


Dysfunctional Factor VII Variant (FVII Tondabayashi) with R79Q: Determination of Mutated Site with Monoclonal Anti-Human Factor VII Antibody (B101/B1), Osamu Takamiya1* and Shigeru Takeuchi 2

Inherited factor VII (FVII) deficiency is a rare autosomal recessive disorder that occurs in roughly 1 in 50,000 people. Although many patients with homozygous FVII deficiency have a life-long tendency to bleed, a coincident thromboembolic complication has been reported (1). In general, the bleeding tendency of FVII deficiency is not as severe as that of hemophilia. The clinical features are quite variable, with a rather poor correlation between measured coagulation activity and clinical bleeding tendency (2). In laboratory testing, FVII deficiency has been divided into three types: cross-reactive material (CRM) type, with decreased synthesis of the FVII molecule; CRM+, with decreased FVII activity but an antigen concentration within reference values; and CRM−, with reduced synthesis of FVII (3). Specific FVII variants have been characterized as FVII molecules that give different procoagulant activities using tissue factor (TF) from various sources, such as human, rabbit, and bovine brain TF (4–6). Some of the dysfunctional FVII variants possess either an Arg 79-to-Gln substitution in the first epidermal growth factor (EGF)-like domain or an Arg 353-to-Gln substitution in the catalytic domain of FVII (7–11).

In the present study, using an ELISA with the monoclonal antibody that recognizes a specific epitope located in the three-dimensional structure near position 79 in the first EGF-like domain of human FVII (12), we determined the mutated site of a dysfunctional FVII variant that possessed different FVII activity with human placental TF than with rabbit and ox brain TF.

The propositus was a 12-year-old girl with FVII deficiency. She was born at 36 weeks gestation, had no hemorrhagic problems, and showed no evidence of abnormal hepatic function either at birth or later. Her parents were not consanguineous, and the family history did not reveal any bleeding disease. Decreased Normotest values were noted at her health examination at the age of 1 month. She was admitted to our Department of Pediatrics because of a suspected deficiency of vitamin K-dependent coagulation factors. The Normotest did not increase after the administration of vitamin K₂ (3.5 mg). Hemostatic examination revealed a prolonged prothrombin time (Simiplastin, Organon Teknika), whereas the bleeding time, platelet count, activated partial thromboplastin time, and fibrinogen were within reference ranges. The coagulation factor activities were within health-related reference values except for FVII, and her plasma showed no inhibitors of coagulation factors. However, the levels of FVII activity (FVII:c) exhibited different activation patterns when TFs from different sources were used. Her FVII:c was 7.2% of the healthy control when rabbit TF was used, and 40% and 65% when human placenta and bovine brain TF, respectively, were used. Abnormal coagulation values were observed constantly over a period of 10 years.

The FVII:c and FVII:ag levels of the propositus and her family members are shown in Table 1. The FVII:c level using recombinant human TF was similar to that using human placenta TF. Both the father and sister of the propositus showed different activation patterns with human TF and rabbit TF, although their FVII:c levels were not as low as that of the propositus. The mother’s activation pattern did not vary with the TFs used. The FVII:ag level of the propositus was only 5% of healthy controls when determined by ELISA-1 binding with hVII-B101/B1 as the solid phase, although it was in the lower limit of the health-related reference range when determined by ELISA-2 and ELISA-3, respectively. As measured by qualitative immunoelectrophoresis (QIEP) (13), the FVII:ag level was 64% of healthy controls. The ELISA/QIEP ratio was 0.078 in ELISA-1, 0.984 in ELISA-2, and 1.016 in ELISA-3. The FVII:ag levels of the father and sister were...
The ability of TF to bind to FVII mother were within reference values with ELISA-1 and reference values with ELISA-3, respectively. Those of her below reference values with ELISA-1, and within reference values with ELISA-3, respectively. Those of her father, and her sister had both a 310-bp band pro-
duced by the loss of the \( MspI \) restriction site and a 245-bp adjacent intron. The amplified DNA fragment is 310 bp. The expected sizes of DNA fragments after digestion with the restriction endonuclease \( MspI \) are shown. The mutation in FVII Tondabayashi abolishes a \( MspI \) site by changing the sequence from CCGG to CCAG. (B) \( MspI \) analysis of the family with FVII Tondabayashi. PCR fragments spanning exons 3 and 4 from the family members were digested with \( MspI \) and analyzed on a 1.8% agarose gel. Lane M, DNA size markers; lane 1, healthy control; lane 2, propositus; lane 3, father; lane 4, mother; lane 5, sister.

**Fig. 1.** \( MspI \) restriction map and analysis of mutations in FVII Tondabayashi.

(A) The \( MspI \) restriction map of a PCR fragment spanning exons 3 and 4 and adjacent introns. The amplified DNA fragment is 310 bp. The expected sizes of DNA fragments after digestion with the restriction endonuclease \( MspI \) are shown. The mutation in FVII Tondabayashi abolishes a \( MspI \) site by changing the sequence from CCGG to CCAG. (B) \( MspI \) analysis of the family with FVII Tondabayashi. PCR fragments spanning exons 3 and 4 from the family members were digested with \( MspI \) and analyzed on a 1.8% agarose gel. Lane M, DNA size markers; lane 1, healthy control; lane 2, propositus; lane 3, father; lane 4, mother; lane 5, sister.

**Table 1.** FVII:c using different TFs and FVII:ag using different FVII antibodies.

<table>
<thead>
<tr>
<th></th>
<th>Propositus</th>
<th>Father</th>
<th>Mother</th>
<th>Sister</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rabbit brain TF</td>
<td>6</td>
<td>38</td>
<td>72</td>
<td>44</td>
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<td>Recombinant human TF</td>
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<td>95</td>
<td>69</td>
<td>95</td>
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<td>80</td>
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<td>Bovine brain TF</td>
<td>68</td>
<td>110</td>
<td>100</td>
<td>120</td>
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<td>Simian brain TF</td>
<td>35</td>
<td>65</td>
<td>69</td>
<td>65</td>
</tr>
<tr>
<td>FVII:ag, %</td>
<td></td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>ELISA-1</td>
<td>5</td>
<td>32</td>
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<td>34</td>
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<td>ELISA-2</td>
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<td>100</td>
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<tr>
<td>ELISA-3</td>
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<td>105</td>
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<tr>
<td>QIEP</td>
<td>64</td>
<td>100</td>
<td>78</td>
<td>110</td>
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\( ^a \) FVII:c was measured by a one-stage method using FVII-depleted plasma as a substrate and various TFs: rabbit brain TF (CRB thromboplastin, Roche Diagnostics), recombinant human TF (Innovin, Baxter Diagnostics), human placenta TF (Thromborel S, Behringwerke), bovine brain TF (kindly provided by Dr. H. Hiura, International Reagents Co., Kobe, Japan), and simian brain TF (Thromboplastin a, Diagnostica Stago).

\( ^b \) FVII:ag was measured by ELISA using monoclonal antibodies hVII-B101/B1, hVII-DC2/D4, hVII-DC6/3D8, or anti-human FVII rabbit serum (polyclonal antibody) as the solid phase. ELISA-1 used hVII-B101/B1 as the solid phase; ELISA-2 used hVII-DC2/D4 and hVII-DC6/3D8 as the solid phase; and ELISA-3 used anti-human FVII rabbit serum as the solid phase. QIEP used \( ^{125}I \)-labeled anti-human FVII IgG (15).

below reference values with ELISA-1, and within reference values with ELISA-3, respectively. Those of her mother were within reference values with ELISA-1 and ELISA-3. The ability of TF to bind to FVII (14) of the propositus was 14% of healthy controls; the values for the father, sister, and mother were 29%, 34%, and 72%, respectively. These findings strongly suggested that the mutation in the abnormal FVII of the propositus was in the structure near position 79 in human FVII. Therefore, a fragment spanning exons 3 and 4 and the adjacent intron of the FVII genes of the propositus and her family members were amplified by PCR. The PCR fragments were digested with the restriction endonuclease \( MspI \) and analyzed by agarose gel electrophoresis. The propositus, her father, and her sister had both a 310-bp band produced by the loss of the \( MspI \) restriction site and a 245-bp band, indicating that they were heterozygous for the mutation (Fig. 1), which could be concluded to consist of base substitutions in the CCGG from nucleotide position 6053 to 6056. Single-strand conformational polymorphism analysis (15) of PCR products of five exons and their adjacent intron junctions (exons 2–8) of the FVII genes of the propositus revealed no difference in mobilities compared with healthy controls analyzed in parallel except with PCR fragments that spanned exons 3 and 4. The nucleotide sequence of exon 4 was determined by directly sequencing the PCR fragments. A heterozygous G-to-A point mutation was found at nucleotide position 6055, producing the substitution of Arg 79 (CGG) by Gln (CAG).

It is difficult to determine molecular defects in FVII by protein analysis because FVII is present in plasma at extremely low concentrations. Only a few studies have detected the sites of mutations in coagulation factors by using monoclonal antibodies, although monoclonal antibodies for coagulation factors have been useful for studying the structure of coagulation proteins and developing sensitive immunoassays. Fair et al. (16) reported that immunochemical assays using a polyclonal antibody or 46 monoclonal antibodies against Factor X failed to reveal any structural deviation for the dysfunctional Factor X Friuli variant. Protein C Yonago, which contains the substitution of Arg 15 by Gly, was nonreactive to monoclonal antibodies JTC-1 and -3, which recognize only the calcium-dependent conformation of the \( \gamma \)-carboxyglutamic acid domain (17). We know of no report that has determined the mutational site of a dysfunctional FVII variant with monoclonal antibodies. It was speculated that the FVII of the propositus, which has different activation patterns for TFs from different sources, might possess substitution of Arg 79 or Gln 304. The FVII-TF binding in the propositus was lower than in healthy controls. The level of the FVII:ag of the propositus was 65% of healthy controls in immunoelectrophoresis using a polyclonal antibody as the probe for determining FVII. The FVII:ag level determined by ELISA using a polyclonal
antibody or a mixture of monoclonal antibodies that recognize the light and heavy chains of FVIIa was similar to that determined by immunoelectrophoresis. However, the level of the FVIIa:Ag, using the monoclonal antibody recognizing the specific epitope located in the three-dimensional structure near position 79, was remarkably low compared with QIEP or ELISA using a polyclonal antibody or a mixture of monoclonal antibodies that recognize the light and heavy chains of FVIIa. The levels of FVIIa:Ag of the father and sister revealed a similar pattern to that of the propositus, although they were not as low. These findings strongly suggested that the mutation in the abnormal FVII of the propositus, her father, and her sister was in the structure near position 79 in the first EGF-like domain of human FVII. DNA sequencing revealed a G-to-A point mutation that was found at nucleotide position 6055 in exon 4 of the FVII gene. This produces a substitution in the CCG codon for Arg 79 in the first EGF-like domain such that it is changed to CAG.

Clark et al. (18) reported that the first EGF-like domain of FVII is essential for binding TF, as analyzed by the reaction of monoclonal antibodies with amino acid residues 51–88 of the first EGF-like domain of human FVII, which was mapped with fusion protein fragments. Interaction between FVIIa and TF involves direct contact between TF, the first EGF-like domain of FVIIa, and the catalytic domain (19). O’Brien et al. (20) showed that the first EGF-like domain of FVII plays a key role in FVII complex formation with TF, as analyzed by surface plasmon resonance of the interaction between TF and recombinant FVII-R79Q. We reported (7) that the loss of charge associated with the substitution of Arg by Gln at position 79 in FVII Shinjo has a direct effect on the TF-binding site in this part of the first EGF-like domain of FVII. Recently, Banner et al. (21) determined the x-ray crystal structure of the complex of active site-inhibited FVIIa with subtilisin-treated soluble TF and showed that Arg 79 was close to Glu 24 and Glu 56 in the N-terminal domain of TF. The amino acid residues close to Arg 79 of FVIIa in the complex are conserved in human, rabbit, and bovine TFs. Why FVII with the substitution of Arg by Gln at position 79 gives different procoagulant activities using TF from various species is still unknown and will require additional studies. Our ELISA system can be used to check the abnormal FVII molecules that give different procoagulant activities using TF from various sources.

References


Serum Carcinoembryonic Antigen, Cancer Antigen 125, Cancer Antigen 15-3, Squamous Cell Carcinoma, and Tumor-associated Trypsin Inhibitor Concentrations during Healthy Pregnancy, Marie-Hélène Schlageret, Jérôme Larghero, Bruno Cassinat, Marie-Elisabeth Toubert, Caroline Borschneck, and Jean-Didier Rain (Service de Médecine Nucléaire, Hôpital Saint-Louis, 75475 Paris Cedex 10, France; * author for correspondence: fax 33 (0)1 42 49 94 05, e-mail schlageret@chu-stlouis.fr)

In the management of cancer patients, tumor-associated antigens are measured in serum as noninvasive tests for relapse detection [reviewed in Ref. (1)]. The tests have limited specificity because the serum concentrations of the