

Msp I Polymorphism of the Coagulation Factor VII Gene in Patients with Ischemic Cerebrovascular Disease in Han Population of Henan, China

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Abstract: Objective. To determine whether there is any relationship between polymorphisms in gene of coagulation factor VII and ischemic cerebrovascular disease in Han population of Henan in China. **Methods.** Coagulation factor VII (R353Q) genotypes were screened in 512 patients with ischemic cerebrovascular disease by PCR and restriction fragment length polymorphisms (PCR-RFLP) assay. **Results.** The R353Q genotypes of factor VII distribution was in accordance with Hardy-Weinberg equilibrium. The distribution of allele and genotype in R353Q had significant difference between the control group and CVD group. **Conclusion.** The Q allele of the R353Q polymorphism of the factor VII gene may be a protective genetic factor against ischemic cerebrovascular disease in Han population of Henan, China. [Life Science Journal. 2006;3(4):54-56] (ISSN: 1097-8135).

Keywords: coagulation factor VII; ischemic cerebrovascular disease; polymorphism; R353Q

Abbreviations: CAD: coronary artery disease; ICVD: ischemic cerebrovascular disease; IHD: ischemic heart disease; FVII: coagulation factor VII

1 Introduction

Coagulation factor VII (FVII) is the initial factor in the extrinsic pathway of the coagulation cascade and FVII levels in plasma are usually related to ischemic heart disease (IHD). The updated result of the NPH study showed that FVIIc was strongly related to fatal events of ischemic heart disease^[1]. In two Japanese reports, FVIIc was also proposed to be the independent risk factor for coronary artery disease (CAD)^[2,3]. Ischemic cerebrovascular disease (ICVD) shares many risk factors related to IHD. ICVD has a complex etiology and pathophysiology generated by the combined effects of genes and the environment. Genetic variation played an important role in the determination of plasma FVII levels. In the previous study, the polymorphism at exon 8 of the FVII gene (designated R353Q polymorphism) was consistently reported to have a genotype effect on plasma FVIIc levels. Our main purpose was to determine whether there is any relationship between R353Q polymorphism in gene of coagulation FVII and ischemic cerebrovascular disease in Han population of Henan, China.

2 Materials and Methods

2.1 Subjects

A total of 512 patients with ischemic cere-

brovascular disease were admitted to the First Affiliated Hospital of Zhengzhou University. There were two groups in the study (1) CVD group: 512 subjects (310 males and 202 females, aged 60 ± 10.2 years); (2) control group: 560 subjects (294 males and 266 females, aged 56 ± 9.8 years). The control group were healthy subjects clinically free of vascular disease. All of them were unrelated, and were the Chinese Han population.

2.2 Gene polymorphism

Genomic DNA were extracted from peripheral-blood lymphocytes by the standard phenol-chloroform method. Primers for PCR amplification were made according to the sequence reported by Green *et al*^[4], and the conditions of the PCR reaction were 94°C for 5 minutes, followed by 35 cycles of 1 minute at 94°C , 1 minute at 56°C , 1 minute at 72°C , and the final cycle was at 72°C for 5 minutes. PCR reaction mixture of $25\ \mu\text{l}$ contains 100 ng genomic DNA, $10\ \mu\text{mol}$ of the primer, $1.5\ \text{mol/L}$ MgCl_2 , $50\ \text{mol/L}$ KCl , $10\ \text{mol/L}$ Tris-HCl (PH 8.3), $0.2\ \text{mol/L}$ of dNTP each and $2.0\ \text{U}$ DNA polymerase. Following amplification, a restriction digestion was performed to detect the FVII sequence polymorphism with the enzyme *MspI* at 37°C for 12 hours. These products and PCR products were separated using electrophoresis through 2% agarose gel and stained with ethidium bromide and visualized under UV light.

2.3 Statistical analysis

The frequencies of the alleles and genotypes were counted and compared by the Chi-square test. Odds Ratios(OR) and their 95% confidence intervals (95% CI) were used to estimate the risk association to the genotype. All statistical procedures were performed with SPSS 10.0 software package. $P < 0.05$ was set statistically significant.

3 Results

Three genotypes of R353Q(RR, RQ and QQ) were found in our study (Figure 1). The genotype

distribution for both males and females was in accordance with Hardy-Weinberg equilibrium by Chi-square test ($P > 0.05$). Due to low number of the 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) genotype were significantly higher in control group than those in CVD group ($P = 0.025$, Odds ratios = 0.657, 95% CI, 0.454 - 0.951 for the Q allele and $P = 0.016$, Odds ratios = 0.625, 95% CI, 0.426 - 0.917). This group of Q carriers had a 35% reduction of the risk of CVD as compared with carrier of R allele (Table 1).

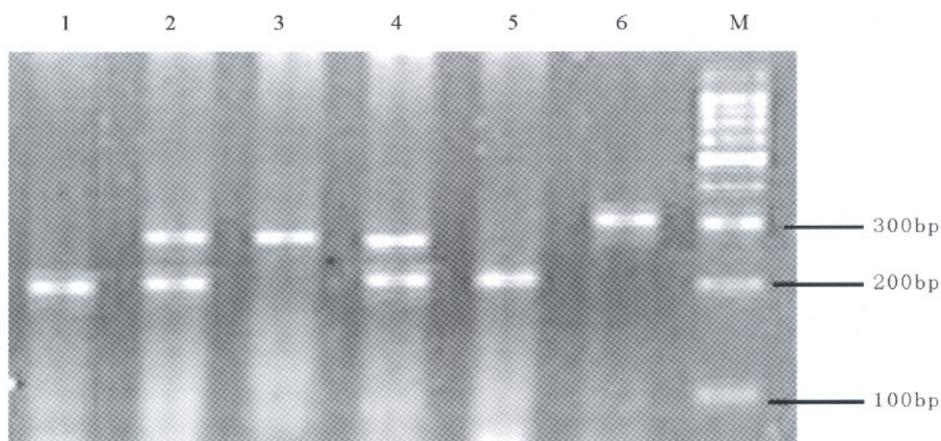


Figure 1. PCR products from amplification of the polymorphic region of the R353Q gene
Lane M: 100 bp DNA Marker; Lanes 1 and 5: RR homozygote with a fragment of 205 bp; Lanes 2 and 4: RQ heterozygote with a fragment of 205 bp and 272 bp; Lane 3: QQ homozygote with fragments of 272 bp; Lane 6: PCR product.

Table 1. Distribution frequency of R353Q genotype and allele in patients with CVD and controls

Genotype	CVD (n = 512)	Control (n = 560)	P value	OR(95% CI)
RR	465(90.8)	482(86.4)		1.00
RQ	46(9.0)	76(13.2)	0.018	0.627(0.426 - 0.924)
QQ	1(0.2)	2(0.4)		
RQ + QQ	47(9.2)	78(13.6)	0.016	0.625(0.426 - 0.917)
Alleles				
R	976(95.3)	1042(93.0)		1.00
Q	48(4.7)	78(7.0)	0.025	0.657(0.454 - 0.951)

4 Discussion

FVII is a vitamin K-dependent protease and it is the initial factor in the extrinsic pathway of the coagulation cascade. The prospective Northwick Park Heart Study (NPHS) found that raised FVII coagulant activity (FVIIc) was an independent risk factor for CAD^[1]. Epidemiological evidence sug-

gested that ICVD shared many risk factors with IHD, such as age, hypertension, and smoking, etc. It is also possible that the features of the hemostatic system may influence the development of ICVD, as has been suggested for IHD. Genetic variations play a role in the determination of plasma FVII levels. In the previous study, the polymorphism at exon 8 of the FVII gene (designated R353Q polymorphism) was consistently reported to have a

genotype effect on plasma FVIIc levels. These polymorphisms arised from a basic substitution of A to G in the second position of the codon 353, leading to substitution of arginine with glutamine (alleles were designated as R and Q, respectively). Green^[4] first reported that heterozygous European subjects were 22% lower on FVIIc levels than those in group mean. In some of these studies, the Q allele was also found to be associated with lower FVIIc levels^[5,6]. Girelli^[7] reported that Q allele had a protective effect against MI. Differences in allelic frequencies had been reported in different ethnic groups. In our study, the frequency of Q allele was 7.0%. The Q allele frequency of the R353Q polymorphism in Chinese was significantly lower than that of the Dravidian Indians (0.29).

It was also lower than frequencies of the Europeans(0.14 - 0.28)^[4,6], but higher than that of the Japanese(0.034)^[5] and it was similar to that of Korean(0.060)^[8], which suggested that the prevalences of gene mutation of R353Q vary with different ethnic groups or geographic regions. The mechanism of R353Q's effect on plasma FVII levels is unknown, and several possible explanations have been discussed. Lower plasma levels of the FVII protein seen with the Q allele might be due to a conformational change produced by the Arg→Gln substitution that affected the processing of FVII in the hepatocytes, resulting in reduced secretion of the protein^[9]. In line with this hypothesis, the Q variant was shown *in vitro* to be associated with reduced secretion of FVII compared with the R variant, thus accounting for the reduction on plasma FVII levels^[10]. Our result was similar to the results reported by Girelli^[7]. Our study has shown that Q allele of the R353Q polymorphism in the FVII gene may play a protective role against ICVD in Han population of Henan, China.

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