Factor VII Padua₂: Another Factor VII Abnormality With Defective Ox Brain Thromboplastin Activation and a Complex Hereditary Pattern

By A. Girolami, G. Cattarozzi, R. Dal Bo Zanon, G. Cella, and F. Toffanin

A new factor VII abnormality is presented. The propositus was a 9-yr-old child who presented a mild bleeding tendency characterized by epistaxis and easy bruising. The parents were not consanguineous, but they came from the same area. The laboratory features were mild prolongation of prothrombin time and P.P. test and normal partial thromboplastin and Stypven cephalin clotting times. The thrombotest was moderately prolonged. Factor VII was 40%-50% of normal using rabbit or human brain thromboplastin, but only 13%-24% using ox brain thromboplastin. Factor VII cross-reacting material (CRM) was about 50% of normal. The father, a paternal aunt, and a paternal cousin showed similar clinical and laboratory findings. The brother of the propositus, the mother, and other members of her family showed about 50% factor VII activity and CRM and were considered to be heterozygotes for true factor VII deficiency. Similar findings were also present in the father and in the brother of the affected cousin. The defect in the propositus seems to consist of a double heterozygosity between abnormal factor VII and heterozygous factor VII true deficiency. The factor VII abnormality appears to consist of abnormal reactivity toward ox brain tissue thromboplastins and appears to be different from previously described factor VII abnormalities. The name factor VII Padua₂ is proposed for this condition.

During the past decade, congenital clotting disorders due to molecular or structural abnormalities of clotting factors have become an interesting field of investigation in hematology. Several dysfibrinogenemias, at least five hemophilia B variants, six dysprothrombinemias, and factor X Friuli have been extensively studied and are now accepted clinical entities.¹⁻¹⁰ Congenital factor VII deficiency was first described by Alexander¹¹ in 1951. In recent years several patients belonging to different kindreds have been reported who have presented a discrepancy between factor VII activity and factor VII antigen or cross-reacting material (CRM).¹²⁻¹⁶ Therefore the name dysproconvertinemia was introduced. In one case a different activation pattern was also demonstrated (factor VII Padua).¹⁷ All these studies suggest heterogeneity of the factor VII defects. Our purpose is to report another complex factor VII abnormality.

CASE REPORTS

Patient 1

The propositus was a 9-yr-old boy who was seen for the first time on November 24, 1977. The parents were not consanguineous, but they came from the same area. The family history was positive for...
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or ○ = double heterozygote, symptomatic (male and female);
☑ or ○ = heterozygote for abnormal factor VII, asymptomatic;
☑ or ☐ = heterozygote for factor VII deficiency, usually asymptomatic;
☐ or ☐ = normal; ☑ or ☐ = not studied, normal ?.

Fig. 1. Family pedigree. Besides the propositus, 3 other members on the paternal side, including the father, were affected. There was no consanguinity in the family, but all patients came from the same area of the eastern Venetian region. The mother of the propositus, the maternal grandmother, and an uncle were found to be heterozygotes for true factor VII deficiency. The same was true for the husband of patient 3 who was also born in the same area.

bleeding disorders; some relatives on the paternal side, including the father, were known to have a mild bleeding tendency (Fig. 1). The propositus was referred for further studies because of persistent mild prolongation of the prothrombin time. The bleeding tendency was mild and was characterized by easy bruising and occasional epistaxis. At the age of 3 yr the patient had undergone surgery for adenoidectomy and had been noted to bleed excessively. The patient had never been transfused.

Patient 2

This patient was the 40-yr-old father of the propositus. He also presented a mild bleeding tendency characterized by occasional epistaxis and gingival bleeding. He had never undergone surgery and had never been transfused.

Patient 3

A 42-yr-old paternal aunt of the propositus had presented easy bruising, epistaxis, and bleeding after tooth extractions throughout her life. Epistaxis on two occasions required admission to a local hospital for packing. At the age of 11 yr the patient had undergone surgery for acute appendicitis, and no bleeding had been reported. Menses had always been normal.

Patient 4

This patient was a 15-yr-old cousin of the propositus and daughter of the preceding patient. Her bleeding tendency was characterized by epistaxis, abundant menses, easy bruising, and bleeding after tooth extractions. At the age of 4 yr the patient had undergone tonsillectomy and had been noted to bleed more than usual, but at the age of 7 yr she had undergone surgery for acute appendicitis and had shown no abnormal bleeding. This patient also had never received blood transfusion.

MATERIALS AND METHODS

Materials and methods have been described in detail in previous reports. Only the most pertinent data will be supplied here. Several tissue thromboplastins were used in a factor VII assay system: rabbit brain and lung thromboplastin (Simplastin, lot 4K059, Warner-Lambert, Morris Plains, N.J.); rabbit brain thromboplastin (Ortho brain thromboplastin, lot 12P 645, Ortho Diagnostics,
Raritan, N.J.); simian brain thromboplastin (Ca thromboplastin, lot 126624, Stago-Biochemia Laboratories, Milan, Italy); human brain thromboplastin (British comparative thromboplastin, lot 047, as received from Dr. Poller, Withington Hospital, Manchester, England); human placental thromboplastin (Thromborel, lot 1105C, Behringwerke Laboratories, Marburg, Germany); ox brain thromboplastin was kindly prepared by Nyegaard Laboratories; porcine brain thromboplastin was kindly prepared by Stago Laboratories, Asnières, France. The substrate used was composed of equal parts of lyophilized factor-VII-deficient plasma (Dade Laboratories, Miami, Fla.) and adsorbed normal plasma.17

The K test, trypsin clotting time, was carried out using the reagents supplied by Stago Laboratories: 0.1 ml of adsorbed normal plasma was mixed with 0.1 ml of a 1:5 Michaelis buffer dilution of test or normal plasma; 0.1 ml of the trypsin and calcium solution was then added, and the clotting time was measured.9

Normotest was carried out using the reagent supplied by Nyegaard Laboratories, Oslo, Norway: 0.025 ml of noncontacted citrated whole plasma was added to 0.25 ml of the reagent, and the clotting time was measured.

Thrombotest was carried out using the reagent supplied by Nyegaard Laboratories: 0.05 ml of noncontacted whole plasma was added to 0.25 ml of the reagent, and the clotting time was measured.

The Thrombotest mixing experiment was carried out by adding to 0.25 ml of the reagent 0.05 ml of mixture containing increasing quantities (10%, 20%, 40%, and 80%) of patient plasma with normal plasma, and the clotting time was measured.

The neutralization test was carried out as previously reported.17 The anti-human-factor-VII antiserum used in these studies was kindly supplied by Dr. H. Heimbungen, Behringwerke Laboratories, Marburg, Germany. This antiserum was raised in rabbits and has neutralizing activity. The antiserum failed to yield satisfactory factor VII precipitates in immunodiffusion, electroimmunoassay, and other immunologic systems. However, in the neutralization test the preparation appeared to be specific, since it did not inhibit other clotting factors.

Patients 1 and 3 were retested after daily intramuscular administration of 10 mg of vitamin K, for 5 days.

RESULTS

The results are summarized in Tables 1 and 2. The prothrombin times using human or rabbit brain and rabbit brain and lung thromboplastins were slightly prolonged, whereas partial thromboplastin, Stypven cephalin, and trypsin clotting

<table>
<thead>
<tr>
<th>Test</th>
<th>Patient</th>
<th>Normal Values</th>
</tr>
</thead>
<tbody>
<tr>
<td>Platelet count</td>
<td>200,000/cu mm</td>
<td>150,000–350,000/cu mm</td>
</tr>
<tr>
<td>Bleeding time</td>
<td>3 min</td>
<td>&lt;5 min</td>
</tr>
<tr>
<td>Stypven cephalin clotting time</td>
<td>11 sec</td>
<td>11–12 sec</td>
</tr>
<tr>
<td>T.T.P.</td>
<td>33.1 sec</td>
<td>32–42 sec</td>
</tr>
<tr>
<td>T.P. (Simplastin)</td>
<td>16.9 sec</td>
<td>14 sec</td>
</tr>
<tr>
<td>P.P. test (rabbit brain and lung)</td>
<td>33.2 sec</td>
<td>23–28 sec</td>
</tr>
<tr>
<td>Normotest (rabbit brain)</td>
<td>27.3 sec</td>
<td>20–23 sec</td>
</tr>
<tr>
<td>Thrombotest (ox brain)</td>
<td>66 sec</td>
<td>38–43 sec</td>
</tr>
<tr>
<td>Factor VII</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Rabbit brain</td>
<td>40%</td>
<td>80%–120%</td>
</tr>
<tr>
<td>Rabbit brain and lung</td>
<td>40%</td>
<td>80%–120%</td>
</tr>
<tr>
<td>Simian brain</td>
<td>46%</td>
<td>80%–120%</td>
</tr>
<tr>
<td>Human brain</td>
<td>40%</td>
<td>80%–120%</td>
</tr>
<tr>
<td>Human placenta</td>
<td>42%</td>
<td>80%–120%</td>
</tr>
<tr>
<td>Ox brain</td>
<td>20%</td>
<td>80%–120%</td>
</tr>
<tr>
<td>Porcine brain</td>
<td>25%</td>
<td>80%–120%</td>
</tr>
<tr>
<td>Factors II, V, IX, X</td>
<td>Normal</td>
<td>80%–120%</td>
</tr>
<tr>
<td>Factors VIII, XI, XII</td>
<td>Normal</td>
<td>60%–160%</td>
</tr>
<tr>
<td>Factor XIII</td>
<td>Clot insoluble in 5-M urea</td>
<td>Clot insoluble in 5-M urea</td>
</tr>
</tbody>
</table>

*Similar results were obtained in the other 3 patients.
times were perfectly normal. The P.P. test and Normotest were also slightly prolonged, but the Thrombotest was definitely prolonged. Partial thromboplastin time, prothrombin consumption, thromboplastin generation, and other routine plasma and platelet tests were all within normal limits. The father, a paternal aunt, and one of her two children (II-6, II-3, III-5) presented similar findings.

Fig. 2. Thrombotest mixing experiment in the propositus. No inhibitor was present, since the presence of even 10%–20% normal plasma is able to correct the abnormal clotting time. For comparison, the results obtained in a patient with inhibitor (hemophilia Bα) and in other patients without inhibitors (coumarin treatment, liver disease) are shown.
Factor VII activities as determined by rabbit brain and human brain thromboplastins varied between 40% and 50% of normal in the 4 affected members. Thromboplastins prepared from human placenta and simian brain yielded similar levels. On the contrary, using ox brain thromboplastin, factor VII activities varied between 13% and 24% of normal. Using porcine thromboplastins, almost equally decreased levels were observed in all 4 affected patients.

The brother of the propositus (III-7) and the son of patient 3 (III-4) had approximately 50% factor VII activity, regardless of the thromboplastin used. Three siblings of the father of the propositus showed factor VII levels 55%–70% of normal (II-1, II-2, II-5). The levels were slightly higher or near normal using rabbit brain and lung thromboplastin. All remaining patients had normal factor VII activity.

No inhibitor was present in these 4 patients, since addition of small amounts of normal plasma fully corrected the Thrombotest clotting time (mixing experiments) (Fig. 2). Administration of vitamin K failed to correct the abnormality.

![Graph showing residual factor VII activity in stage 3.](image)

**Fig. 3.** Neutralization tests in the propositus and his father (open triangles), in the mother (dark triangle) who was heterozygous for true factor VII deficiency, and in a paternal aunt (dark square) who was heterozygous for the abnormality. In every instance, with the exception of the mother, there was a clear difference between clotting activity in test plasma and CRM. In the case of the mother there was no significant discrepancy between clotting activity and CRM (true heterozygous deficiency). Only the factor VII activity observed with ox brain thromboplastin was taken into consideration. The other symbols refer to 2 patients with factor VII Padua defect (open circles) and 3 patients with classic true factor VII deficiency (open squares). In the former there was a wide discrepancy between activity and CRM whereas in the latter there was no significant discrepancy.
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By means of the neutralization test it was shown that all 4 patients had approximately 50\% CRM (Fig. 3). The brother (III-7) of the propositus and one of his paternal cousins (III-4) also showed approximately 50\% CRM. All the remaining subjects on the paternal side had approximately 100\% factor VII CRM.

The mother of the propositus (II-7) showed approximately 50\% factor VII activity, regardless of the thromboplastin used, and approximately 50\% CRM. Similar results were found in two other members on the maternal side (grandmother and uncle) and in the husband of patient 3 (I-1, II-8, II-4).

DISCUSSION

The data suggest that these patients have a mild factor VII defect providing that an ox brain thromboplastin is used in the prothrombin time or factor VII assay systems. Since the factor VII CRM is definitely higher than the clotting counterpart, as determined by ox brain thromboplastin, it seems probable that the factor VII is abnormal.

The 4 patients probably have abnormal factor VII that may be poorly activated by ox brain thromboplastin, whereas it may be almost normally activated by rabbit brain or human brain thromboplastins. The presence in the 4 patients of factor VII CRM approximately 50\% of normal also suggests that the patients are heterozygous for true factor VII deficiency. As a consequence, they appear to be double heterozygous for two distinct defects: abnormal factor VII and factor VII heterozygous true deficiency. This interpretation is consistent with the following observations: (1) The defect is not corrected by vitamin K administration, and this rules out an acquired condition. (2) The mother of the propositus and two other members of her family are heterozygotes for true factor VII deficiency. (3) The father of the propositus is himself affected, namely, doubly heterozygous. (4) Three genetically different individuals are present among the siblings on the paternal side (normals, double heterozygotes, and heterozygotes for the abnormal factor VII). The latter patients probably have two populations of factor VII in their plasma (normal and abnormal), whereas the former have only one population of factor VII (either normal or abnormal). (5) The factor VII levels in the brother of the propositus and in one of his paternal cousins vary between 42\% and 46\% of normal, regardless of the thromboplastin used; since these patients have approximately 50\% CRM, they
are probably heterozygotes for true factor VII deficiency. The complex hereditary pattern is diagrammatically depicted in Fig. 4.

The possibility that the results are secondary to serum contaminants contained in the thromboplastins used may be ruled out, since the same thromboplastins yielded similarly depressed levels in severe true congenital deficiency.

Factor IX activity and factor IX antigen were normal in our patients. However, since this factor has recently been shown to be a possible substrate for factor VII and tissue factor, a defect involving this pathway cannot be completely ruled out.

The observation that porcine brain thromboplastin behaves toward this abnormal factor VII like ox brain thromboplastin is of interest. It is known that this thromboplastin is almost as sensitive as ox brain thromboplastin to the hemophilia Bₜₜ inhibitory effect. Furthermore, it is also known that factor VII Padua, which may be fully activated by ox brain thromboplastin, may also be almost completely activated by porcine brain thromboplastin. The similarity between these two thromboplastins is now further confirmed.

The possibility that this abnormal factor VII may act as an inhibitor toward the ox brain thromboplastin in a way similar to an abnormal factor IX (hemophilia Bₜₜ) may be ruled out. The mixing experiment indicated that this new defect does not behave like hemophilia Bₜₜ plasma.

The name factor VII Padua₂ is proposed to define this new abnormality. The factor VII Padua defect consists of an abnormality at the site of interaction with rabbit brain thromboplastin, whereas reactivity to ox brain thromboplastin is normal. The inverse may be true for this new defect.

Our findings suggest heterogeneity of factor VII defects. On the basis of the reported cases (Table 3), a tentative classification of factor VII abnormalities into five groups may be proposed: factor VII, or with normal antigen; factor VII reduced; factor VII, Padua (rabbit brain); factor VII, Verona (rabbit and human brain); factor VII, Padua₂ (ox brain). Indication of the tissue thromboplastin that yields the lowest factor VII level is necessary; otherwise, all these abnormalities could not be properly differentiated.

<p>| Table 3. Tentative Classification of Factor VII Abnormalities on the Basis of Reported Cases* |
|---------------------------------|-----------------|-----------------|</p>
<table>
<thead>
<tr>
<th>Author</th>
<th>Year</th>
<th>Factor VII Activity (%)</th>
<th>Factor VII Antigen or CRM (%)</th>
</tr>
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<tbody>
<tr>
<td>Factor VII, or factor VII reduced</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Goodnight et al.</td>
<td>1971</td>
<td>10</td>
<td>35†</td>
</tr>
<tr>
<td>Denson et al.</td>
<td>1972</td>
<td>15</td>
<td>50</td>
</tr>
<tr>
<td>Briët et al.</td>
<td>1977</td>
<td>&lt;1</td>
<td>20</td>
</tr>
<tr>
<td>Mazzucconi et al.</td>
<td>1977</td>
<td>1</td>
<td>100</td>
</tr>
<tr>
<td>Factor VII, Verona</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Girolami et al.</td>
<td>1977</td>
<td>20‡</td>
<td>55</td>
</tr>
<tr>
<td>Factor VII, Padua</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Girolami et al.</td>
<td>1978</td>
<td>95§</td>
<td>100</td>
</tr>
<tr>
<td>Present defect</td>
<td></td>
<td>13–24</td>
<td>55</td>
</tr>
</tbody>
</table>

*Patients with factor VII antigen or CRM level of less than 15% were excluded; patients with an activity—antigen difference of less than 15% were also excluded.

†Value given as residual activity.
‡Rabbit or human brain thromboplastin. Level was approximately 40% using ox brain thromboplastin.
§Rabbit brain thromboplastin. Level was normal using ox brain thromboplastin.
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ACKNOWLEDGMENT

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REFERENCES


Factor VII Padua 2: another factor VII abnormality with defective ox brain thromboplastin activation and a complex hereditary pattern

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