A New Variant of Type II von Willebrand Disease With Aberrant Multimeric Structure of Plasma but Not Platelet von Willebrand Factor (Type IIF)

By Pier Mannuccio Mannucci, Rossana Lombardi, Augusto B. Federici, Judith A. Dent, Theodore S. Zimmerman, and Zaverio M. Ruggeri

A patient with a lifelong bleeding disorder was diagnosed as having Type II von Willebrand disease. The larger multimers of von Willebrand factor were absent from her plasma but present in platelets. A high-resolution electrophoretic technique was used to study the complex structure of individual von Willebrand factor multimers. In normal plasma, each multimer could be resolved into five bands: a more intense central one and four less intense, two moving faster and two slower than the central band. In normal platelets, each multimer could also be resolved into five bands. The central one had a mobility similar to that in plasma, whereas the four satellite bands had a mobility that differed from that of the corresponding plasma bands. In the patient, platelet von Willebrand factor antigen content and ristocetin cofactor activity were normal, and von Willebrand factor showed the same structure of individual multimers as seen in normal platelets. On the other hand, plasma von Willebrand factor antigen and ristocetin cofactor activity were decreased, and the structure of individual von Willebrand factor multimers was different from that of normal plasma and similar to that seen in normal and patient's platelets. After infusion of 1-deamino-8-D-arginine vasopressin, the largest von Willebrand factor multimers, as well as new satellite bands with a mobility similar to those in normal plasma, appeared in the patient plasma, and the levels of von Willebrand factor antigen and ristocetin cofactor activity became normal. Yet no relevant change in the prolonged bleeding time was observed. This new variant of von Willebrand disease, therefore, is characterized by the presence of a dysfunctional von Willebrand factor molecule that exhibits unique structural abnormalities in plasma but appears to be normal in platelets. The designation of Type IIF is proposed for this type of von Willebrand disease in accordance with the terminology that has been previously used.

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ANALYSIS OF THE MULTIMERIC STRUCTURE of von Willebrand factor (vWF) has proved essential for a more precise characterization of the molecular abnormalities of this glycoprotein in patients with von Willebrand disease. Improvement in the electrophoretic resolution of the structural organization of individual vWF multimers has recently been accompanied by the identification of new variant forms of von Willebrand disease. It is apparent that the survey of large numbers of patients using refined analytical methods will lead to a better characterization of the congenital molecular defects of vWF and will help to explain the significance of the complex multimeric structure of this protein.

In this study we have used an improved high-resolution electrophoretic technique recently developed by some of us, to define the structural vWF abnormality in a new variant form of Type II von Willebrand disease. The patient described here had a lifelong bleeding disorder and lacked the largest vWF multimers in plasma. These were present in platelets, however, and appeared in the circulation after administration of 1-deamino-8-D-arginine vasopressin (DDAVP). Moreover, the complex banding pattern of each multimer was distinctly aberrant in the patient's plasma but normal in her platelets. In keeping with terminology that has been previously used, this new variant form has been designated Type IIF von Willebrand disease.

MATERIALS AND METHODS

Patient. The propositus was a 55-year-old Caucasian female who was referred to the Angelo Bianchi Bonomi Center in Milan, Italy, in March 1983 because of excessive bleeding during hysterectomy for fibromyoma. A lifelong history of easy bruising, epistaxis, and menorrhagia was reported. Two dental extractions had been accompanied by excessive bleeding five years before referral, but appendectomy and cholecystectomy were carried out without bleeding complications at ages 31 and 45, respectively. The mother, a brother, and a sister of the propositus did not report any bleeding tendency. The father died five years before our evaluation and had no history of bleeding. In May 1983, a surgical procedure was deemed necessary to correct a vaginal fistula diagnosed in the propositus. She was hospitalized and treated with DDAVP and cryoprecipitate to correct her bleeding tendency. DDAVP was also given subsequently on several occasions for experimental purposes. Informed consent was obtained from the patient and all normal controls before performing any experimental procedure, according to the Declaration of Helsinki.

Experimental procedures. The bleeding time was measured by performing two 1-cm long × 1-mm deep incisions in the forearm skin, using a template device (Simplate II, General Diagnostics, Morris Plains, NJ). Blood was drawn from an antecubital vein, through a 19-gauge needle, into a polypropylene syringe and immediately transferred into polypropylene tubes containing 1/50th final volume of 0.129 mol/L trisodium citrate. The anticoagulant also contained protease inhibitors, namely, EDTA, N-ethylmaleimide, and leupeptin (Chemicon, El Segundo, Calif) to give final blood concentrations of 5 mmol/L, 6 mmol/L, and 1 mmol/L, respectively. Platelet-rich plasma was prepared by pooling the supernatants from three successive centrifugation steps, each performed...
amounts of ristocetin (Sigma Chemical Co, St Louis) as previously reported. Platelet-poor plasma was prepared by centrifugation at 3,000 g for 20 minutes at 4°C. It was then rapidly separated from the sedimented cells and either used for assay the same day or stored at –70°C until tested. When necessary, frozen plasma was transported in dry ice. Factor VIII procoagulant activity (VIIIc) was assayed by a one-stage clotting technique, vWF antigen by electroimmunoassay, and the ristocetin cofactor activity of vWF, using formalin-fixed platelets. Concentrations of these three activities were expressed in international units (IU) per deciliter, referred to pooled normal plasma calibrated against the First International Reference Preparation for Factor VIII-Related Activities in Plasma (National Institute for Biological Standards and Control, London). The activity of “average” normal plasma corresponds to 100 IU/dL. Platelets were counted in blood collected in EDTA, using phase contrast microscopy.

Electrophoresis of vWF in agarose gels containing sodium dodecyl sulfate (SDS) was performed as previously described, with the following modifications. Short gels were 22 cm wide x 9 cm long x 1.5 mm thick and were cast using either 1.2% high-gelling temperature agarose (Sigma) on Gel Bond film (FMC Corp, Rockland, Me). The gels were allowed to polymerize at 4°C for three hours before the pouring of the stacking gel, 2.5 cm long, consisting of 0.8% high-gelling temperature agarose (FMC). Ten sample wells, each 10 x 2 mm, were cut I cm from the bottom. After electrophoresis, the gels were fixed and reacted overnight with 125I-labeled rabbit antihuman vWF antibody as described. The platelet content of vWF antigen and ristocetin cofactor activity was measured in platelets that had been washed free of plasma constituents and then lysed by a previously published protocol. 

### RESULTS

The relevant clinical and laboratory findings in the propositus and her family are summarized in Table 1. In the patient, low plasma levels of Factor VIIIc, vWF antigen, and ristocetin cofactor activity, as well as the prolonged bleeding time, were consistent with the diagnosis of von Willebrand disease. The mother, sister, and brother had normal results.

<table>
<thead>
<tr>
<th>Family Member</th>
<th>Bleeding History</th>
<th>Bleeding Time (min)</th>
<th>Factor VIIIc (U/dL)</th>
<th>vWF Antigen (U/dL)</th>
<th>Ristocetin Cofactor (IU/dL)</th>
<th>RIPA</th>
</tr>
</thead>
<tbody>
<tr>
<td>Propositus</td>
<td>Positive</td>
<td>&gt;30</td>
<td>45</td>
<td>36</td>
<td>15</td>
<td>Decreased</td>
</tr>
<tr>
<td>Mother</td>
<td>Negative</td>
<td>6</td>
<td>180</td>
<td>200</td>
<td>160</td>
<td>Normal</td>
</tr>
<tr>
<td>Sister</td>
<td>Negative</td>
<td>5</td>
<td>160</td>
<td>126</td>
<td>100</td>
<td>Normal</td>
</tr>
<tr>
<td>Brother</td>
<td>Negative</td>
<td>6</td>
<td>85</td>
<td>73</td>
<td>66</td>
<td>Normal</td>
</tr>
</tbody>
</table>

Normal subjects

<table>
<thead>
<tr>
<th>Mean</th>
<th>4.16</th>
<th>99</th>
<th>96</th>
<th>90</th>
</tr>
</thead>
<tbody>
<tr>
<td>Range</td>
<td>2.31–6.01</td>
<td>57–150</td>
<td>49–145</td>
<td>51–140</td>
</tr>
</tbody>
</table>

RIPA (ristocetin-induced platelet agglutination) was measured by adding varying amounts of ristocetin to platelet-rich plasma and determining the concentration (expressed in mg/mL) that induced aggregation with an initial velocity corresponding to 30% of maximum (see ref 10). The results of VIIIc, vWF antigen, and ristocetin cofactor activity are expressed in international units (IU) per deciliter, where 100 units is the amount present in 1 dL of pooled normal plasma. Results in normal subjects represent either arithmetic mean ± SD (bleeding time, RIPA) or geometric mean with 95% confidence limits (all other measurements).
of plasma, was also seen in the patient's platelet vWF (Fig 2). The complex structure of individual multimers was further resolved by using longer agarose gels of high resolving power. In this system, the three to five smaller multimers appeared to consist of five bands, two migrating above and two below a most intense central band (Fig 3). The technique did not allow resolution of the complex structure of larger multimers, which barely entered this type of gel. In the plasma from more than 100 normal individuals, the mobility of satellite bands was consistently reproducible (data not shown; see also ref 4). When normal and patient plasma were compared side by side on the same gel, it became apparent that the central band of each vWF multimer had the same mobility in both cases, whereas the four satellite bands in the patient sample reproducibly migrated closer to the central band than did the normal counterparts (Fig 3). In this regard, the vWF structure in the patient's plasma was remarkably similar to that seen in platelet vWF. This finding was constantly observed on repeated analysis of two samples of the patient's plasma. On the contrary, the banding patterns of normal and patient platelet vWF were similar (Fig 3). The total protein content and vWF antigen levels were normal in the patient's platelets (0.1 IU of vWF antigen per milligram of platelet protein in the patient v 0.1 1 U/mg in a normal control run at the same time; see also refs 14 and 16 for normal ranges). The ristocetin cofactor activity of the patient platelet vWF was also normal (0.12 IU/mg v 0.11 IU/mg). High-resolution gels demonstrated that the multimeric structure of plasma vWF in the patient's brother and mother was similar to normal (Fig 4). Therefore, the abnormality seen in the patient was not found in the other members of the family studied.

The patient bled abnormally at surgery despite the administration of DDAVP, and satisfactory surgical hemostasis was achieved only after cryoprecipitate was given. No relevant shortening of the bleeding time was observed after
DDAVP, even though vWF antigen and ristocetin cofactor activity were normalized (Table 2). Multimeric analysis of plasma vWF performed on short gels showed that the largest multimers appeared in the circulation immediately after the infusion of DDAVP and persisted for two to four hours (Fig 5). The complex structure of each multimer, however, remained abnormal. On long gels, additional bands previously not clearly detected became evident immediately after DDAVP infusion (Fig 6) and remained detectable for at least eight hours (not shown). Their mobility was similar to that of the satellite bands seen in normal plasma and contrasted with that of the abnormal bands still present in the post-DDAVP samples.

**DISCUSSION**

This patient, who had a lifelong bleeding tendency and a prolonged bleeding time, had low vWF antigen and ristocetin cofactor activity in plasma, contrasting with normal platelet levels of both moieties. In addition, there was a distinctive abnormality of plasma vWF multimeric structure, which became clearly evident using a combination of different agarose gels of varying resolving power.

Because of the absence of larger vWF multimers in plasma, this patient belongs to the category previously designated as Type II von Willebrand disease. Five subtypes have been identified so far within this group (IIA, IIB, IIC, IID, and, recently, IIE), each of them characterized by distinct structural and functional abnormalities of vWF. A discrepancy between the abnormal multimeric structure of vWF in plasma and the normal structure in platelets has been previously reported in two instances: Type IIB, as described by Ruggeri et al, and Type IIA-3, reported by Weiss et al. Type IIB patients are clearly different from this case because of the typical hyper-responsiveness of their platelet-rich plasma to low doses of ristocetin. The opposite finding was observed in this patient, who had a decreased ristocetin-induced platelet agglutination. The cases called IIA-3 by Weiss et al also exhibited lack of larger vWF multimers in plasma, contrasting with a normal set of multimers in platelets. Because Weiss et al used low-resolution gels only and have not further characterized their variant, it cannot be established whether their patients are in some ways similar to the one described here.

The use of long agarose gels of high resolving power has...
TYPE II F von WILLEBRAND’S DISEASE

allowed a more precise definition of the complex banding pattern of individual vWF multimers in normal and patient’s plasma and platelets. Five distinct bands could be resolved in each of the three to five smaller vWF multimers. The structure of the larger multimers could not be appreciated because they barely entered the small-pore long gels. Of the five bands, only the central, most intense one (sometimes resolved into a closely spaced doublet) showed similar mobility in both normal and patient’s plasma vWF. The four satellite bands, on the contrary, had a distinctly altered mobility in the patient’s plasma vWF as compared with normal. It is important to consider that the pattern seen in normal individuals was extremely reproducible, as demonstrated in well over 100 subjects. Therefore, the abnormal mobility of satellite bands seen in the plasma vWF of the patient reflects a unique, distinctive abnormality of this variant molecule. Also of interest is the fact that normal vWF of platelet origin exhibited a mobility of satellite bands different from that of the normal plasma counterparts but similar to that seen in the patient’s plasma vWF. The patient’s platelet vWF, in this regard, was identical to normal platelet vWF. At the present time, we cannot explain why plasma and platelet vWF show different mobility of the satellite bands seen in individual multimers, or why plasma vWF in the patient described here appears to have a structure similar to that seen in platelet vWF.

After infusion of DDAVP, additional satellite bands with a mobility similar to the satellite bands of normal plasma appeared transiently in patient’s plasma, coexisting with the abnormal bands. Hence, vWF with an intact multimeric structure appears to be mobilized from storage sites by an appropriate stimulus such as DDAVP. At the present time, it remains unexplained why the multimeric structure of vWF becomes abnormal after release into the circulation. The abnormality could be detected despite addition to the plasma samples of a mixture of protease inhibitors that has been shown to partially prevent, in some cases, the absence of intermediate and larger vWF multimers in the plasma of patients with Type IIA von Willebrand disease. At the present time, it is not possible to define the correlation, if any, between structural alteration of individual multimers and lack of the largest vWF multimers in plasma, despite their presence in platelets. In any case, our results demonstrate that the vWF molecule present in storage sites and mobilized by DDAVP was impaired in its function after release into the circulation. Larger vWF multimers, as well as satellite bands with a mobility identical to that of bands present in normal plasma, appeared in the patient’s circulation and persisted for several hours after DDAVP infusion, but the bleeding time and the hemorrhagic tendency were not corrected, indicating that a dysfunctional protein was present.

The modality of genetic transmission of this newly described abnormality of vWF remains unclear. The mother and the two siblings of the propositus had a negative bleeding history and normal vWF. The father also had a negative bleeding history but had died before the patient came to our attention, and no data were available as to the structure of his vWF. The propositus had a lifelong bleeding tendency, so that the possibility of an acquired von Willebrand syndrome is remote. She had no descendants, however, to allow further insights into the mode of inheritance of this alteration. Altogether, the most likely possibility is that this new variant is transmitted as a phenotypically recessive trait.

In conclusion, these studies define a new variant form of von Willebrand disease, characterized by a distinctive aberration of vWF multimeric structure and impaired function of the molecule. In keeping with terminology that has been used previously, we propose that this variant be designated Type II F von Willebrand disease.

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REFERENCES


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