THROMBOSIS AND HEMOSTASIS

VWF propeptide and ratios between VWF, VWF propeptide, and FVIII in the characterization of type 1 von Willebrand disease

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Key Points

• VWFpp/VWF:Ag and FVIII:C/VWF:Ag ratios define the pathophysiological mechanisms that play a role in VWD and various VWF mutations.
• A high VWFpp/VWF:Ag ratio indicates increased clearance of VWF and a high FVIII:C/VWF:Ag ratio decreased synthesis of VWF.

Introduction

von Willebrand disease (VWD) is a bleeding disorder caused by inherited quantitative (types 1 and 3) or qualitative (type 2) defects of von Willebrand factor (VWF). VWF supports platelet adhesion and carries factor VIII (FVIII). After cleavage of the signal peptide, the pro-VWF dimerizes in the endoplasmic reticulum (ER) via disulfide bridges at the carboxy-terminal cysteine knot (CK). In the Golgi, pro-VWF dimers multimerize via disulfide bridges between D’D3 domains. Subsequently, the VWF propeptide (VWFpp) is cleaved but stays noncovalently attached to VWF, only to become dissociated after release into the circulation. VWF and VWFpp are secreted in equimolar amounts.

Because of the different half-lives of VWFpp (2 hours) and VWF (8-12 hours), the ratio between VWFpp and VWF antigen (VWF:Ag) in plasma can be used to assess synthesis, secretion, and clearance rates of VWF. We have reported an increased VWFpp/VWF:Ag ratio in 19 individuals of the Molecular and Clinical Markers for the Diagnosis and Management of Type 1 VWD (MCMDM-1VWD) study in whom the response to desmopressin showed a decreased VWF half-life. Some other type 1 VWD patients with increased clearance of VWF could be identified by an increased VWFpp/VWF:Ag ratio. 3,10

During posttranslational modifications of von Willebrand factor (VWF), the VWF propeptide (VWFpp) is cleaved. The ratio between VWFpp and VWF antigen (VWF:Ag) and the ratio between factor VIII (FVIII:C) and VWF:Ag may be used to assess synthesis and clearance of VWF. We analyzed the contribution of VWFpp and ratios of VWFpp/VWF:Ag and FVIII:C/VWF:Ag in the pathophysiological characterization of type 1 von Willebrand disease (VWD) in the Molecular and Clinical Markers for the Diagnosis and Management of Type 1 VWD (MCMDM-1VWD) study. The VWFpp/VWF:Ag and FVIII:C/VWF:Ag ratios were increased among patients compared with unaffected family members and healthy controls. The VWFpp/VWF:Ag ratio was higher in individuals heterozygous for missense mutations than in those heterozygous for null alleles. In contrast, the FVIII:C/VWF:Ag ratio was highest among heterozygotes for VWF null alleles. The ratios of VWFpp/VWF:Ag and FVIII:C/VWF:Ag indicate that the pathophysiological mechanisms of type 1 VWD include reduced production and accelerated clearance of VWF, but that often a combination of both mechanisms is implicated. (Blood. 2013;121(12):2336-2339)

Methods

In the European MCMDM-1VWD study, 744 individuals, including index cases (ICs), affected family members (AFMs), and unaffected family
members (UFMs), from 154 families previously diagnosed with type 1 VWD as well as healthy controls (HCs) were recruited. Local ethical review committees approved the study. Written informed consent was obtained in accordance with the Declaration of Helsinki. The evaluation of phenotype and genotype was reported previously. VWF:Ag, VWF ristocetin cofactor activity (VWF:RCo), FVIII:C, VWF multimers, ABO blood group genotypes, and VWF:RCo in blood group O and no suggestion of preferential group O compared with non-O (supplemental Table 2). Blood clearance or proteolysis of large multimers in HCs with blood group O. In HCs, the VWFpp levels were not influenced by blood group, whereas VWFpp/VWF:Ag was clearly increased for blood group O in line with more rapid clearance of mature VWF.

VWFpp and FVIII are cleared as a complex.13 No difference was observed for FVIII:C/VWF:Ag, supporting the view that VWF and FVIII levels via VWF synthesis, was seen in carriers of null alleles (Table 1, Figure 1). In the MCMDM-1VWD cohort, subtle multimer abnormalities were reported in 38% of the ICs, and these individuals had more severe phenotypes, higher penetrance, a greater extent of linkage to the VWF gene locus, and mutations identified in all cases.14,19,21 The increased VWFpp/VWF:Ag ratio was particularly raised (median 4.3) in patients with abnormal multimers and mutations (Table 1). An increased VWFpp/VWF:Ag ratio was a good predictor of VWD patients with mutations in the VWF gene (Figure 1A): a VWFpp/VWF:Ag >3 had a positive predictive value for the presence of a VWF mutation of 98% with a specificity of 99% in the entire cohort of patients and family members. The VWF:RCo/VWF:Ag ratio was decreased in the group with abnormal multimers (median 0.6, P < .001); however, the large coefficient of variation for VWF:RCo assays reduces the significance of this ratio when VWF levels are low. Among the UFMs, there were 31 individuals classified as unaffected but with a VWF mutation identified (Table 1). Those nonpenetrant cases had slightly higher VWFpp/VWF:Ag and FVIII:C/VWF:Ag ratios than UFMs without a mutation (P values .013 and .008 respectively, Table 1). Patients with mutations in families showing complete cosegregation between VWF gene and disease phenotype had higher VWFpp/VWF:Ag and FVIII:C/VWF:Ag ratios than patients from families with incomplete cosegregation (Table 1).

VWFpp in heterozygotes for VWF null mutations was approximately half of that in HCs, reflecting reduced synthesis (Table 1). A high FVIII:C/VWF:Ag ratio (median 2.4), identifying reduced VWF synthesis, was seen in carriers of null alleles (Table 1, Figure 1B). The FVIII:C/VWF:Ag ratio was above the upper limit of the normal range (1.9) in 80% of these heterozygotes. Missense mutations demonstrated the highest VWFpp/VWF:Ag ratio, reflecting faster clearance of abnormal mutant VWF, although not all missense

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**Table 1. Stratification by multimer pattern and mutation status, linkage, and type of mutation**

<table>
<thead>
<tr>
<th>Multimer pattern†</th>
<th>Mutation identified</th>
<th>n</th>
<th>VWFpp,U/dL</th>
<th>VWFpp/VWF:Ag</th>
<th>FVIII:C/VWF:Ag</th>
<th>VWF:RCo/VWF:Ag</th>
</tr>
</thead>
<tbody>
<tr>
<td>IC + AFM Normal</td>
<td>No</td>
<td>113</td>
<td>92 (81-108)</td>
<td>1.6 (1.3-1.9)</td>
<td>1.4 (1.1-1.7)</td>
<td>1.1 (0.9-1.2)</td>
</tr>
<tr>
<td></td>
<td>Yes</td>
<td>134</td>
<td>81 (64-101)</td>
<td>2.0 (1.5-2.4)</td>
<td>1.7 (1.2-2.3)*</td>
<td>1.0 (0.9-1.2)</td>
</tr>
<tr>
<td>Abnormal</td>
<td>No</td>
<td>150</td>
<td>79 (65-100)</td>
<td>4.3 (2.9-7.0)</td>
<td>1.8 (1.2-2.5)</td>
<td>0.6 (0.3-0.9)†</td>
</tr>
<tr>
<td></td>
<td>Yes</td>
<td>255</td>
<td>111 (98-128)</td>
<td>1.2 (1.0-1.5)</td>
<td>1.2 (0.9-1.4)</td>
<td>1.0 (0.8-1.2)</td>
</tr>
<tr>
<td>UFM</td>
<td>No</td>
<td>31</td>
<td>115 (97-132)</td>
<td>1.4 (1.1-1.8)</td>
<td>1.3 (1.1-1.7)</td>
<td>0.9 (0.8-1.2)</td>
</tr>
<tr>
<td></td>
<td>Yes</td>
<td>228</td>
<td>89 (73-109)</td>
<td>2.8 (1.8-5.3)</td>
<td>1.6 (1.1-2.1)†</td>
<td>0.8 (0.5-1.1)†</td>
</tr>
</tbody>
</table>

**Type of mutation**

| Missense mutations | 228 | 89 (73-109) | 2.8 (1.8-5.3) | 1.6 (1.1-2.1)† | 0.8 (0.5-1.1)† |
| "Null" mutations# | 20  | 72 (63-85)  | 2.0 (1.7-2.4) | 2.4 (2.0-2.8)  | 1.1 (1.0-1.3)  |
|                  | 387 | 118 (104-136)| 1.2 (1.0-1.5)| 1.1 (1.0-1.3)*| 1.1 (0.9-1.3)  |

**P values**

| missense vs null | 0.001 | 0.007 | <0.001 | 0.001 |

Results are indicated as median (25th to 75th percentile).

*P < 133.

†n = 149.

#Comprise premature stop codons caused by nonsense mutations, frame shifts (small deletions and insertions), and out-of-frame splice site mutations.

‡Linkage was defined as complete cosegregation (pedigrees with no phenocopies and fully penetrant) or incomplete cosegregation (pedigrees with either phenocopies or nonpenetration).14

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**Results and discussion**

It has been described in a selected subset of VWD patients that the VWFpp/VWF:Ag ratio can identify VWF with decreased half-life. We now report on VWFpp/VWF:Ag and FVIII:C/VWF:Ag in the entire MCMDM-1VWD study (supplemental Table 1, available on the Blood website). VWFpp was lower in ICs and AFMs than in HCs (P < .001), although less marked than the VWF:Ag reduction. VWFpp/VWF:Ag was increased for ICs and AFMs compared with HCs and this was also observed for FVIII:C/VWF:Ag (P < .001).

ABO blood group determines VWF and FVIII levels via VWF synthesis, was seen in carriers of null alleles (Table 1, Figure 1). In the MCMDM-1VWD cohort, subtle multimer abnormalities were reported in 38% of the ICs, and these individuals had more severe phenotypes, higher penetrance, a greater extent of linkage to the VWF gene locus, and mutations identified in all cases.14,19,21 The increased VWFpp/VWF:Ag ratio was particularly raised (median 4.3) in patients with abnormal multimers and mutations (Table 1). An increased VWFpp/VWF:Ag ratio was a good predictor of VWD patients with mutations in the VWF gene (Figure 1A): a VWFpp/VWF:Ag >3 had a positive predictive value for the presence of a VWF mutation of 98% with a specificity of 99% in the entire cohort of patients and family members. The VWF:RCo/VWF:Ag ratio was decreased in the group with abnormal multimers (median 0.6, P < .001); however, the large coefficient of variation for VWF:RCo assays reduces the significance of this ratio when VWF levels are low. Among the UFMs, there were 31 individuals classified as unaffected but with a VWF mutation identified (Table 1). Those nonpenetrant cases had slightly higher VWFpp/VWF:Ag and FVIII:C/VWF:Ag ratios than UFMs without a mutation (P values .013 and .008 respectively, Table 1). Patients with mutations in families showing complete cosegregation between VWF gene and disease phenotype had higher VWFpp/VWF:Ag and FVIII:C/VWF:Ag ratios than patients from families with incomplete cosegregation (Table 1).
mutations lead to increased clearance (Table 1, Figure 1B). In heterozygotes for VWF missense mutations, there was an intermediate reduction in VWFpp level and increase in FVIII:C/VWF:Ag ratio (median 1.6) indicating that missense mutations partly also cause reduced synthesis and/or secretion. Thus, missense mutations can cause a combined defect of reduced synthesis and increased clearance (Figure 1B). The extent of increased clearance was not the same for all missense mutations, and the highest VWFpp/VWF:Ag ratios clustered in the VWF D3 and A1 domains (Figure 1C). Overall, the ratios in most patients with no mutation fell within the normal range (Figure 1A-B) and no clear mechanism could be deduced, but in some of those patients the defect was mainly of reduced synthesis whereas 1 patient had primarily increased clearance (Figure 1B). For a group of other mutations, including putative splice site mutations and changes in the 5′ untranslated region where the pathogenic mechanism had not been fully characterized at the molecular level, a defect in synthesis could be deduced in the majority (Figure 1B).

In conclusion, the VWFpp/VWF:Ag and FVIII:C/VWF:Ag ratios define the pathophysiological mechanisms that play a role in VWD and various types of VWF mutations. Clinical implications of these findings range from identifying increased clearance of VWF, which is important when considering desmopressin treatment, to discrimination between low VWF levels due to blood group O versus VWF null alleles.

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Authorship

Contribution: J.E. designed the study, collected data, performed laboratory analyses, analyzed and interpreted results, and reviewed and approved the final manuscript; A.B.F., R.J.D., U.B., R.S., J.B., M.T.C., and J.G. designed the study, collected data, performed laboratory analyses, and reviewed and approved the final manuscript; G.C. designed the study, collected data, performed laboratory analyses, revised manuscript drafts, and reviewed and approved the final manuscript; F.R. initiated, coordinated, and designed the study, collected data, performed laboratory analyses, revised manuscript drafts, and reviewed and approved the final manuscript; I.P. initiated, coordinated, and designed the study, collected data, performed laboratory analyses, reviewed and interpreted results, revised manuscript drafts, and reviewed and approved the final manuscript; A.G. initiated, coordinated, and designed the study, collected data, performed laboratory analyses, analyzed and interpreted results, revised manuscript drafts, and reviewed and approved the final manuscript.

Conflict-of-interest disclosure: The authors declare no competing financial interests.

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A list of the the MCMDM-1VWD Study Group members appears in the “Appendix.”

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Appendix: study group members

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References

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