R353Q Polymorphism, Activated Factor VII, and Risk of Premature Myocardial Infarction in Japanese Men

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Background The association between myocardial infarction (MI) and the R353Q polymorphism of the Factor VII (FVII) gene, which reportedly influences FVII concentrations, activated Factor VII (FVIIa), or FVII antigen (FVIIag), remains controversial.

Methods and Results The present case–control study in 127 Japanese men with their first MI at or before 45 years of age and 150 matched healthy controls was designed to clarify this association in premature MI. R353Q polymorphism was determined by polymerase chain reaction, and plasma concentrations of FVIIa and FVIIag were assayed. The distribution of the RR, RQ, and QQ genotypes with respect to R353Q polymorphism was 117, 10, and 0 in the patients, and 131, 17, and 2 in the controls. The Q allele was negatively associated with premature MI (odds ratio=0.41, $p=0.038$). The plasma concentration of FVIIa was slightly higher in patients (55.1 ± 40.9 U/L) than in controls (44.8 ± 20.2 U/L), but not significantly ($p=0.078$); the plasma concentration of FVIIag did not differ between patients ($88.7\pm 15.7\%$) and controls ($87.0\pm 9.0\%$) ($p=0.557$). Plasma FVIIa concentrations were influenced by R353Q polymorphism ($p<0.001$).

Conclusions The Q allele may be protective against premature MI. (Circ J 2004; 68: 520–525)

Key Words: Coronary risk factor; Factor VII; Genotype; Premature myocardial infarction; R353Q polymorphism

Factor VII (FVII) is the first enzyme in the extrinsic pathway of the blood coagulation system. Activation of the extrinsic coagulation pathway plays a key role in hemostasis,¹ and thus FVII contributes to the occurrence of thrombotic events. Although most FVII circulates in plasma in the zymogen form, small but significant amounts of activated FVII (FVIIa) also are present, and appear to serve as a primer for triggering the extrinsic cascade.^{2–5} The Northwick Park Heart Study suggested that FVII coagulant activity is independently associated with risk of coronary events in middle-aged men,^{6,7} and several additional studies have linked elevated concentrations of FVII in plasma to coronary heart disease.^{8–14} Thus, FVII has become recognized as a hemostatic coronary risk factor.

Plasma FVII concentrations are influenced by both genetic and environmental factors. Green et al reported a strong association between a common polymorphism in exon 8 of the FVII gene (R353Q polymorphism) and plasma FVII,¹⁵ which has been confirmed by several other studies,^{8,9,16–23} especially with respect to FVIIa.²⁴ However, the association

between R353Q polymorphism of the FVII gene and myocardial infarction (MI) remains controversial. The contribution of the coagulation system to the pathogenesis of MI can be studied most readily in patients with premature MI, who have less atherosclerotic coronary stenosis than elderly patients.^{25–28}

We performed a case–control study to determine whether plasma FVIIa concentrations and R353Q polymorphism are associated with risk of premature MI.

Methods

Study Population

We investigated 129 consecutive Japanese male patients treated as outpatients at Kagoshima Coronary Care Unit Network (2000–2003), with a first MI occurring before the age of 45 years (mean $\pm$ SD, 40.4 ± 4.5 years old) and giving informed consent. We angiographically confirmed occlusion or significant stenosis of a coronary artery in all patients. Of the 129 patients, 2 patients, one had Kawasaki disease and another had a coronary artery anomaly, were excluded. The remaining 127 patients (premature MI patients) had a mean age of 43.9 ± 5.1 years at study entry, 3.3 ± 3.8 years after initial MI. Coronary angiography showed single-vessel disease in 93 (73.2%), and multivessel disease in 34 (26.8%). Oral anticoagulant agents were administered to 33 patients. Control subjects were 150 consecutive age-matched healthy Japanese men (43.8 ± 5.1 years old at entry), who underwent a medical checkup at Kagoshima Prefectural Comprehensive Health Center and

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Table 1 Characteristics of the Premature MI Patients and Control Subjects

Characteristics	Premature MI patients	Control subjects	p value
No.	127	150	
Age at MI, years	40.4±4.5		
Age at study entry, years	43.9±5.1	43.7±5.1	NS
Total number of coronary risk factors*	1.9±0.8	1.4±0.8	<0.001
Hypertension present (%)	17 (13.4)	24 (16.0)	NS
Hypercholesterolemia present (%)	79 (62.2)	66 (44.0)	0.003
Diabetes mellitus present (%)	29 (22.8)	9 (6.0)	<0.001
Smoking history (%)	110 (86.6)	110 (73.3)	0.006

MI, myocardial infarction. Values are means ± SD for continuous variables. NS, not significant.

*Total number of coronary risk factors represents the sum of hypertension, hypercholesterolemia, diabetes mellitus, and smoking (maximum, 4).

gave informed consent. None of the control subjects had coronary heart disease according to their medical history or electrocardiography, and none took an oral anticoagulant. This study protocol was approved by the Ethics Committee of Kagoshima University.

Identification of Conventional Coronary Risk Factors

Hypertension, hypercholesterolemia, diabetes mellitus, and smoking history were evaluated as conventional coronary risk factors. Hypertension was defined by a systolic blood pressure at entry of at least 140 mmHg, a diastolic pressure at least 90 mmHg, a past history of hypertension, or receiving antihypertensive medication. Hypercholesterolemia was defined by a serum total cholesterol concentration at entry of at least 220 mg/dl, a past history of hypercholesterolemia, or receiving lipid-lowering medication. Diabetes mellitus was defined by a fasting plasma glucose concentration at entry of at least 126 mg/dl, a past history of diabetes mellitus, or receiving hypoglycemic medications.

Laboratory Measurements and Techniques

Blood sampling was performed gently by 3 expert physicians at study entry. Samples were collected from all subjects between 07.00 and 11.00 h after an overnight fast, and also 20 min later in a separate syringe. Blood samples were centrifuged for 10 min (3,000G, 4°C) and divided into plasma, serum, and blood cells. Each was dispensed into a plastic tube respectively and frozen at -80°C until analysis. Plasma concentrations of FVIIa and FVII antigen (FVIIag) were measured using a double-antibody enzyme-linked immunosorbent assay (Roche Diagnostics, Basel, Switzerland)⁴ and a coagulation time method (Roche Diagnostics), respectively.

Detection of R353Q Polymorphism

R353Q polymorphism was detected as described by Green et al¹⁵. Amplified fragments were digested with 5U of *MspI* (New England BioLabs, Beverly, MA, USA) and then subjected to electrophoresis on a 2% agarose gel. Fragments of 205 bp (the R allele) and 272 bp (the Q allele) were detected. Genotypes were defined as RR, RQ, and QQ.

Statistical Analysis

Differences in baseline characteristics between the premature MI patients and control subjects were assessed using chi-square test for categorical variables and by unpaired t-test for continuous variables. Because the distributions of the plasma concentrations of FVIIa and FVIIag were skewed, logarithmic transformation was performed.

Log FVIIa and log FVIIag were compared between groups by unpaired t-test. To estimate the contribution of various risk factors to the occurrence of premature MI, multivariate logistic regression analysis was performed with FVIIa, FVIIag, hypertension, hypercholesterolemia, diabetes mellitus, and smoking status as independent variables. The frequencies of genotypes in the premature MI and control groups were compared using the chi-square test with the values predicted by Hardy-Weinberg equilibrium. Frequencies of alleles and genotypes (RR genotype vs RQ+QQ genotypes) were compared using the chi-square test between the premature MI and control groups. The odds ratio (OR) was calculated with a 95% confidence interval (95%CI). Multivariate logistic regression analysis including hypertension, hypercholesterolemia, diabetes mellitus, and smoking status was performed to assess the association between the R353Q polymorphism and the occurrence of premature MI. The effect of the Q allele, with the R allele chosen as the reference allele, was analyzed by the introduction of 3 dummy variables (0, 1, and 2) coding respectively for the number of Q alleles. Categorical independent variables (hypertension, hypercholesterolemia, diabetes mellitus, and smoking status) also were coded as dummy variables (0 for absence and 1 for presence). The association of plasma FVIIa and FVIIag concentrations with each genotype was analyzed with one-way analysis of variance.

Values for continuous variables are expressed as means ± SD. A p-value of less than 0.05 was considered to be statistically significant. All computations were carried out with the STAT View-J 5.0 (SAS Institute, NC, Cary, USA) or STATA 7.0 (Stata Corporation, College Station, USA).

Results

Characteristics of the Study Population

Characteristics of the premature MI patients and control subjects are shown in Table 1. There was no significant difference in age at entry between the 2 groups. Patients showed significantly higher prevalences of hypercholesterolemia, diabetes mellitus, and smoking than control subjects (p<0.01), and had a greater number of conventional coronary risk factors than control subjects (p<0.001). Family history was recognized in 7.1% of MI patients.

R353Q Polymorphism and Premature MI

The frequencies of the genotypes and alleles of the R353Q polymorphism are shown in Table 2. The distribution of genotypes was virtually identical to that predicted by the Hardy-Weinberg equilibrium, in both the MI patients (p=0.644) and control subjects (p=0.113). Because

Table 2 Plasma FVIIa and FVIIag Concentrations, and the Distribution of Genotypes and Alleles of R353Q Polymorphism of the Factor VII Gene Between the Premature MI Patients and Control Subjects: Risk of Premature MI

Variable	Premature MI patients	Control subjects
FVIIa (U/L)	55.1±40.7	44.8±20.2
FVIIag (%)	88.7±15.7	87.0±9.0
Genotype, no. (%)		
RR	117 (92.1)	131 (87.3)
RQ	10 (7.8)	17 (11.3)
QQ	0 (0)	2 (1.3)
RQ + QQ	10 (7.8)	19 (12.7)
Alleles, no. (%)		
R	244 (96.1)	279 (93.0)
Q	10 (3.9)	21 (7.0)

FVIIa, activated factor VII; FVIIag, factor VII antigen; MI, myocardial infarction.

Values are means±SD for continuous variables.

Table 3 Risk of Premature Myocardial Infarction for R353Q Polymorphism by Multivariate Logistic Regression Analysis

Variable	OR (95% CI)	p value
Q allele	0.41 (0.18–0.95)	0.038
Hypertension	0.86 (0.42–1.76)	NS
Hypercholesterolemia	1.87 (1.13–3.10)	0.016
Diabetes mellitus	5.37 (2.27–12.74)	<0.001
Smoking status	2.43 (1.25–4.70)	0.009

OR, odds ratio; CI, confidence interval.

The multivariate logistic regression analysis included the Q allele of R353Q polymorphism and hypertension, hypercholesterolemia, diabetes mellitus and smoking history. All independent variables were coded as dummy variables (for R353Q polymorphism, 0 for RR, 1 for RQ, and 2 for QQ; for hypertension, hypercholesterolemia, diabetes mellitus, and smoking history: 0 for absence and 1 for presence).

Fig 1. Plasma concentrations of FVIIa (A) and FVIIag (B) according to R353Q polymorphism. QQ, RQ, and RR refer to the respective genotypes. Values are means±SD. FVIIa, activated factor VII; FVIIag, factor VII antigen; MI, myocardial infarction.

the QQ genotype was not seen in premature MI patients, the distribution of genotypes was compared between the RR and the RQ+QQ genotypes. No significant difference in frequency of the RQ+QQ genotype or the Q allele was seen between the 2 groups.

The results of logistic regression analysis including the Q allele, hypertension, hypercholesterolemia, diabetes mellitus, and smoking are shown in Table 3. In the multivariate analysis including the 4 conventional coronary risk factors, the Q allele was significantly associated with the occurrence of premature MI (OR 0.41; $p=0.038$, $r^2=0.01$). There were no significant interactions between the Q allele and conventional risk factors.

Ten premature MI patients with the Q allele had 2.7 ± 1.2 conventional risk factors, significantly more ($p=0.002$) than 19 control subjects with the Q allele (1.5 ± 0.7). All premature MI patients with the Q allele had a history of smoking,

and none had hypertension. No significant difference in prevalence of diabetes mellitus or hypercholesterolemia was noted (data not shown).

Plasma FVII and Premature MI

Plasma concentrations of FVIIa and FVIIag were compared between MI patients not receiving anticoagulant agents and control subjects. The plasma FVIIa concentrations in the patients (55.1 ± 40.7 U/L) were slightly higher than those in control subjects (44.8 ± 20.2 U/L), but not significant ($p=0.078$). Plasma FVIIag concentrations showed no significant difference between the 2 groups ($88.7\pm15.7\%$ vs $87.0\pm9.0\%$, $p=0.557$) (Table 2).

In multivariate logistic regression analysis including plasma concentrations of FVIIa and FVIIag and the presence of hypertension, hypercholesterolemia, diabetes mellitus, and past or present smoking status with respect to

premature MI, plasma FVIIa and FVIIag concentrations showed no significant association with risk of premature MI ($p=0.102$ and 0.810 , respectively).

R353Q Polymorphism and FVII

Plasma concentrations of FVIIa and FVIIag in the various genotypes are compared in Fig 1. In the control subjects, plasma FVIIa concentrations for the QQ, RQ, and RR genotypes were 9.7 ± 0.7 U/L, 26.0 ± 13.2 U/L, and 47.8 ± 12.3 U/L, respectively. Plasma FVIIag concentrations were $72.5\pm10.6\%$, $83.6\pm7.2\%$, and $87.6\pm9.0\%$, respectively. R353Q polymorphism, then, showed significant associations with FVIIa ($p<0.001$) and FVIIag ($p=0.015$). In MI patients, plasma FVIIa concentrations for the RQ genotype (21.0 ± 9.8 U/L) were significantly lower ($p<0.001$) than those with the RR genotype (58.3 ± 41.0 U/L). However, plasma FVIIag concentrations showed no significant difference between the 2 genotypes ($87.5\pm15.7\%$ vs $88.8\pm15.8\%$, respectively, $p=0.815$).

Discussion

The findings of the multivariate logistic regression analysis that included the conventional coronary risk factors of hypertension, hypercholesterolemia, diabetes mellitus, and smoking suggest that R353Q polymorphism is significantly associated with the risk of premature MI in the present study population. Plasma FVIIa concentrations in the patients were slightly higher than those in control subjects, but not significantly. Therefore, the Q allele of R353Q polymorphism might protect against premature MI in Japanese men.

Several studies have suggested that FVII is a coronary risk factor⁶⁻¹⁴ but it has not been established by consensus that R353Q polymorphism of the FVII gene is associated with the risk of MI. Therefore, we conducted the present case-control study analyzing FVII genotypes. Although most FVII circulates in the zymogen form, a priming role in triggering the coagulation cascade has been assigned to the smaller amounts of circulating FVIIa²⁻⁵. Because FVIIa is the first active protease in the extrinsic pathway of the coagulation cascade, plasma FVIIa concentrations may be important for determining the occurrence of thrombotic events such as thrombosis after rupture of an atherosclerotic plaque. Kario et al reported that plasma FVIIa concentrations were increased in patients with cardiovascular disease⁹, and Philippou et al observed increased FVIIa in patients with acute coronary syndromes.¹⁰ However, the increase of FVIIa has not been sufficiently established as a hemostatic coronary risk factor. In our previous study, we found activation of the coagulation cascade, evaluated by the ratio of tissue factor and tissue factor pathway inhibitor in premature MI, but could not confirm the increase of plasma FVIIa.²⁹

In the present study of premature MI in Japanese men, we observed significant associations for R353Q polymorphism, but not for plasma FVIIa ($p=0.078$). Many studies have not recognized associations between plasma R353Q polymorphism or FVIIa and cardiovascular disease.^{23,30-34} The Framingham Heart Study reported that R353Q polymorphism was not significantly associated with cardiovascular disease, and suggested that R353Q polymorphism might be in linkage disequilibrium with other polymorphisms of the FVII gene or with another as-yet-unidentified gene located near the FVII gene.²³ Recently, significant

associations between polymorphisms of the FVII gene and MI, coronary artery disease, or cardiac events after intervention have been confirmed.³⁵⁻⁴⁰ Iacoviello et al suggested that R353Q and the hypervariable region 4 polymorphisms influenced the risk of MI in families with a history of thrombosis.³⁵ Girelli et al reported that there were significantly more heterozygotes and homozygotes for the Q and A2 alleles among those who had not had a MI than among those who had had an infarction ($p=0.01$ for R353Q).³⁶ However, there have been no studies showing a significant association between R353Q polymorphism of FVII and premature MI.

Among there are many studies of the polymorphisms of genes and coronary heart disease in Japanese,⁴¹⁻⁴³ as far as we know there have been only 2 studies investigating the genotypes of coagulation factor VII and coronary heart disease. Tamaki et al reported that polymorphisms of factor VII gene were not associated with the risk of MI,³⁴ whereas Shimokata et al found a significant association between FVII polymorphism and coronary artery disease, but not MI.⁴⁰ Therefore, we are the first to recognize a significant association with the risk of MI in Japanese.

Many studies have reported fewer stenotic atheromatous lesions on the coronary angiograms of younger MI patients compared with the elderly.²⁵⁻²⁸ Because atherosclerosis plays a reduced role in premature MI, prothrombotic factors become relatively important.^{27,44-47} Therefore, we selected relatively young Japanese men as study subjects. In the present study, the relation of R353Q polymorphism to the risk of premature MI was significant when considered together with the presence of conventional coronary risk factors. The discrepancy between our results and previous Japanese studies may be caused by the difference in the age of the study subjects. The mean ages of patients in studies by Tamaki et al and Shimokata et al were 59 years and 63 years, respectively. Because the role of FVII in the pathogenesis of MI might be less significant than conventional coronary risk factors in elderly Japanese, the previous 2 Japanese studies that included many elderly patients did not recognize the significant associations between R353Q polymorphism and MI. In contrast to hypercholesterolemia, diabetes mellitus, and smoking, all of which increased risk in this study, the Q allele seemed to be protective against premature MI. In contrast to the present results, when Moor et al and Ardissino et al assessed R353Q polymorphism in young survivors of MI, they found no significant differences in the prevalence of R353Q polymorphism between patients with premature MI and control subjects.^{8,32} In Taiwan, Li et al reported that R353Q polymorphism was an independent risk predictor of subsequent cardiac events in the young survivors of MI, but there was no difference in the prevalence of this polymorphism between patients and controls.³⁹ The reason for disagreement between these study findings is not clear, but may involve differences of ethnicity and the prevalence of established coronary risk factors in the subjects.

Premature MI patients with the Q allele who otherwise might have had a reduced risk of MI had a significantly greater number of conventional coronary risk factors than control subjects with the Q allele. Recently, Iacoviello et al reported that smoking doubled the risk of MI in subjects with the Q allele.⁴⁸ In the present study, all premature MI patients with the Q allele were current or former smokers. The Q allele presumably could not counter the increased risk of MI from smoking.

Study Limitations

The study was retrospective in design and had small statistical power (0.28) because of its small scale. We need more than 450 young MI patients to obtain results with sufficient statistical power (type II error <0.20) and we will continue the present study protocol to obtain a sufficient number of patients. Although the distribution of the Q allele in the control subjects was similar to that previously described in Japanese (12.6% by Tamaki et al; 17.6% by Shimokawa et al)^{20,21,35,40} the number of controls in our study was also low. Therefore, we cannot establish the FVII gene as the protective factor of MI. Large prospective epidemiologic studies are necessary to clarify the associations between polymorphism of the FVII gene, plasma FVIIa, and risk of MI.

In addition, among several polymorphisms we examined only the R353Q polymorphism of the FVII gene. Other polymorphisms of the FVII gene such as the hypervariable region 4⁴⁹ and the decanucleotide insertion/deletion polymorphism,^{37,50–52} which is located in the promoter region of the FVII gene and influences its transcription, may have influenced the association. Recently, Peyvandi et al reported that a novel polymorphism in intron 1a of the FVII gene (G73A) might protect against premature MI.⁵³ Therefore, these polymorphisms of the FVII gene also need to be analyzed. Because antiplatelet agents do not have direct effects on the coagulation cascade, we measured factor VII concentrations without interrupting these agents, which were being administered to the majority of cases. To strictly exclude the effects of antiplatelet agents, it is necessary to take blood samples from patients not receiving antiplatelet agents.

In conclusion, R353Q polymorphism of FVII, which importantly influences plasma FVIIa concentrations, may protect against premature MI.

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