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Our results showed that 3 (9.4%), 3 (4.4%), and 1 (4%) individuals were heterozygous for prothrombin G20210A and 3 (9.4%), 5 (7.4%), and 1 (4%) individuals were heterozygous for factor V Leiden in the AMI, UA, and control groups, respectively. The following genotype frequencies for the factor VII R353Q polymorphism were identified: 25 (78.1%), 56 (82.4%), and 18 (72%) with RR and 7 (21.9%), 12 (17.6%), and 7 (28%) with RQ in the AMI, UA, and control groups, respectively. No QQ homozygotes were identified. For the HVR4 size polymorphism, the following genotypes were identified: 3 (9.4%), 4 (5.9%), and 5 (20%) individuals with H7H7; 11 (34.4%), 33 (48.5%), and 12 (48%) with H6H7; and 18 (56.2%), 31 (45.6%), and 8 (32%) with H6H6 genotypes in the AMI, UA, and control groups, respectively. There were no H7H5 and H6H5 genotypes found in this study.

Conclusions.

Although the frequency differences of these polymorphisms in patients with AMI and UA were not statistically significant from those in controls, several trends are consistent with what has been reported in the literature. Although any of these or other undefined genetic abnormalities may result in IHD, it is possible that phenotypic predisposition to IHD initially presents as UA. A larger population study addressing the significance of these polymorphisms in the sequence of events that lead to IHD, including cases of UA, is warranted.

Polymorphisms in the Genes for Coagulation Factors II, V, and VII in Patients With Ischemic Heart Disease

Yue Jin Feng, MD; Andrew Draghi, BS; Douglas R. Linfert, BS; Alan H. B. Wu, PhD; Gregory J. Tsongalis, PhD

• Background.—Cardiovascular disease remains the leading cause of mortality in the United States, accounting for approximately 33% of all deaths in this country. Of these deaths, most are due to acute myocardial infarctions (AMIs), which are associated with thrombotic coronary artery obstruction and/or occlusion. These events could potentially be due to alterations in genes coding for coagulation factors. Several polymorphisms have been described in the factor II, V, and VII genes, which may predispose one to increased risk for ischemic heart disease (IHD).

Objective.—To determine if mutations in 3 coagulation factor genes could predispose an individual to increased risk for arterial thrombosis as a mechanism for developing unstable angina (UA) or AMI.

Methods.—We examined 125 hospitalized patients (mean age, 53 ± 6 years, 79 men and 46 women), including 32 with AMI, 68 with UA, and 25 noncardiac controls, for a genetic predisposition for increased risk of IHD. EDTA-anticoagulated whole blood was collected at the time of hospital admission. DNA was extracted, and the polymorphisms were detected by polymerase chain reaction amplification of these genes with subsequent restriction enzyme digestion and gel electrophoresis.

Results.

Our results showed that 3 (9.4%), 3 (4.4%), and 1 (4%) individuals were heterozygous for prothrombin G20210A and 3 (9.4%), 5 (7.4%), and 1 (4%) individuals were heterozygous for factor V Leiden in the AMI, UA, and control groups, respectively. The following genotype frequencies for the factor VII R353Q polymorphism were identified: 25 (78.1%), 56 (82.4%), and 18 (72%) with RR and 7 (21.9%), 12 (17.6%), and 7 (28%) with RQ in the AMI, UA, and control groups, respectively. No QQ homozygotes were identified. For the HVR4 size polymorphism, the following genotypes were identified: 3 (9.4%), 4 (5.9%), and 5 (20%) individuals with H7H7; 11 (34.4%), 33 (48.5%), and 12 (48%) with H6H7; and 18 (56.2%), 31 (45.6%), and 8 (32%) with H6H6 genotypes in the AMI, UA, and control groups, respectively. There were no H7H5 and H6H5 genotypes found in this study.

Conclusions.—Although the frequency differences of these polymorphisms in patients with AMI and UA were not statistically significant from those in controls, several trends are consistent with what has been reported in the literature. Although any of these or other undefined genetic abnormalities may result in IHD, it is possible that phenotypic predisposition to IHD initially presents as UA. A larger population study addressing the significance of these polymorphisms in the sequence of events that lead to IHD, including cases of UA, is warranted.

Polymorphisms in Coagulation Factor Genes—Feng et al

MATERIALS AND METHODS

Patient Population

In this study, 125 subjects were evaluated. We retrospectively screened 100 consecutive patients presenting to the Hartford Hospital, Hartford, Conn.

Accepted for publication July 28, 1999.

From the Department of Pathology and Laboratory Medicine, Hartford Hospital, Hartford, Conn.


Reprints: Gregory J. Tsongalis, PhD, Department of Pathology and Laboratory Medicine, Hartford Hospital, 80 Seymour St, Hartford, CT 06102 (e-mail: gtsonga@harthosp.org).
Hospital Emergency Department, Hartford, Conn, from February 1997 to May 1997 with chest pain suspected to be due to acute coronary syndromes and 25 patients with noncardiac events. The diagnosis of AMI was made for 32 chest pain patients by attending physicians as defined by the World Health Organization electrocardiographic and laboratory criteria. Thirty-eight of these patients were classified as having UA defined as a new onset of severe or accelerated angina and subacute or acute angina at rest. Control patients ($n = 25$) were selected from patients with any clinical presentation as described. DNA extraction was added, and the sample was allowed to rehydrate overnight in 100% isopropanol to precipitate the DNA. DNA hydration solution was incubated in a total reaction volume of 50 mL that contained 40 ng of the forward and reverse gene-specific primers (Table 2), 2.0 U of AmpliTaq Gold (Perkin-Elmer, Norwalk, Conn), 200-μmol/L each deoxynucleotide triphosphate, 1.5-mmol/L MgCl₂, 10-mmol/L Tris-HCl (pH 8.3), 50-mmol/L KCl, and 0.001% gelatin. A multiplex PCR reaction was performed with both the prothrombin and factor V Leiden primers as we previously described. Separate PCR reactions were performed with the factor VII primers for the R353Q and HVR4 polymorphisms. The PCR amplification was performed in the GeneAmp 2400 Thermal Cycler (Perkin-Elmer, Foster City, Calif) using the following conditions: 94°C for 11 minutes; 35 cycles of 30-second denaturation at 94°C, 30-second annealing at 55°C, and 30-second extension at 72°C. The final cycle included a 3-minute extension step at 72°C. Following amplification, a restriction digestion was performed to detect the factor II, V, and VII sequence polymorphisms in a total volume of 50 mL and consisted of 30 mL of PCR product, 13 to 14 mL of dH₂O, 10 U of each enzyme (HindIII [factor II], MnlI [factor V], and MspI [factor VII] [New England Biolabs, Beverly, Mass]), and 5 mL of 10× buffer. Samples were then incubated for a minimum of 3 hours or overnight at 37°C.

Restriction fragment size analysis was performed by visualization of digested PCR products after separation by gel electrophoresis in a 10% polyacrylamide gel and staining with ethidium bromide. The HVR4 polymorphism was detected by PCR amplification and polyacrylamide gel electrophoresis. All gels were examined under UV light.

### Statistical Analysis

The frequencies of the factor II, V, and VII polymorphisms in patients and controls were determined. The frequencies of alleles and genotypes among the groups were compared by the $\chi^2$ test, and the values were predicted on the basis of the assumption of Hardy-Weinberg equilibrium. A $P$ value <.05 was considered statistically significant.

### RESULTS

Polymerase chain reaction analysis for detecting the factor II, factor V, and factor VII polymorphisms was used to screen 100 hospitalized patients with chest pain and 25 age-matched controls (Table 1). In this study, the factor II and factor V polymorphisms were detected by a multiplex PCR-mediated restriction fragment length polymorphism assay. The characteristic banding patterns of the homozygous normal or wild-type alleles and mutant alleles for the factor II and factor V polymorphisms by the multiplex PCR reaction are shown in Figure 1.

Separate PCR amplifications were performed for the factor VII gene regions that contained a sequence polymorphism (R353Q, exon 8) (Figure 2) and a size polymorphism (HVR4, intron 7) (Figure 3). The R353Q sequence polymorphism was detected by digesting a 312-}

<table>
<thead>
<tr>
<th>Table 1. Patient Demographic Data</th>
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<tbody>
<tr>
<td>Variable</td>
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<tr>
<td>---------</td>
</tr>
<tr>
<td>n</td>
</tr>
<tr>
<td>Male/Female ratio</td>
</tr>
<tr>
<td>Age, y (mean ± SD)</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Table 2. Factors II, V, and VII Polymorphisms</th>
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<tbody>
<tr>
<td>Gene</td>
</tr>
<tr>
<td>------</td>
</tr>
<tr>
<td>Factor II prothrombin</td>
</tr>
<tr>
<td>Factor V (Leiden)</td>
</tr>
<tr>
<td>Factor VII (R353Q)</td>
</tr>
<tr>
<td>Factor VII (HVR4)</td>
</tr>
</tbody>
</table>
bp amplified fragment (exon 8) with MspI. The common allele (R) consisted of a 205-bp, 67-bp, and 40-bp banding pattern, whereas the rare allele (Q) was detected as having a 272-bp and 40-bp banding pattern. Two HVR4 alleles were identified: a common allele (H6) of 443 bp with 6 monomers of the 37-bp repeat and a less frequent allele (H7) of 480 bp with 7 monomers.

In the present study, we identified 3 (9.4%), 3 (4.4%), and 1 (4%) individuals who were heterozygous for the factor II polymorphism and 3 (9.4%), 5 (7.4%), and 1 (4%) individuals who were heterozygous for factor V Leiden in the AMI, UA, and control groups, respectively (Table 3). No homozygous individuals were detected for either polymorphism. The frequency of these polymorphisms among the 3 groups did not show statistically significant differences.

The genotypes for the factor VII R353Q and HVR4 polymorphisms are shown in Table 4. The 353Q allele was very rare and only detected in 7 (9.5%), 12 (8.8%), and 7 (14%) individuals in the AMI, UA, and control groups, respectively. No 353Q homozygotes were detected in this study. The homozygous 353R genotype was very common, but no statistically significant differences were observed among the 3 groups (Table 4). In this study, we also did not identify the H5 allele, which is a very rare allele in the general population.12–13 The frequencies of genotypes iden-
Figure 3. Schematic representation of the factor VII HVR4 polymorphism.

Table 3. Patients Heterozygous for the Factor II (Prothrombin) and Factor V Leiden Polymorphisms

<table>
<thead>
<tr>
<th>Polymorphism</th>
<th>AMI (n = 32)</th>
<th>UA (n = 68)</th>
<th>Control (n = 25)</th>
<th>P Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>G20210A</td>
<td>3 (9.4%)</td>
<td>3 (4.4%)</td>
<td>1 (4%)</td>
<td>0.56</td>
</tr>
<tr>
<td>G1691A</td>
<td>3 (9.4%)</td>
<td>5 (7.4%)</td>
<td>1 (4%)</td>
<td>0.74</td>
</tr>
</tbody>
</table>

Table 4. Genotype Frequencies for the Factor VII Polymorphisms R353Q and HVR4

<table>
<thead>
<tr>
<th>Genotype</th>
<th>AMI (n = 32)</th>
<th>UA (n = 68)</th>
<th>Control (n = 25)</th>
<th>P Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>RR</td>
<td>25/32 (78.1%)</td>
<td>56/68 (82.4%)</td>
<td>18/25 (72%)</td>
<td>0.54</td>
</tr>
<tr>
<td>RQ</td>
<td>7/32 (21.9%)</td>
<td>12/68 (17.6%)</td>
<td>7/25 (28%)</td>
<td></td>
</tr>
<tr>
<td>QQ</td>
<td>0/32</td>
<td>0/68</td>
<td>0/25</td>
<td></td>
</tr>
<tr>
<td>H7H7</td>
<td>3/32 (9.4%)</td>
<td>4/68 (5.9%)</td>
<td>5/25 (20%)</td>
<td></td>
</tr>
<tr>
<td>H6H6</td>
<td>11/32 (34.4%)</td>
<td>33/68 (48.5%)</td>
<td>12/25 (48%)</td>
<td></td>
</tr>
<tr>
<td>H6H5</td>
<td>18/32 (56.2%)</td>
<td>31/68 (45.6%)</td>
<td>8/25 (32%)</td>
<td>0.16</td>
</tr>
<tr>
<td>H7H5</td>
<td>0/32</td>
<td>0/68</td>
<td>0/25</td>
<td></td>
</tr>
<tr>
<td>H6H6</td>
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<td>0/68</td>
<td>0/25</td>
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Coagulation involves numerous gene products that interact in a series of reactions that constitute both the intrinsic and extrinsic coagulation pathways. Genetic abnormalities in these genes can result in an increased risk for developing a thromboembolic event due to quantitative increases in coagulation factor proteins. Most recently, increased levels of factor VII have been associated with an increased risk for AMI. In this study, we evaluated genetic risk for arterial thrombosis associated with AMI by examining the genes of 3 coagulation factors (II, V, and VII) for described polymorphisms.

Prothrombin (factor II), factor V, and factor VII contain a serine protease domain that is highly homologous to trypsin. Prothrombin, a precursor of the serine protease thrombin, is a key enzyme in the processes of hemostasis and thrombosis, along with factors V and VII. Factor V is a single-chain glycoprotein that accelerates factor Xa-catalyzed conversion of prothrombin into thrombin. A single base change in the factor V gene, known as factor V Leiden, results in the substitution of adenine for guanine at position 1691. This results in a change in the amino acid sequence (Arg→Gln) of the factor V gene product, which now becomes resistant to degradation by the activated protein C complex. Several studies have shown that resistance to activated protein C is the most common risk factor associated with venous thrombosis and is present in 20% to 60% of the patients with a history of thrombophilia. 

Prothrombin or factor II is coded for by a 21-kb gene, containing 14 exons, and is localized to chromosome 11p11-q12. Previous studies by Poort et al and Hillarp et al demonstrated that the prothrombin gene is a candidate gene for venous thrombosis and found a G→A transition at nucleotide position 20210, which increased the risk of venous thrombosis in patients with a personal and family history of venous thrombophilia. Similar results were also reported by Bentolila et al and Cumming et al. Recently, several studies have indicated that there may be a relationship between heterozygous carriers of the genetic variant (G→A transition) in the factor II, V, and VII genes and coronary artery disease. It has been suggested that these genetic defects may be associated with the increased risk of myocardial infarction.

We and others have described multiplex PCR-mediated assays for the simultaneous detection of the factor V Leiden and prothrombin G20210A polymorphisms. These assays rely on restriction endonuclease digestion to detect either mutant or wild-type alleles. These multiplex assays allow the laboratory to test for both polymorphisms simultaneously, thus providing a result with faster turnaround time and lower expense. In this study, we have...
identified heterozygosity for the factor II polymorphism (20210A) in 6 (6%) of 100 patients who were admitted because of AMI or UA. The factor V Leiden allele (1691A) was detected in 8 (8%) of 100 of these same patients (AMI or UA). Only 1 (4%) of 25 controls was found to be heterozygous for the factor V Leiden or prothrombin G20210A polymorphisms. These findings suggest that these two polymorphisms may be associated with arterial thrombosis more often than was once thought. Our findings are consistent with other studies that evaluated the factor II 20210 and factor V Leiden 1691 polymorphisms. Rosendaal et al33 reported that the factor II 20210 and factor V Leiden 1691 G→A transitions were present more often in women with myocardial infarction (15.1% and 9.5%) than among control women (1.6% and 4.1%). Wang et al noted that the 1691A allele was present in 2.2% to 2.5% of hospitalized, angiographically diagnosed patients. Marz et al found that mutant factor V was more frequent in 89 patients who had myocardial infarctions before 55 years of age (9%) than in controls (4%). Another study found the factor V Leiden polymorphism more often in patients with myocardial infarction (5.7%) than in healthy controls (2.9%). Samani et al and Ardissino et al, however, evaluated young survivors (<45 years) of myocardial infarction for factor V Leiden and found that the factor V polymorphism was not a factor for the early development of coronary artery disease. The discrepancies among these studies may well be the result of differences in patient populations with respect to sex, since the cause of myocardial infarction may in part differ between men and women. Because activated protein C resistance associated with factor V Leiden is a common genetic defect, it offered a unique opportunity to study the possible association of this defect with other genetic risk factors for coronary artery disease.

Several studies, including the Northwick Park Heart Study and the Prospective Cardiovascular Munster Study, reported that increased levels of factor VII are associated with increased risk for ischemic heart disease. In contrast to these studies, the Edinburgh Artery Study showed no differences in factor VII levels between men and women with myocardial infarction and healthy controls. Factor VII is a vitamin K-dependent single-chain plasma glycoprotein that functions in the extrinsic pathway of blood coagulation. The heavy chain of factor VIIa contains the 3 principal residues involved in the catalytic activity, including serine at position 344. A single-base polymorphism (R353Q), first described by Green et al, results in an Arg to Gln substitution at position 353, which lies within the serine loop, close to the active serine 344 site. This amino acid substitution may substantially alter the conformation of factor VII by a change in charge within a region of the enzyme whose tertiary structure is necessarily constrained. The relationship between this factor VII polymorphism and risk of myocardial infarction has been reported in several studies. Iacoviello et al examined patients with familial myocardial infarction for this polymorphism and reported that patients with the QQ genotype and lowest factor VII levels were associated with the lowest risk of myocardial infarction, followed by the RQ and RR genotypes. Similarly, Doggen et al demonstrated high levels of factor VII in patients with the RR genotype. However, these patients were at lowest risk for developing myocardial infarction.

A second size polymorphism (HVR4) has also been described in the factor VII gene, which may account for predisposition to heart disease. Iacoviello et al showed that the H7H7 and H6H5 genotypes had highest risk, followed in descending order by the H6H6, H7H7, and H7H5 genotypes, for developing myocardial infarction. The genotypic findings in our study with respect to the distribution of genotypes in patients versus controls were similar to those of Iacoviello et al. The one exception was that we did not find the rare genotypes in any of our patients (QQ and H5 alleles) that were found in a larger clinical population.

Despite contradicting results that associated a simple nucleotide change in several coagulation factor genes with a genetic predisposition for developing myocardial infarction, further prospective clinical investigations are warranted to determine if there is a true genetic component for IHD. It is possible that genetic polymorphisms in coagulation factor genes may be independent risk factors for coronary artery disease. However, many environmental and epigenetic variables exist for developing coronary artery disease among different populations around the world.

In this study, which evaluated the possibility of a sole genetic component for increased risk of AMI, our data showed that the differences in allele and genotype frequencies for factor VII polymorphisms in patients with AMI and UA were not statistically significant from those in hospitalized control patients without cardiovascular events. Of the two factor VII polymorphisms evaluated, the HVR4 H6H6 genotype, known to be associated with highest factor VII levels, was more common in AMI patients than in UA or healthy control patients. In contrast, the H7H7 genotype associated with lower factor VII levels was least common in AMI and UA patients compared with healthy controls. Further prospective population-based studies are needed to establish a definitive genetic predisposition for IHD based on the 3 coagulation factor gene polymorphisms described herein.

References


