Asymptomatic Factor VII Deficiency in African Americans

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Abstract

African Americans with factor VII (FVII) deficiency, as defined by clinical laboratory values, are frequently asymptomatic. To date the genotypes underlying this FVII defect in asymptomatic African Americans have not been established. We show in 3 unrelated African American patients that the defect is due to a G to A nucleotide change resulting in an arginine to glutamine mutation in Factor VII amino acid 304. This defect results in low FVII coagulant activity levels using rabbit brain thromboplastin but not using human thromboplastin. This report may aid transfusion and hematology specialists to evaluate patient results and prevent unnecessary transfusions to treat patients with abnormal laboratory values.

Asymptomatic Factor VII deficiency in African Americans was described by Triplett et al9 in 1985 before the publication of the factor VII DNA sequence. However, no further study has reported the genetic basis of apparent FVII:C deficiency in the African American population. We describe FVII Padua in 3 African American patients and demonstrate that the genetic cause of FVII Padua in these patients is a guanine to adenine mutation (CGG→CAG) in the nucleotide sequence encoding arginine at FVII amino acid 304, a change that has been reported by James et al.7 We also show that the use of currently available human thromboplastins and molecular sequencing in asymptomatic

The activated form of factor VII (FVIIa) interacts with cofactor tissue factor (TF) to play a critical role in initiating blood coagulation.1 Deficiency of FVII causes a bleeding disorder of variable severity owing to a decreased level of this vital trigger of blood clotting. FVII coagulant activity (FVII:C) in patient plasma is evaluated using a modified prothrombin time calibrated with FVII-deficient plasma. FVII antigen (FVII:Ag) measurements provide further information about the presence or absence of the FVII protein.

Congenital FVII deficiency, which occurs at a frequency of approximately 1 per 500,000, is caused by a mutation in the FVII gene on the long arm of chromosome 13.2,3 Symptomatology in congenital human FVII deficiency with FVII:C levels of less than 1% ranges from asymptomatic to severe hemorrhagic problems.4 In addition, FVII:C levels differ markedly depending on the source of TF in the thromboplastin used for measuring FVII:C.1 This difference is exemplified by FVII Padua, a defect originally reported from patients in Italy5,6 and later shown to be associated with an arginine (R) to glutamine (Q) mutation at FVII amino acid 304.7,8
patients can verify a diagnosis of FVII Padua and prevent unnecessary blood product transfusions.

Materials and Methods

Reporting the patients from this study was approved by the institutional review board of the University of Pennsylvania, Philadelphia. Subjects were selected from patients in whom a decreased FVII:C was detected in the clinical laboratory at the Hospital of the University of Pennsylvania, Philadelphia.

Genotyping

Chronologically, in the first 3 patients, FVII genotyping was performed on genomic DNA purified from whole blood leukocytes. As previously described, DNA sequencing was performed following polymerase chain reaction of all 8 exons and exon-intron junctions, as well as 200 bases of the 3' and 5' untranslated regions of the entire factor VII gene.10 The specific sequences were analyzed using an ABI DNA sequence analyzer (Applied Biosystems, Foster City, CA) at the Children’s Hospital of Philadelphia. No DNA was available for genotyping for the subsequent 12 patients in whom assays were performed for clinical purposes.

Factor VII Assays

In the coagulation laboratory at the Hospital of the University of Pennsylvania, the normal range for FVII:C is 50% to 135% when using a TF reagent derived from rabbit brain (Simplastin Excel, Biomerieux, Durham, NC). A normal plasma pool was constructed by mixing equal volumes of plasma from more than 20 healthy control subjects. The plasma from patients was obtained using 3.2% sodium citrate. FVII:C levels were performed using 1-stage clotting assays with rabbit brain thromboplastin (Simplastin Excel) or lyophilized recombinant human TF (Innovin, Dade Behring, Newark, DE). FVII:Ag levels were measured using an enzyme-linked immunosorbent assay (American Bioproducts, Parsippany, NJ). The patient samples were analyzed between July 1999 and June 2005.

Case Histories

Information about age, ethnicity, and medical condition was provided by the patient’s referring physician. All patients were referred for an abnormal prothrombin time, a decreased FVII:C level, or for family studies.

Results

We studied 15 African American patients for FVII deficiency. The first 3 patients were referred to us to identify the cause of an FVII abnormality associated with the laboratory finding of decreased FVII:C (E.S.P.). In these patients, we identified a G to A single point mutation at nucleotide position 10828 in the FVII gene. This causes a CGG to CAG change in exon 8 at amino acid 304, a mutation consistent with the diagnosis of FVII Padua.7,8 Figure 1. We also verified the lack of mutations in the entire FVII coding sequence, intron-exon junctions, and critical untranslated regions that could cause the observed clinical phenotype. The laboratory clinical diagnosis of FVII Padua also was confirmed on the basis of plasma coagulation values using thromboplastins derived from rabbit brain (Simplastin Excel) and recombinant human TF (Innovin) Table 1.

Case Histories

Case 1 was a 14-year-old African American boy with a history of minor, occasional nosebleeds. The patient’s mother is Caucasian and his father, African American. The patient’s FVII...
deficiency was noted in a hospital workup for management after a motor vehicle accident. The prothrombin time remained prolonged despite treatment with vitamin K and fresh frozen plasma (FFP). Case 2 involved a 27-year-old African American woman with an elevated prothrombin time found during a preoperative workup for tonsillectomy. The patient previously was treated prophylactically with blood products owing to abnormal laboratory values. Case 3 was a 52-year-old African American woman with diabetes. A FVII:C level of 34% was found after an elevated prothrombin time was noted by the hematologist during a routine checkup.

We further tested blood samples from an additional 12 African American patients in whom an elevated prothrombin time was noted during clinical testing. Ten of these patients had laboratory results consistent with FVII Padua (Table 2). In an additional 2 asymptomatic African American patients, special coagulation studies were ordered owing to mildly elevated prothrombin times. However, the results were not consistent with FVII Padua because the prothrombin time and FVII:C were similar using rabbit brain and recombinant human TF thromboplastins (Table 2, cases 14 and 15).

**Discussion**

We report coagulation testing for FVII Padua in 15 African American patients. In the 3 unrelated African American patients in whom we evaluated the FVII genotype, we identified a G to A change within the nucleotide sequence encoding the arginine at amino acid 304, a mutation shown to cause FVII Padua (Table 1 and Figure 1).

We further identified a probable clinical diagnosis of FVII Padua in 10 asymptomatic African American patients identified as “FVII deficient” owing to elevated prothrombin times and decreased FVII:C levels using rabbit brain but not recombinant human TF thromboplastins. Two additional asymptomatic African American patients had coagulation results of mild FVII deficiency in the clinical laboratory but lacked the specific laboratory values seen in FVII Padua (Table 2).

In a mutation update of FVII deficiency, McVey et al showed that the FVII:Ag correlates best with patient symptomatology, as was the case in the present study. A study by Triplet et al from 1985 concluded that the closest correlation of patient FVII-deficient clinical symptomatology with...

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**Table 1**

<table>
<thead>
<tr>
<th>Case No./ Sex/Age (y)</th>
<th>PT (s)</th>
<th>FVII:C (%)</th>
<th>FVII:C (%)</th>
<th>FVII:Ag (%)</th>
<th>Factor VII Exon 8 Genotype</th>
<th>Phenotype</th>
</tr>
</thead>
<tbody>
<tr>
<td>1/M/14</td>
<td>15.6</td>
<td>33</td>
<td>61</td>
<td>57</td>
<td>Heterozygote for R304Q</td>
<td>Minor nosebleeds; consistently elevated PT after FFP transfusions</td>
</tr>
<tr>
<td>2/F/27</td>
<td>Prolonged</td>
<td>26</td>
<td>54</td>
<td>67</td>
<td>Compound heterozygote for R304Q and R315W</td>
<td>Asymptomatic for bleeding; elevated PT preoperatively for tonsillectomy</td>
</tr>
<tr>
<td>3/F/52</td>
<td>14.5</td>
<td>21</td>
<td>69</td>
<td>NA</td>
<td>Heterozygote for R304Q</td>
<td>Asymptomatic for bleeding; diabetes mellitus</td>
</tr>
</tbody>
</table>

FFP, fresh frozen plasma; FVII:Ag, factor VII antigen; FVII:C, factor VII coagulant activity (normal range, 50%-135%); NA, not available; PT, prothrombin time (normal range with Simplastin Excel, 11.3-13.3 s).

1. With Simplastin Excel, Biomerieux, Durham, NC.
2. With InnoViva, Dade Behring, Newark, DE.

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**Table 2**

<table>
<thead>
<tr>
<th>Case No./ Sex/Age (y)</th>
<th>PT (s)</th>
<th>FVII:C (%)</th>
<th>FVII:C (%)</th>
<th>FVII:Ag (%)</th>
<th>Genotype</th>
<th>Phenotype</th>
</tr>
</thead>
<tbody>
<tr>
<td>4/F/51</td>
<td>25.1</td>
<td>&lt;1</td>
<td>32</td>
<td>61</td>
<td>NA</td>
<td>Asymptomatic; preoperative fibroid removal</td>
</tr>
<tr>
<td>5/M/18</td>
<td>22.0</td>
<td>&lt;1</td>
<td>33</td>
<td>NA</td>
<td>NA</td>
<td>Asymptomatic; football team eligibility</td>
</tr>
<tr>
<td>6/M/6</td>
<td>25.3</td>
<td>&lt;1</td>
<td>34</td>
<td>NA</td>
<td>NA</td>
<td>Asymptomatic; preoperative for orthopedic procedure</td>
</tr>
<tr>
<td>7/F/7</td>
<td>14.8</td>
<td>32</td>
<td>54</td>
<td>NA</td>
<td>NA</td>
<td>Asymptomatic; SS; preoperative for elective surgery</td>
</tr>
<tr>
<td>8/M/10</td>
<td>16.3</td>
<td>16</td>
<td>26</td>
<td>NA</td>
<td>NA</td>
<td>Asymptomatic; s/p appendectomy, abscess drainage</td>
</tr>
<tr>
<td>9/M/12</td>
<td>23.8</td>
<td>&lt;1</td>
<td>33</td>
<td>NA</td>
<td>NA</td>
<td>Asymptomatic; preoperative for elective tonsillectomy</td>
</tr>
<tr>
<td>10/F/20</td>
<td>13.9</td>
<td>44</td>
<td>74</td>
<td>NA</td>
<td>NA</td>
<td>Asymptomatic; preoperative for kidney donation</td>
</tr>
<tr>
<td>11/F/61</td>
<td>16.4</td>
<td>22</td>
<td>29</td>
<td>NA</td>
<td>NA</td>
<td>PE; received FFP transfusion before testing</td>
</tr>
<tr>
<td>12/F/38</td>
<td>10.6</td>
<td>36</td>
<td>53</td>
<td>NA</td>
<td>NA</td>
<td>Asymptomatic; family study for case 11</td>
</tr>
<tr>
<td>13/F/59</td>
<td>17.5</td>
<td>15</td>
<td>38</td>
<td>NA</td>
<td>NA</td>
<td>Asymptomatic; family study for case 11</td>
</tr>
<tr>
<td>14/M/13</td>
<td>13.3</td>
<td>57</td>
<td>59</td>
<td>NA</td>
<td>NA</td>
<td>Asymptomatic; preoperative</td>
</tr>
<tr>
<td>15/M/15</td>
<td>15.8</td>
<td>29</td>
<td>31</td>
<td>NA</td>
<td>NA</td>
<td>Asymptomatic; preoperative for elective tonsillectomy</td>
</tr>
</tbody>
</table>

FFP, fresh frozen plasma; FVII:Ag, factor VII antigen; FVII:C, factor VII coagulant activity (normal range, 50%-135%); NA, not available; PE, pulmonary embolus; PT, prothrombin time (normal range with Simplastin Excel, 11.3-13.3 s); SS, sickle cell disease.

1. With Simplastin Excel, Biomerieux, Durham, NC.
2. With InnoViva, Dade Behring, Newark, DE.
laboratory studies was by measurement of FVII activity using human thromboplastin. The study reported FVII deficiency in 26 patients, including 16 Caucasians, 1 Latin American, and 9 blacks. Of these patients, all 9 black patients were asymptomatic, whereas the other 17 patients had a clinically relevant bleeding disorder. There have been several further reports of a mild to asymptomatic phenotype in African Americans. However, there have been no reports describing the genotypic cause of FVII deficiency in asymptomatic African Americans.

FVII R304 mutations have been described in the literature in patients from Italy, England, Brazil, and Asia. Patients are asymptomatic or exhibit very mild bleeding problems, even with a FVII:C level less than 1%. The nucleotide codon for FVII R304 (CGG), represents a CpG hotspot, a sequence sensitive to frequent nucleotide substitutions. In vitro, dysfunction of FVII Padua also has been reported to be consistent with R304Q. The amino acid at position Arg304 (R304) is highly conserved in FVII from different species but is not well conserved among other vitamin K–dependent proteins. This positively charged residue is solvent exposed and a likely site for interaction between factor X and TF.

Biochemical and molecular genetic data support a role for the R304 residue in mediating interactions with TF and possibly factor X. An alanine scanning mutagenesis approach led to the hypothesis that several other residues such as Pro303, Leu305, Met306, and Asp309 are directly involved in making contacts with TF. These biochemical predictions eventually were borne out by structural data, which indeed revealed direct contacts between TF and FVIIa in the vicinity of R304. These observations also nicely explain why an amino acid change at position 304 could give markedly different clotting time values depending on the source of the TF.

We demonstrated that FVII Padua underlies asymptomatic FVII deficiency in many African Americans. We showed that the FVII deficiency in 3 patients at our specialty laboratory was caused by a CGG→CAG change. This has not been reported specifically in the African American population in the United States. An additional 10 patients had coagulant activity values consistent with the laboratory phenotype of FVII Padua (Table 2). In addition, in several patients mentioned in this report, FVII Padua was diagnosed before a surgical procedure, which allowed surgery to be performed safely, omitting prophylactic transfusions. However, other patients in this study had previously received transfusions owing solely to the laboratory abnormality.

The use of currently available human thromboplastins in asymptomatic African American patients with FVII deficiency could prevent unnecessary replacement therapy associated with a laboratory abnormality. Prothrombin complex concentrates, FFP, rFVIIa, and e-caproic acid are the treatments of choice for managing a coagulopathy due to FVII deficiency when correction is necessary. Unfortunately, blood products may be given with a prolonged prothrombin time due to low FVII:C, but correction is not clinically necessary. In clinically documented FVII deficiency, correction may be required prophylactically and with surgery.

Patient histories and symptomatology are critical in the evaluation of FVII:C and/or FVII:Ag when evaluating transfusion requirements. This article shows the importance of knowing the specific thromboplastins used by the clinical laboratory when making a transfusion recommendation based on the prothrombin time and the FVII:C results. We hope that awareness of FVII Padua in the African American population as described in this report will help prevent inappropriate, nonemergency transfusion of FFP and rFVIIa to patients for asymptomatic problems due solely to a laboratory abnormality. The current trend in clinical laboratories toward replacement of thromboplastins derived from animal tissue with those synthesized with recombinant human TF protein/protein fragments likely will reduce this problem in the near future.

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References


