von Willebrand Disease "Vicenza" With Larger-Than-Normal (Supranormal) von Willebrand Factor Multimers

By Pier Mannuccio Mannucci, Rossana Lombardi, Giancarlo Castaman, Judith A. Dent, Antonella Lattuada, Francesco Rodeghiero, and Theodore S. Zimmerman

When normal volunteers or patients with type I von Willebrand disease (VWD) are given desmopressin (DDAVP), a set of larger-than-normal (supranormal) von Willebrand factor (VWF) multimers, similar to those present in VWF-containing cells such as platelets megakaryocytes and endothelial cells, appear transiently in postinfusion plasma. In two kindreds with mild lifelong bleeding symptoms transmitted as an autosomal dominant trait, all ten symptomatic members (but none of the five asymptomatic members) had a supranormal multimeric structure for plasma VWF, apparently identical to that seen for postdesmopressin normal plasma. Plasma factor VIII coagulant activity (VIII:C), VWF antigen (VWF:Ag), ristocetin-induced platelet agglutination, and ristocetin cofactor (RiCof) activity were low. Platelet VWF:Ag and RiCof levels (tested for three patients only) were normal. Bleeding times were normal or slightly prolonged. The patients' platelet multimeric structure was the same as that for normal platelets. After desmopressin infusion the plasma VWF multimeric structure remained supranormal as for preinfusion plasma, with VIII:C VWF:Ag and RiCof increasing markedly over baseline values and disappearing at a normal rate. Examination of the VWF subunit composition from three of these patients indicated that proteolytic processing of their VWF did not differ from normal. This study describes the first variant of VWD with a supranormal multimeric structure.

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Till T12

Not tested. positive history

Asymptomatic members of the two families are in italics.

Fig 1. Pedigrees of kindreds G. and T.

Table 1. Bleeding Times, Plasma Factor VIII and von Willebrand Levels, and Multimeric Structure in Kindreds G. and T.

<table>
<thead>
<tr>
<th>Kindred, Generation, and Patient</th>
<th>Bleeding Time (min)</th>
<th>RIPA (mg/mL)</th>
<th>Platelet Count</th>
<th>VWF:Ag (U/dL)</th>
<th>RiCof (U/dL)</th>
<th>VIII:C (U/dL)</th>
<th>Multimeric Structure</th>
<th>Bleeding Symptoms</th>
</tr>
</thead>
<tbody>
<tr>
<td>G.IV 1</td>
<td>9</td>
<td>&gt;2.0</td>
<td>267,000</td>
<td>9</td>
<td>7</td>
<td>14</td>
<td>Supranormal</td>
<td>PO bleeding</td>
</tr>
<tr>
<td>G.IV 2†</td>
<td>4</td>
<td>0.7</td>
<td>236,000</td>
<td>84</td>
<td>85</td>
<td>120</td>
<td>Normal</td>
<td>None</td>
</tr>
<tr>
<td>G.IV 3</td>
<td>6</td>
<td>&gt;2.0</td>
<td>216,000</td>
<td>16</td>
<td>10</td>
<td>14</td>
<td>Supranormal</td>
<td>PO bleeding</td>
</tr>
<tr>
<td>G.IV 4</td>
<td>12</td>
<td>1.5</td>
<td>174,000</td>
<td>12</td>
<td>9</td>
<td>31</td>
<td>Supranormal</td>
<td>PO bleeding</td>
</tr>
<tr>
<td>G.IV 5</td>
<td>5</td>
<td>1.8</td>
<td>258,000</td>
<td>8</td>
<td>8</td>
<td>12</td>
<td>Supranormal</td>
<td>PO bleeding and epistaxis</td>
</tr>
<tr>
<td>G.V 1</td>
<td>5</td>
<td>1.2</td>
<td>202,000</td>
<td>81</td>
<td>70</td>
<td>80</td>
<td>Normal</td>
<td>None</td>
</tr>
<tr>
<td>G.V 2</td>
<td>6</td>
<td>1.2</td>
<td>199,000</td>
<td>58</td>
<td>60</td>
<td>78</td>
<td>Normal</td>
<td>None</td>
</tr>
<tr>
<td>G.V 3</td>
<td>5</td>
<td>1.3</td>
<td>244,000</td>
<td>10</td>
<td>10</td>
<td>14</td>
<td>Supranormal</td>
<td>Epistaxis</td>
</tr>
<tr>
<td>G.V 4</td>
<td>7</td>
<td>0.9</td>
<td>359,000</td>
<td>150</td>
<td>82</td>
<td>128</td>
<td>Normal</td>
<td>None</td>
</tr>
<tr>
<td>G.V 5</td>
<td>7</td>
<td>1.4</td>
<td>207,000</td>
<td>6</td>
<td>6</td>
<td>11</td>
<td>Supranormal</td>
<td>PO bleeding</td>
</tr>
<tr>
<td>G.V 6</td>
<td>8</td>
<td>1.8</td>
<td>213,000</td>
<td>8</td>
<td>8</td>
<td>16</td>
<td>Supranormal</td>
<td>Epistaxis</td>
</tr>
<tr>
<td>T.II 1</td>
<td>14</td>
<td>&gt;2.0</td>
<td>300,000</td>
<td>10</td>
<td>7</td>
<td>9</td>
<td>Supranormal</td>
<td>Menorrhagia</td>
</tr>
<tr>
<td>T.II 2</td>
<td>11</td>
<td>1.5</td>
<td>208,000</td>
<td>12</td>
<td>13</td>
<td>22</td>
<td>Supranormal</td>
<td>Postpartum bleeding</td>
</tr>
<tr>
<td>T.III 1</td>
<td>ND</td>
<td>ND</td>
<td>268,000</td>
<td>8</td>
<td>6</td>
<td>9</td>
<td>Supranormal</td>
<td>PO bleeding</td>
</tr>
<tr>
<td>T.III 2</td>
<td>ND</td>
<td>ND</td>
<td>250,000</td>
<td>98</td>
<td>60</td>
<td>70</td>
<td>Normal</td>
<td>None</td>
</tr>
</tbody>
</table>

Normal range 3-7 0.7-1.2 150,000-400,000 52-160 53-135 53-162

Abbreviations: ND, assay not done; PO, postoperative.

*Supranormal multimeric structure denotes the presence of larger-than-normal high–molecular weight multimers.
†Asymptomatic members of the two families are in italics.
A

B

C

Fig 2. Autoradiograph pattern of plasma VWF for kindred G. in 0.8% low-gelling temperature agarose (low-resolution gel resolving each smaller multimer in one band only). VWF is detected by $^{125}$I-labeled, affinity-purified antibody. The origin of the running gel is at the top (indicated by an arrow), and the anode is at the bottom. The intensity of VWF multimers does not correspond closely to the VWF:Ag concentration in plasma because different plasmas were variously diluted and autoradiographs variously exposed in the attempt to achieve similar intensities of staining. (A) From left to right: normal plasma, plasma from patients G.V 6, G.IV 4, G.V 5, and normal plasma collected immediately after desmopressin infusion. The parentheses indicate the larger-than-normal high-molecular weight multimers present in the affected members of the kindred. (B) From left to right: normal plasma, normal plasma after desmopressin, and plasma from patients G.V 3 and G.IV 3. (C) From left to right: plasma from patient G.IV 5, normal plasma, normal plasma after desmopressin, and plasma from unaffected kindred members G.IV 2 and G.V 1.

analyzed on SDS–agarose gel electrophoresis with a low-resolution system that permits partial resolution of high-molecular weight multimers, a set of larger-than-normal multimers were seen (regardless of whether or not the plasma was collected with protease inhibitors) (Figs 2 and 3) but not for plasma from five unaffected members of both kindreds (Fig 2, panel C). The patients' platelet multimeric structure was the same as that for normal platelets and for platelets from unaffected kindred members (Fig 4). In high-resolution gels that resolved each smaller multimer into five discrete bands but did not resolve high–molecular weight multimers, the patients' plasma multimeric structure was the same as that for normal plasma (Fig 5).

Effects of desmopressin on plasma VWF levels and multimeric structure. For the six patients studied, VWF:Ag and RiCof (and VIII:C) increased markedly over baseline values and reached normal values 30 to 60 minutes after the infusion started. After desmopressin the mean peak levels of VIII/VWF measurements were not significantly different from those for seven congenital type I vWD patients taken as a control group (Table 2). Similarly, the mean half-disappearance times of VIII/VWF were not significantly different in the two groups (Table 2). The

Fig 3. Autoradiograph pattern of plasma VWF for kindred T. From left to right: normal plasma after desmopressin, plasma from patients T.II 1 and T.II 2, and normal plasma. For more details, see Fig 2.
to 5.5%). The 140-kd fragment was between 1.5% and 1.8% (normal, 2.0% to 7.4%). Hence, the relative concentration of the 189- and 176-kd fragments was marginally greater than that in normal individuals, and the intact 225-kd subunit and the 140-kd fragment were marginally less.

DISCUSSION

The symptomatic members of the two kindreds had congenital VWD, as indicated by low plasma VWF levels in all

bleeding time shortened from 14 to nine minutes and from ten to six minutes in the only two patients (T.II 1 and T.II 2) in whom it was prolonged before desmopressin administration. The multimeric structure remained identical to that for patients' resting plasma and to that for postdesmopressin normal plasma (Fig 6). There was no postdesmopressin change in platelet count (not shown).

Subunit composition of plasma VWF. Examination of the subunit composition of plasma VWF from three of the affected family members demonstrated the presence of the same 189-, 176-, and 140-kd fragments as are present in normal plasma. No new fragments were demonstrated, nor was there any evidence of uncleaved pro-VWF (not shown). The intact 225-kd subunit composed between 68.2% and 69.8% of the total VWF in the patients, whereas it constituted between 70.3% and 84.5% in 25 normal individuals. The 189-kd fragment was between 7.2% and 9.4% (normal, 2.1% to 5.5%). The 176-kd fragment was between 20.6% and 21.4% (normal, 8.2% to 19.7%). The 140-kd fragment was between 1.5% and 1.8% (normal, 2.0% to 7.4%). Hence, the relative concentration of the 189- and 176-kd fragments was marginally greater than that in normal individuals, and the intact 225-kd subunit and the 140-kd fragment were marginally less.

Table 2. Quantitative Changes and Half-Disappearance Times (t 1/2) of VIII/VWF (Measured as VIII:C, VWF:Ag, and RCoF) After Desmopressin Infusion in Patients With VWD Vicenza Compared With Patients With Type I VWD (platelet normal)

<table>
<thead>
<tr>
<th></th>
<th>VIII:C</th>
<th>VWF:Ag</th>
<th>RCoF</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Peak Level</td>
<td>t 1/2</td>
<td>Peak Level</td>
</tr>
<tr>
<td>VWD Vicenza (n = 6)</td>
<td>10.2 ± 4.9</td>
<td>76 ± 8</td>
<td>8.3 ± 3.0</td>
</tr>
<tr>
<td>Type I VWD (n = 7)</td>
<td>10.3 ± 2.3</td>
<td>68 ± 13</td>
<td>9.0 ± 2.3</td>
</tr>
<tr>
<td>P value</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
</tr>
</tbody>
</table>

*Expressed as ratios of peak postdesmopressin levels to baseline levels (mean ± SD).
†Expressed in minutes (mean ± SD).
nir or thrombotic complications were observed in our patients.

The mechanism responsible for the concomitance of supranormal VWF multimers in plasma and low concentrations of total plasma VWF in these families is uncertain at present. Enhanced plasma clearance is unlikely to explain the low plasma concentration because the mean half-disappearance time of VWF (and VIII:C) after desmopressin infusion was not significantly different from that for type I VWD patients taken as a control group. A decreased rate of synthesis or secretion from endothelial cells is not excluded by the currently available data, however.

It has recently been reported that a loss of large multimers in types II A and II B VWD is associated with increased proteolysis. Because other variants of VWD show reduced proteolysis of their plasma VWF, it seemed reasonable to postulate that a reduced rate of proteolysis might account for the presence of supranormal multimers in plasma of the patients reported here. However examination of the subunit structure of plasma VWF for three of these patients provided no evidence of reduced proteolytic fragmentation. The relative concentration of 189-kd and 176-kd VWF fragments was indeed marginally greater than that in normals, but the difference was not statistically significant, probably because of the small number of cases.

Several lines of evidence indicate that large multimers are most important for the role of VWF in supporting primary hemostasis. Therefore, it is puzzling to find that supranormal multimers are associated with a mild but definite hemorrhagic tendency when one considers also that the bleeding time, an important determinant of the bleeding tendency in vWD, was normal or borderline. The mild hemorrhagic tendency in our patients is probably explained by the severe quantitative defects of plasma VIII:C, another important determinant of the bleeding tendency of VWD.

According to the classification very recently proposed by Ruggeri, this new variant of VWD should be classified among quantitative defects with no functional abnormality of VWF. This allocation, however, is tentative because we have not carried out experiments to rule out the possibility that the supranormal multimers made by these patients are functionally abnormal and do not associate with platelets or subendothelial structures as do larger VWF forms synthesized and secreted by the endothelial cells of normal individuals.

REFERENCES


von Willebrand disease "Vicenza" with larger-than-normal (supranormal) von Willebrand factor multimers

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