A New Variant of Dominant Type II von Willebrand’s Disease With Aberrant Multimeric Pattern of Factor VIII-Related Antigen (Type IID)

By Seiji Kinoshita, Janet Harrison, Jack Lazerson, and Charles F. Abildgaard

A new type II variant form of von Willebrand’s disease has been recognized in a mother and daughter who have bleeding manifestations typical of von Willebrand’s disease. Laboratory findings include consistently prolonged bleeding times, with normal levels of factor VIII procoagulant and antigen, but decreased ristocetin cofactor activity. Electrophoresis in SDS 1.5% agarose gel and reaction with 125I-labeled anti-factor VIII-related antigen rabbit IgG, followed by autoradiography, revealed that both plasma and platelets lack the large multimers of factor VIII-related antigen. In 2.5% gel, the propositus plasma lacked the normal “triplet” pattern. In 3.0% gel, a 5-band pattern was observed in normal, type IIA, and type IIB plasma, whereas type IIC plasma revealed a 2-band pattern. The patient’s plasma revealed a 4-band pattern distinctly different from normal or other type II variants. We suggest that this new variant be labeled type IID, until a more appropriate nomenclature is developed.

Factor VIII-related antigen (FVIII:Ag) exists in normal plasma as a series of multimers with molecular weights from 0.9 x 10^6 to 20 x 10^6 daltons, as demonstrated by use of sodium dodecyl sulfate (SDS) agarose electrophoresis.1,2 By use of this technique, patients with von Willebrand’s disease (vWD) have been classified into several types. In the “classic” form, or type I vWD, the multimer pattern is identical to normal plasma, but the total amount of antigen is decreased, ranging from complete absence (homozygote or double heterozygote) to a mild decrease. Type II vWD is characterized as lacking the large multimers, with two subtypes based on differences in reactivity to ristocetin (IIA normal response, IIB increased response), and one subtype, IIC, characterized by an abnormally migrating multimeric pattern in 2.5% agarose gel.3,4

Using similar methods, we have identified a new type II vWD, characterized by a multimeric pattern in 3.0% agarose gel that has a slightly slower mobility of the major bands and three abnormally migrating subbands. The pattern of inheritance of the abnormality appears to be dominant, in that the propositus and her daughter have identical findings.

Materials and Methods

Materials

Seakem agarose HGT(P) (high gelling temperature, ultrapure), and Gel Bond Film were purchased from Marine Colloid Division, FMC Corp., Rockland, ME; SDS from Bio-Rad Laboratories, Richmond, CA; and urea (ultra pure grade) from Mallinckrodt Chemical Works, St. Louis, MO. All other reagents were purchased from Sigma Chemical Co., St. Louis, MO.

Procedures

Blood samples were collected, prepared, and stored as previously described.3 Bleeding times were performed using a modified Ivy method, and factor VIII procoagulant activity (FVIII:C) was measured using a one-stage assay.3 FVIII:Ag was measured by the Laurell technique, and factor VIII ristocetin cofactor (FVIII:RCof) by a macroscopic agglutination technique using formalin-fixed platelets.3 Ristocetin-induced platelet aggregation was measured in platelet-rich plasma using a Chronolog aggregometer.4 SDS-agarose gel electrophoresis was performed according to the method of Ruggeri,2 with minor modifications. Thirteen microliters of a 1:20 dilution of citrated plasma with sample buffer containing 2% SDS, incubated at 56°C for 15 min, was electrophoresed on 1.5%, 2.5%, or 3.0% agarose gel containing 0.1% SDS. Urea (8 M) was added to the sample buffer when using 3.0% agarose gel. In order to prepare 3.0% gels successfully, it was necessary to prewarm all materials. Using the “sandwich set” method, the agarose was applied with a syringe to the Gel Bond Film via small plastic tubing inserted at the base of the set. The gel was cooled and allowed to polymerize at 4°C. The remainder of the procedure followed that previously described for the 1.5% and 2.5% gels. The gel was fixed and reacted with 125I-labeled, affinity-purified, antihuman FVIII:Ag rabbit IgG, produced according to the method of Zimmerman.7 The gel was washed, dried, and autoradiographed at –70°C for 8–48 hr.

Platelet lysate was prepared as follows. A platelet pellet, prepared by centrifugation from platelet-rich plasma, was washed 4 times with 0.05 M Tris-buffered saline (pH 7.35), containing 0.02 M EDTA, and resuspended with the platelet count adjusted to 10^9 cells/ml. The suspension was frozen at –70°C and thawed 5 times. After the final thawing, the suspension was centrifuged, and the supernatant was used for agarose gel electrophoresis.

Results

The propositus, a 23-yr-old female with a lifelong history of easy bruising, epistaxis, and prolonged bleeding from minor injuries, was initially diagnosed

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as having von Willebrand’s disease when she presented with menorrhagia and postpartum hemorrhage. Since her initial diagnosis, she has been treated on two occasions with cryoprecipitate, once for menorrhagia and once during a hysterectomy procedure. The patient’s mother, one sister, and daughter also have easy bruising. Other family members (father and four siblings) deny hemorrhagic symptoms. Hemostatic measurements of the propositus and her daughter are summarized in Table 1.

Plasma and platelets from both individuals were analyzed by SDS-agarose gel electrophoresis, using 1.5%, 2.5%, and 3.0% gels. For comparison, plasma from other types of von Willebrand’s disease were also analyzed on 1.5% and 3.0% gels.

The results obtained using 1.5% gel are shown in Fig. 1. Plasma from a patient with type I von Willebrand’s disease contains all sizes of FVIIIR:Ag multimers, with a generalized decrease in quantity, whereas plasma from a homozygous patient has only trace amounts of the smallest multimer. In contrast, plasma from patients with type II variants (A, B, C) lack the slow-moving large multimers, and a similar pattern is present in plasma from the propositus. The pattern obtained with platelets from the propositus appears similar to that of her plasma and also lacks the large forms, as compared to normal platelets.

On 2.5% gel, the FVIIIR:Ag of normal plasma appears as a repeating series of three bands (see markers), designated as a “triplet” pattern by Ruggeri et al. (Fig. 2). In comparison, the propositus plasma reveals more prominent major bands, lacks the “triplet” pattern, and has an apparent complex subband between the major bands.

On 3.0% gel, normal plasma reveals a repeating series of multimers with one major band and four subbands (Fig. 3, see markers). Type IIA and type IIB plasma also have five bands, with an apparent increase in the density of the outermost subbands. Type IIC plasma has a more prominent major band, with slightly decreased mobility (compared to normal, IIA, and IIB), and has only a single subband, as described by Ruggeri et al. Our patient’s plasma is similar to that of IIC in regard to the major band, but there are three distinct intervening subbands (see markers). This pattern is repeated throughout the gel, and similar findings are present in plasma from the patient’s daughter and in the patient’s platelets.

Examination of plasmas in our laboratory from 30 normal subjects and from 72 patients known to have the characteristics of von Willebrand’s disease, classified as type I or type IIA, failed to reveal any abnormalities similar to the pattern seen in the propositus and her daughter.

**DISCUSSION**

The FVIIIR:Ag multimer of normal plasma has been reported to consist of a triplet pattern in 2.0% and 2.5% agarose gel electrophoresis. In comparison, using 3.0% gel, we observed the multimer pattern of normal plasma to consist of one major band and four subbands. Similar five-band patterns were observed in plasmas from patients with type IIA and type IIB, differing from normal only in the density of the subbands. In contrast, the FVIIIR:Ag multimer pattern in 3.0% gel in our patients consists of a repeating series of one sharply defined major band and three distinct subbands. Type IIB variants have been previously reported to have a triplet pattern in 2.5% gel (similar to normal), with increased ristocetin-induced aggregation and the presence of large FVIIIR:Ag multimer in their platelets. In addition to the different multimer pattern of the propositus and her daughter, both have decreased ristocetin-induced aggregation.

### Table 1. Hemostatic Values

<table>
<thead>
<tr>
<th></th>
<th>Bleeding Symptoms</th>
<th>Bleeding Time</th>
<th>FVIII:C</th>
<th>FVIII:Ag</th>
<th>FVIII:RCof</th>
<th>Ristocetin-Induced Platelet Aggregation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal</td>
<td>—</td>
<td>&lt;9 min</td>
<td>50%~150%</td>
<td>50%~150%</td>
<td>50%~150%</td>
<td>Decreased</td>
</tr>
<tr>
<td>Propositus</td>
<td>Menorrhagia</td>
<td>&gt;20 min</td>
<td>95%</td>
<td>102%</td>
<td>44%</td>
<td>Decreased</td>
</tr>
<tr>
<td></td>
<td>Bruising</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
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<td></td>
<td>Epistaxis</td>
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<tr>
<td>Daughter</td>
<td>bruising</td>
<td>&gt;20 min</td>
<td>62%</td>
<td>56%</td>
<td>28%</td>
<td>Decreased</td>
</tr>
</tbody>
</table>
aggregation, and large multimers are lacking in the platelets of the propositus.

More recently, an autosomal recessive variant (type IIC) has been described in which a "doublet" pattern is seen using 2.5% gel. Although the present cases are similar to this IIC variant, electrophoresis of our patient's plasma on 2.5% gel reveals a more complex intervening band. When analysis is performed in 3.0% agarose gel, this complex band clearly resolves into three distinct subbands of different mobility compared to normal, type I, IIA, IIB, or IIC plasmas. Lastly, the inheritance of type IIC is autosomal recessive compared to an apparent dominant pattern in our family.

Following the approach used by previous investigators, we propose that the present variant form of vWD be labeled type IID, until better nomenclature is developed based on the actual molecular abnormality.

As indicated by the more complex FVIIIIR:Ag multimer patterns obtained in normal, type IIA, and type IIB plasma using carefully prepared 3.0% agarose gels for electrophoresis, further refinements of analytic methods may lead to increased resolution of multimer patterns and, possibly, to recognition of additional von Willebrand's disease variants.

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REFERENCES

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