0.65 to 0.93), whereas the QQ genotype had more protection (OR = 0.53, 95% CI, 0.27 to 1.03) (Wu, Tsongalis, 2001). However, these data are a matter of controversy. Song *et al*. found no increased risk of developing CAD resulting from FVII R353Q polymorphisms in 139 controls and 158 patients in Korean (Song *et al*., 2000).

We found the frequency of the Q allele is only 5.9% in controls, about one third of that in European (14% – 28%). No association was observed between the R353Q polymorphism and risk of coronary artery disease with angiography demonstrated. It was a crucial finding of our study that the R353Q polymorphism was associated with the risk of MI. Compared to the CAD patients with a history of MI, the significantly higher of the Q allele and the (RQ + QQ) genotype in the CAD patients without a history of MI suggested that the Q allele provides protection from MI. This may explain why some patients do not have myocardial infarction despite the presence of severe coronary artery disease. Our results agreed with those of a study of 444 angiographically diagnosed patients by Girelli *et al*., who described a protective effect against MI in carries of the Q allele (Girelli *et al*., 2000). In our study, we did not identify the H5 allele, which was a very rare allele in the general population. We only detected H6 and H7 alleles and showed that the FVII HVR4 polymorphism was not correlated with CAD and/or MI.

In conclusion, our preliminary study indicated that neither the FIIG20210A nor the FVG1691A gene polymorphisms are associated with CAD and/or MI in Chinese. We show that the Q allele of the R353Q polymorphism in the FVII gene may be a protective factor against MI in Chinese and may help identify patients with CAD who might benefit from oral anticoagulant to prevent the risk for cardiovascular events. Further studies are needed to define the role of the coagulation factor gene polymorphism in coronary artery disease.

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