Korean J Biol Sci 7: 255-259, 2003

Genotype Distribution of the Mutations in the Coagulation Factor V Gene in the Korean Population: Absence of Its Association with Coronary Artery Disease

Seung Ho Hong*

Department of Science Education, Jeju National University of Education, Jeju 690-061, Korea

Key Words:

Coronary artery disease
Factor V mutation
Korean population

Mutations in the factor V gene are major risk markers for venous thrombosis. Several factors for blood coagulation have been related with cardiovascular disease. I investigated genotype distribution for three mutations (G1691A, A2379G and G2391A) of the factor V gene in the Korean population. Genotype frequencies were examined by polymerase chain reaction in 135 patients with coronary artery disease (CAD) and 116 healthy subjects. For the G1691A mutation (factor V Leiden), no mutation was detected in either group. Allele frequencies of A2379G and G2391A mutations were not significantly different between CAD patients and controls. Non-Caucasian populations have a considerably lower factor V Leiden allele frequency than Caucasian populations. Thus, it may be due to differences in the genetic background as well as environmental factors.

Factor V has been considered as a candidate which may contribute to the pathogenesis of venous thrombosis. Factor V can be inactivated by activated protein C (APC) for maintenance of coagulation-antithrombosis homeostasis.

The gene encoding human factor V has been cloned (Jenny et al., 1987; Cripe et al., 1992) and assigned to chromosome 1q21-25 (Wang et al., 1988). A number of mutations within the factor V gene have been detected and associations have been suggested between some mutations and resistance to APC, although the results have not always been concordant in all populations. The G1691A mutation (factor V Leiden) at a putative APC cleavage site of factor V has been reported (Bertina et al., 1994). This mutation detected by restriction enzyme *Mnl*I is located in exon 10. Several studies have reported that the factor V Leiden mutation may be a major cause of inherited thrombophilia (Bertina et al., 1994; Greengard et al., 1994; Voorberg et al., 1994). This mutation has been associated with resistance to APC. And also the factor V Leiden mutation was associated with susceptibility to preeclampsia (Grandone et al., 1997), factor V level in plasma (Lunghi et al., 1996), severe pre-eclamptic women (Nagy et al., 1998), coronary artery disease (CAD) (Redondo et al., 1999).

Frequency of the factor V Leiden mutation has been reported to vary among different populations (Burick et al., 1997; Rees, 1996; Rees et al., 1995; Pepe et al., 1997). In addition, two mutations in exon 13 have been found (Zoller and Dahlback, 1994). These mutation sites cleaved by *Taq*I and *Eco*RI are at positions 2391 (G/A substitution) and 2379 (A/G substitution), respectively.

The prevalence of CAD widely differs by genetic and environmental factors among different populations. The factor V Leiden mutation study was performed in Korean healthy population by Ishida et al. (1995) previously. To determine the differences of allele frequency for the factor V mutation among different races or ethnicities, I investigated allele frequencies of three mutations (G1691A, A2379G and G2391A) of the factor V gene in Korean patients with CAD versus controls.

Materials and Methods

Study subjects

I have selected 135 patients with CAD documented by coronary angiography from Seoul National University Hospital, Korea. The control group consisted of 116 individuals within the same age range as the patients, who were randomly selected via health screening. None of the controls had a history of CAD, and all had normal electrocardiograms. Mean age was 56.1±9.3 and 55.4±8.1 for the study and control populations, respectively; the age difference was not statistically significant.

*Tel: 82-064-720-0822, Fax: 82-064-755-5061
E-mail: shhong@jejue.ac.kr

Table 1. Primer sequences of factor V polymorphism markers

Locus	Primer Sequence (5'→3')	Annealing Tm
*Mnl*I	5'-GGGCTAATAGGACTACTTCTAATC-3'	55°C
	5'-TCTCTTGAAGGAAATGCCCCATTA-3'	
*Taq*I, *Eco*RI	5'-GAACTTGGATGTTAACTTCC-3'	55°C
	5'-GGCTTCACTTCTTAGAGGGTG-3'	

DNA analysis

I isolated genomic DNA from 10 ml of peripheral blood in the study subjects (Lahiri and Nurnberger, 1991). The factor V gene was amplified by the polymerase chain reaction (PCR) technique using genomic DNA (0.1 μg) as a template. PCR conditions for factor V mutations have previously been described (Bertina et al., 1994; Zoller and Dahlback, 1994). Primer sequences of factor V polymorphism markers are shown in Table 1. Amplification reactions were performed in a 25 μl volume containing 0.2 mM each of dCTP, dATP, dGTP, dTTP, 10pmol of each oligonucleotide primer, and 1 unit of *Taq* DNA polymerase, 10 mM Tris-HCl (pH 8.4), 50 mM KCl, 1.5 mM $MgCl_2$ and 0.01% gelatin. The amplification condition for each locus is as follows: 30 cycles for 94°C for 30 sec, 55°C for 30 sec, and 72°C for 1 min were performed.

PCR products of the mutation sites were digested with *Mnl*I, *Taq*I and *Eco*RI restriction enzymes. The digested samples were resolved on 2% agarose gels. Alleles of each mutation site were classified to (+) or (−) according to the presence or absence of cutting site of a restriction enzyme, respectively.

Data analysis

The counting method was used for estimation of the factor V gene frequencies. Statistical significance was assessed using χ^2 analysis. The distribution of factor V genotypes between CAD and control groups was compared using 2×2 contingency tables. The odds ratio (OR) and the associated 95% confidence interval (CI) were calculated for the allele frequencies between the case and control populations. Two diallelic mutations of the factor V gene were used for haplotyping. Haplotype frequencies were determined according to the method described by Thompson et al. (1998). Statistical significance was accepted at the $P<0.05$ level.

Results and Discussion

Genotype and allele frequencies of three mutations of the factor V gene in Korean CAD patients and control subjects are given in Table 2. For the factor V Leiden (A1691G) mutation, no individual was identified with the mutation in either group. Genotype and allele frequencies of the A2379G and G2391A mutations did not show a significant difference between the CAD and control groups. The genotype distributions of these two mutations did not differ from those expected for Hardy-Weinberg proportions in the CAD and normal groups. Haplotypes based on the genotypes of the A2379G and G2391A mutations are shown in Table 3. The frequencies of haplotypes are not significantly different between the CAD and control groups.

Table 2. Comparison of genotype and allele frequencies of the factor V mutations in the CAD patients and controls

	Genotype[a]			Allele	
	−/−	+/−	+/+	−	+
G1691A(*Mnl*I)					
CAD	0	0	135	0.00	1.00
Controls	0	0	116	0.00	1.00
A2379G (*Eco*RI)[b]					
CAD	130	5	0	0.98	0.02
Controls	107	9	0	0.96	0.04
G2391A (*Taq*I)[c]					
CAD	91	41	3	0.83	0.17
Controls	66	47	3	0.77	0.23

CAD = coronary artery disease.
[a]Genotypes were classified to the + or − according to presence or absence for the cutting site of the restriction enzyme, respectively.
[b]The odds ratio for allele frequencies between CAD and normals was 2.14 (95% CI, 0.71-6.48; $P = 0.14$).
[c]The odds ratio for allele frequencies between CAD and normals was 1.40 (95% CI, 0.91-2.18; $P = 0.08$).

Several factors related with coagulation or fibrinolysis are involved in cardiovascular disease. It has been suggested that factor V mutations may be risk factors for CAD. Therefore I investigated if factor V mutations are associated with Korean CAD patients. Interestingly, none of the 251 Koreans studied had the factor V Leiden mutation. Allele frequencies of the factor V Leiden mutation have been reported to vary among racial or ethnic groups (Table 4). The factor V Leiden mutant percentage

Table 3. Haplotype frequencies between *Eco*RI and *Taq*I polymorphisms of the factor V gene

Haplotype[a]		Controls	CAD
*Eco*RI	*Taq*I		
+	+	49	46
+	−	173	223
−	+	0	1
−	−	4	0
Total chromosome		226	270

CAD = coronary artery disease.
[a]Individuals having the same heterozygote of each polymorphism was excluded from the haplotype analysis. The odds ratio for haplotype frequencies between CAD and normals was 0.93 (95% CI, 0.72-1.19; $P = 0.30$).

Table 4. Comparison of mutation allele frequency of the factor V gene from various healthy populations

Population	Sample No	Mutation allele	References
European			
English	237	0.04	Cooper (1994)
English	381	0.03	Beauchamp et al. (1994)
Icelandic	96	0.03	Dahlback et al. (1993)
Greek	187	0.07	Koster et al. (1993)
German	49	0.02	Rosendaal et al. (1995)
German	1043	0.04	Schroder et al. (1996)
Swedish	101	0.06	Holm et al. (1996)
Austrian	104	0.02	Gisslinger et al. (1995)
Spanish	72	0.01	Pepe et al. (1997)
Italian	268	0.02	Pepe et al. (1997)
Italian	49	0.01	Bertina et al. (1994)
Italian	1207	0.01	Rees et al. (1995)
Dutch	474	0.02	Rosendaal et al. (1995)
Irish	133	0.01	Maat et al. (1996)
French	51	0.02	Maisonneuve et al. (1995)
French	1384	0.02	Emmerich et al. (1995)
African			
Kenya	60	0.00	Bertina et al. (1994)
Senegalese	96	0.00	Rosendaal et al. (1995)
Zambian	95	0.00	Koeleman et al. (1994)
Sub-saharan African	308	0.00	Helley et al. (1996)
Sub-saharan African	202	0.00	Pepe et al. (1997)
Ethiopian	114	0.00	Pepe et al. (1997)
Asian			
Indonesian	105	0.00	Rosendaal et al. (1995)
Indonesian	100	0.00	Pepe et al. (1997)
Pakistani	36	0.00	Bertina et al. (1994)
Mongolian	36	0.00	Rosendaal et al. (1995)
Indian(Gujerati)	32	0.02	Bertina et al. (1994)
Indian(Punjab)	36	0.00	Bertina et al. (1994)
Indian(Sikh)	29	0.02	Bertina et al. (1994)
Indian	203	0.01	Rees et al. (1995)
Sri Lanka	47	0.00	Bertina et al. (1994)
Chinese	254	0.00	Ishida et al. (1995)
Chinese	24	0.00	Pepe et al. (1997)
Chinese	330	0.00	Ko et al. (1996)
Taiwanese	83	0.00	Rosendaal et al. (1995)
Hong Kong Chinese	48	0.00	Rosendaal et al. (1995)
Japanese	270	0.00	Ishida et al. (1995)
Korean	93	0.00	Ishida et al. (1995)
Korean	116	0.00	*Present study*
North American			
Canadian Indian	36	0.00	Rosendaal et al. (1995)
Canadian	90	0.09	Liu et al. (1995)
American white	704	0.03	Ridker et al. (1995)
American black	307	0.01	Dilly et al. (1995)
American Indian	114	0.00	Pepe et al. (1997)
American black	168	0.00	Pepe et al. (1997)
American white	2468	0.03	Ridker et al. (1997)
American Hispanic	407	0.01	Ridker et al. (1997)
American black	650	0.01	Ridker et al. (1997)
American Asian	442	0.00	Ridker et al. (1997)
American Indian	80	0.01	Ridker et al. (1997)
American white	197	0.08	Liu et al. (1995)
American white	429	0.08	Liu et al. (1995)
South American			
Peruvian Indian	19	0.00	Flint et al. (1993)
Jamaican	91	0.00	Bertina et al. (1994)
Brazilian Amazonian Indians	83	0.00	Arruda et al. (1995)
Brazilian black	137	0.01	Arruda et al. (1995)
Australasian			
Australian Aboiginal	73	0.00	Rosendaal et al. (1995)
Papua New Guinean	95	0.00	Ridker et al. (1995)
Australian	126	0.02	Bockxmeer et al. (1995)
Middle-East			
Saudi Arabian	55	0.00	Bertina et al. (1994)
Saudi Arabian	255	0.01	Rees et al. (1995)

in Caucasian-descent populations has a frequency of approximately 1.0-9.0%. In contrast, non-Caucasian populations have a considerably lower frequency of the factor V Leiden mutation. Among these non-Caucasian populations, most have zero percent of the mutant frequency except for American Indian (2.0%), Saudi Arabian (1.0%), North American black (1.0%), Asian Indian (2.0%) and Brazilian black (1.0%) populations, suggesting that these populations could reflect a low level from interracial marriage by migration of European-descent populations. The frequency differences of the factor V Leiden mutation among populations may be due to differences in the genetic background as well as environmental factors. That is, it may be due to genetic drift by a founder effect or a selective mechanism. Thus, the factor V Leiden mutation may originate from Caucasian populations. Therefore, it raises the possibility that this mutation distribution may partly explain the rarity of thromboembolic disease in non-Caucasian populations. Indeed, spontaneous deep-vein thromboses are uncommon in African and Asian populations compared to European populations (Burkitt, 1972; Nathwani and Tuddenham, 1992). Thus, a point mutation in a restricted region such as factor V Leiden could provide an important clue to elucidate the migration route of populations.

Although associations between factor V Leiden mutation and CAD have been elucidated in studies of Caucasian populations (Rosendaal et al., 1997; Marz et al., 1995; Holm et al., 1996), other studies failed to detect an association with CAD (Gardemann et al., 1999; Ridker et al., 1995; Prohaska et al., 1995). That is, the associations are neither strong nor consistent in all populations. Therefore, the comparative studies of the factor V Leiden mutation among populations demonstrate the importance of using a homogeneous population in the selection of study samples, making possible the identification of more exact distributions of the mutation among racial or ethnic populations. Mutation studies of the factor V gene are mainly reported on Caucasian populations. There have, however, been few investigations of non-Caucasian groups. Therefore further studies in other racial or ethnic groups will be of great interest.

In conclusion, my results suggest that, at least in the Korean population, mutations of the factor V gene are unlikely to be a useful marker for CAD patients.

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[Received June 10, 2003; accepted July 23, 2003]