Dominant von Willebrand Disease Type 2M and 2U Are Variable Expressions of One Distinct Disease Entity Caused by Loss-of-Function Mutations in the A1 Domain of the von Willebrand F...

Article in Acta Haematologica - February 2009
DOI: 10.1159/000214855
Source: PubMed

5 authors, including:

Alain P Gadisseur
Universitair Ziekenhuis Antwerpen
74 PUBLICATIONS 1,544 CITATIONS

Jan Jacques Michiels
University of Rochester Medical Center
307 PUBLICATIONS 7,395 CITATIONS

Some of the authors of this publication are also working on these related projects:

Project Von Willebrand Disease View project
Project Immuno-Oncology View project
Advances in the Diagnosis and Classification of von Willebrand Disease

Guest Editors
Alain Gadisseur, Edegem
Jan Jacques Michiels, Rotterdam

60 figures, 3 in color, and 40 tables, 2009
Contents

71 Laboratory Diagnosis and Molecular Classification of von Willebrand Disease  
Gadisseur, A. (Edegem); Hermans, C. (Brussels); Berneman, Z.; Schroyens, W. (Edegem);  
Deckmyn, H. (Kortrijk); Michiels, J.J. (Edegem/Rotterdam)

85 Laboratory Diagnosis and Molecular Basis of Mild von Willebrand Disease Type 1  
Michiels, J.J. (Edegem/Rotterdam); Berneman, Z.; Gadisseur, A.; van der Planken, M.;  
Schroyens, W. (Edegem); van Vliet, H.H.D.M. (Rotterdam)

98 C1584: Effect on von Willebrand Factor Proteolysis and von Willebrand Factor Antigen Levels  
Davies, J.A.; Collins, P.W.; Hathaway, L.S.; Bowen, D.J. (Cardiff)

102 Factor VIII-von Willebrand Factor Binding Defects in Autosomal Recessive von Willebrand Disease Type Normandy and in Mild Hemophilia A.  
New Insights into Factor VIII-von Willebrand Factor Interactions  
Jacquemin, M. (Leuven)

106 Autosomal Recessive von Willebrand Disease Type 1 or 2 due to Homozygous or Compound Heterozygous Mutations in the von Willebrand Factor Gene. A Single Center Experience on Molecular Heterogeneity and Laboratory Features in 12 Families  
Castaman, G.; Giacomelli, S.; Rodeghiero, F. (Vicenza)

111 Laboratory and Molecular Characteristics of Recessive von Willebrand Disease Type 2C (2A Subtype IIC) of Variable Severity due to Homozygous or Double Heterozygous Mutations in the D1 and D2 Domains  
Michiels, J.J. (Edegem/Rotterdam); Gadisseur, A.; van der Planken, M.; Schroyens, W.;  
Berneman, Z. (Edegem)

119 Recessive von Willebrand Disease Type 2 Normandy: Variable Expression of Mild Hemophilia and VWD Type 1  
Michiels, J.J. (Edegem/Rotterdam); Gadisseur, A.; Vangenegten, I.; Schroyens, W.;  
Berneman, Z. (Edegem)

128 Laboratory Diagnosis of von Willebrand Disease Type 1/2E (2A Subtype IIIE), Type 1 Vicenza and Mild Type 1 caused by Mutations in the D3, D4, B1–B3 and C1–C2 Domains of the von Willebrand Factor Gene. Role of von Willebrand Factor Multimers and the von Willebrand Factor Propeptide/Antigen Ratio  
Gadisseur, A.; Berneman, Z.; Schroyens, W. (Edegem); Michiels, J.J. (Edegem/Rotterdam)
139 Autosomal Dominant von Willebrand Disease Type 2M
Hermans, C. (Brussels); Batlle, J. (Santiago de Compostela/La Coruña)

145 Dominant von Willebrand Disease Type 2M and 2U Are Variable Expressions of One Distinct Disease Entity Caused by Loss-of-Function Mutations in the A1 Domain of the von Willebrand Factor Gene
Gadisseur, A.; van der Planken, M.; Schroyens, W.; Berneman, Z. (Edegem); Michiels, J.J. (Edegem/Rotterdam)

154 Dominant von Willebrand Disease Type 2A Groups I and II due to Missense Mutations in the A2 Domain of the von Willebrand Factor Gene: Diagnosis and Management
Michiels, J.J. (Edegem/Rotterdam); van Vliet, H.H.D.M. (Rotterdam)

167 Managing Patients with von Willebrand Disease Type 1, 2 and 3 with Desmopressin and von Willebrand Factor-Factor VIII Concentrate in Surgical Settings
Michiels, J.J. (Edegem/Rotterdam); van Vliet, H.H.D.M. (Rotterdam); Berneman, Z.; Schroyens, W.; Gadisseur, A. (Edegem)

177 Causes, Etiology and Diagnosis of Acquired von Willebrand Disease: A Prospective Diagnostic Workup to Establish the Most Effective Therapeutic Strategies
Sucker, C. (Berlin); Michiels, J.J. (Edegem/Rotterdam); Zotz, R.B. (Düsseldorf)

183 ADAMTS13 in Health and Disease
Feys, H.B.; Deckmyn, H.; Vanhoorelbeke, K. (Kortrijk)

186 Author Index Vol. 121, No. 2–3, 2009
186 Subject Index Vol. 121, No. 2–3, 2009
Laboratory Diagnosis and Molecular Classification of von Willebrand Disease

Alain Gadisseur a, Cedric Hermans b, Zwi Berneman a, Wilfried Schroyens a, Hans Deckmyn c, Jan Jacques Michiels a, d

a Hemostasis and Thrombosis Research Center, Antwerp University Hospital, Edegem; b Hemostasis and Thrombosis Center, Hemophilia Clinic, St. Luc University Hospital, Brussels, and c Laboratory for Thrombosis Research, Interdisciplinary Research Center, Catholic University of Leuven, Campus Kortrijk, Kortrijk, Belgium; d Hemostasis Thrombosis Science Center, Goodheart Institute, Rotterdam, The Netherlands

Key Words
Classification of von Willebrand disease • Dominant disease • International Society on Thrombosis and Haemostasis • Laboratory parameters • Molecular classification • Recessive disease • von Willebrand factor

Abstract
A complete set of laboratory investigations, including bleeding time, PFA-100 closure times, factor VIII (FVIII) coagulant activity (FVIII:C), von Willebrand factor (VWF) ristocetin cofactor (VWF:RCo), collagen binding (VWF:CB), antigen (VWF:Ag) and propeptide (VWFpp), ristocetin-induced platelet aggregation (RIPA), multimeric analysis of VWF and the response of FVIII:C and VWF parameters to desmopressin (DDAVP), is necessary to fully diagnose all variants of von Willebrand disease (VWD) and to discriminate between type 1 and type 2 and between severe VWD type 1 and type 3. The response to DDAVP of VWF parameters is normal in pseudo VWD (mild VWF deficiency due to blood group O), in mild VWD type 1 and in carriers of recessive severe VWD type 1 and 3. The response to DDAVP is rather good but restricted followed by increased clearance in dominant type 1/2E, good but transient in mild type 2A group II, good for VWF:CB, with only poor response for VWF:RCo in 2M and 2U, poor in 2A group I, 2B, 2C and 2D, and very poor or non-responsive in severe recessive VWD type 1 and 3. Homozygosity or double heterozygosity for nonsense (null) mutations in the VWF gene result in recessive VWD type 3. The combination of a nonsense and missense mutation or of two missense mutations (homozygous or double heterozygous) may cause recessive severe VWD type 1. Recessive VWD type 2A subtype IIC (2C) is caused by homozygous or double heterozygous gene defects in the D1–D2 domain. Homozygosity or double heterozygosity for a FVIII binding defect of the VWF is the cause of recessive VWD type 2N (Normandy) characterized by low FVIII:C, mild or moderate VWF deficiency and normal VWF multimers. Dominant VWD type 1/2E is a mixed quantitative and qualitative multimerization defect caused by a heterozygous cysteine mutation in the D3 domain resulting in abnormal multimerization with a secretion and clearance defect of VWF not due to increased proteolysis. Dominant VWD type 1 Vicenza is a qualitative defect with normal secretion but rapid clearance with equally low levels of FVIII:C, VWF:Ag, VWF:RCo, VWF:CB and the presence of unusually large VWF multimers in plasma due to a specific mutation (R1205H) in the D3 domain. Dominant VWD type 2M and 2U are caused by loss-of-function mutations in the A1 domain.
resulting in quantitative/qualitative deficiencies with a selectively decreased platelet-dependent function with decreased VWF:RCo but normal VWF:CB, a relative decrease in large VWF multimers and the presence but relative loss of large VWF multimers. VWD type 2A and 2B show loss of large VWF multimers due to increased proteolysis. Dominant type 2A is caused by heterozygous missense mutations in the A2 domain. VWD type 2B is due to gain-of-function mutations in the A1 domain and differs from 2A by a normal VWF multimeric pattern in platelets and increased RIPA. DDAVP response curves and VWFpp/Ag ratios contribute to the diagnostic differentiation of VWD type 1 and 2. Rapid clearance of VWF after DDAVP with increased VWFpp/Ag ratios >10 appears to be diagnostic for VWD Vicenza. VWD type 1/2E due to the mutations in the D3 domain uniformly show increased VWFpp/Ag ratios ranging from 3.2 to 4.69 indicating clearance of the VWF/FVIII complex. Normal VWFpp/Ag ratios in mild VWD type 1 with mutations in the D1-D2 and the D4-B-C domains reflect a synthesis/secretion defect.

Introduction

von Willebrand factor (VWF) is a multimeric plasma glycoprotein that acts as a carrier for coagulation factor VIII (FVIII) in the plasma and as a mediator of platelet adhesion to subendothelial collagen after vascular injury [1–4]. A number of distinct functional domains have been identified within the VWF, including regions involved in binding to FVIII, platelet receptor glycoprotein (GP) Ib, GPIIb-GPIIIa and to components of extracellular matrix such as collagen, regions involved in multimerization and dimerization of VWF, and finally domains involved in proteolysis and clearance of VWF (fig. 1). In this article, we provide historical background information and try to extend the current classification of von Willebrand disease (VWD) [5–10] based on experimental evidence and critical appraisal of the literature regarding the clinical, laboratory and molecular characterization of patients with congenital VWD type 1, 2 and 3.

VWD Type 2A Subtypes IIA, IIB, IIC, IIE and IID: Need to Define 2A, 2B, 2C, 2E and 2D as Distinct Entities

In 1980, Ruggeri et al. [5] discovered a heightened interaction between platelets and functionally abnormal FVIII/VWF protein in a new subtype of VWD type II with increased ristocetin-induced platelet aggregation (RIPA) and labeled it type IIB. VWD type II, in which RIPA was decreased or absent, was reclassified as type IIA. Using high-resolution SDS-agarose multimeric analysis of VWF in plasma in combination with immunoblots of VWF proteolytic degradation products, Zimmerman et al. [6] demonstrated in 1986 that proteolysis of VWF is a normal event in normal individuals, which is however increased in VWD type IIA (2A) and IIB (2B) with pronounced triplet structure of each band as a result of increased proteolysis of large VWF multimers (fig. 2). In contrast, proteolysis of VWF is minimal in recessive type IIC (2C), IID (2D) and dominant IIE (2E) variants with aberrant multimeric structure of individual oligomers (fig. 2) [4, 6, 7]. In dominant type IIA (2A) and IIB (2B), the proportion of 176- and 140-kDa fragments was increased relative to the intact 225-kDa subunit, but in recessive VWD type IIC (2C), IID (2D) and dominant type IIE (2E), these degraded VWF fragment were not detected or only present in trace amounts (fig. 2) [4, 6, 7]. Schnepenheim et al. [4] and Schnepenheim and Budde [7] elucidated the different pathophysiologic mechanisms to explain the aberrant structure of individual oligomers in VWD type II (2) A, B, C, D and E patients (fig. 3, 4) related to different functional domains of the VWF protein.

The current classification of congenital VWD is based on the successive recommendations of the VWF Scientific Standardization Committee (SSC) decided at the annual SSC meetings of the International Society on Thrombosis and Hemostasis (ISTH) between 1994 and 2006 [8–10]. The VWF-SSC classification of VWD patients in table 1 is based on a few laboratory tests, including, FVIII: coagulant activity (C), VWF:antigen (Ag), VWF:ristocetin cofactor (RCo), RIPA and VWF multimers in low-resolution gel. As the tests used thus are in some respects rather insensitive, it is therefore not possible to make a clear distinction between the various variants of VWD type 1 and type 2N, 2M or 2E at VWF levels <0.15 U/ml [1, 2]. For reasons of simplicity, the SSC ISTH used a 'lumping' and/or 'splitting' approach for the classification of VWD type 2 not based on the structure-function relationship of VWF gene mutations and functional domains of the VWF protein.

In this 'lumping' approach, type 2 is defined independently of multimer structure and refers to all loss-of-function variants including (i) loss of high-molecular-weight (HMW) multimers with decreased RIPA (lumping IIA, IIC and IID together as type 2A, fig. 3, 5) or (ii) loss of HMW multimers and increased RIPA (type 2B),
and (iii) FVIII binding defects; * VWF:FVIII binding defect = Normandy = 2N (fig. 3, 5). The latter has normal VWF multimers and shows a typical type 1 phenotype of VWD. Lumpying is easier to apply and to make clinical treatment decisions, however at the expense of grouping VWD variants IIA, IIC, IID, IIE as type 2A characterized by decreased RIPA and loss of large VWF multimers despite various mechanisms: increased proteolysis in dominant VWD type IIA (2A) and 2B, defective multimerization in recessive VWD type IIC (2C), multimerization defect in dominant VWD IIE (2E) and dimerization defect in dominant or recessive VWD IID (2D). Decreased or absent RIPA due to loss of function in the interaction of platelet-GPIb-VWF is a typical feature of VWD 2M and 2U with relative loss of otherwise fairly normal large multimers and may phenotypically present as severe VWD type 1. VWD type Vicenza (R1205H/M740I) with ‘supranormal’ VWF multimers and a type 1 phenotype due to rapid clearance not due to increased proteolysis has been classified as type 2M and type 1 in subsequent SSC ISTH classifications of VWD.

In the ‘splitting’ alternative, VWD type 2 refers to the loss of function (VWF:RCo) attributed to the absence of HMW multimers expressed by decreased RIPA and hereby lumping VWD types II (2) A, C, E and D as variants of type 2A, or with increased RIPA in VWD type IIB (2B). VWD type 2M frequently labeled as 2U, 2A-like or variant 2A refers to loss-of-function variants (VWF:RCo and RIPA) not caused by the loss of HMW multimers in low-

Fig. 1. Structure and function of the normal VWF protein.

Fig. 2. Classification of VWD type II according to Zimmermann et al. [6]. Upper part: SDS-agarose multimeric analysis of plasma VWF in normal plasma (N) and in VWD type IIA, IIB, IIC, IIE and IID. Lower part: Immunoblot of VWF proteolytic degradation products show increased proteolysis in VWD type IIA and IIB, but not in VWD type IIC, IIE and IID.
resolution SDS-agarose gel. Diagnosis of so-called severe VWD type 1 with VWF:Ag and VWF:RCo or VWF:collagen binding (CB) levels <0.15 U/l using the SSC ISTH classification (table 1) includes various variants of type 1 secretion and/or clearance defects as well as VWD type 2M [2]. The original description of dominant VWD type 2E has a laboratory phenotype 1 [6]. Diagnostic differentiation of so-called severe VWD type 1 using the SSC ISTH criteria in table 2 remains a persistent problem in routine daily practice, which can easily be overcome using a complete set of laboratory tools related to VWF and a correct interpretation of VWF and FVIII:C response curves to 1-desamino-8-D-argininevasopressin (DDAVP) [1, 2].

**Laboratory Classification of VWD according to SSC ISTH Guidelines**

We propose that the splitting approach using a more complete set of specific diagnostic tools in view of new molecular data on structure and function of VWF gene defects will be clearly superior to the lumping approach in elucidating the variable phenotypic variants of VWD type 1 and 2 [1–7]. Laboratory diagnosis and classification of VWD patients should be based on a complete set of laboratory measurements including bleeding time (BT), PFA-100 closure time, FVIII:C, VWF:Ag, VWF:RCo, VWF:CB, VWF propeptide (pp) and RIPA, in combination with the response of the different VWF parameters and FVIII:C to DDAVP, and the analysis of VWF multimeric patterns using low- and medium-resolution gels to classify VWD according to established criteria [1, 2, 10–12]. Three main categories of VWD can be distinguished: first recessive type 3 and severe type 1, second dominant type 1 and 2, and third a large group of mild VWD with no or low penetrance of bleeding manifestations [1–10]. In general, VWD type 1 is a quantitative VWF deficiency with equally decreased values of all VWF parameters (<0.70 U/ml), and a VWF:RCo and VWF:CB to VWF:Ag ratio >0.70 before and after DDAVP. VWD type 2 is a qualitative VWF deficiency with normal, near normal or decreased levels of FVIII:C and VWF:Ag and much lower values of VWF:RCo and VWF:

![Image of VWF gene mutations and multimeric patterns](image-url)
CB with deceased ratios for VWF:RCo/Ag and VWF:CB/Ag (<0.70). VWF multimer analysis using low- and medium-resolution gels clearly distinguishes dominant VWD type IIA, dominant 2B, recessive IIC, dominant IIE, and dominant or recessive IID (fig. 5) according to the SSC ISTH recommendations or dominant 2A, 2B, 2M and 2E, recessive 2C and dominant or recessive 2D according to the molecular classification of VWD anno 2008 (fig. 3).

**Laboratory and Molecular Classification of VWD**

**Autosomal Recessive VWD**

The inheritance of VWD type 3 is autosomal recessive [11–16]. VWD type 3 patients with virtual absence of VWF are homozygous or compound heterozygous for two null alleles (gene deletions, stop codons, frame shift mutations, splice site mutations and absence of mRNA) in the majority or, more rarely, are compound heterozygous for a null allele and a missense mutation or homozygous for a missense mutation [17]. Compound heterozygosity for a null allele and a missense mutation or homozygosity or double heterozygosity for missense mutations is common in patients with severe autosomal recessive VWD type 1 [18–24]. Recessive severe VWD type 1 patients have detectable but very low VWF levels and FVIII:C levels may range from very low up to ~30% of normal. A considerable number of missense mutations related to autosomal recessive severe VWD type 1 have been identified in the VWF prosequence (D1 and D2 domains) and the dimerization site (cysteine knot, CK, domain) [18–24]. The detection of even tiny amounts of VWF:Ag after DDAVP or in hidden sites like platelets...
Table 1. Classification of VWD according to the VWF SSC ISTH guidelines [8–10]

(1) Inherited VWD caused by genetic mutations at the VWF locus includes a broad spectrum of recessive and dominant variants of VWD. Carriers of recessive VWD type 3 or severe recessive VWD type 1 are asymptomatic or may manifest mild bleeding in particular when associated with blood group O [1, 2].

(2) Type 1 VWD refers to partial quantitative deficiency in VWF mainly based on a normal VWF:Ag/VWF:RCo ratio, VWD type 2 refers to a qualitative deficiency in VWF documented by a decreased VWF:Ag/VWF:RCo ratio, and type 3 refers to virtual absence of VWF.

(3) Type 2 VWD refers to qualitative variants of the VWF and VWF-platelet function interactions attributed to the absence of HMW VWF multimers. The VWF SSC followed a compromised ‘lumping-splitting’ approach in distinguishing four main categories of VWD type 2A (subtypes IIA, IIC, IIE, IID), 2B, 2M and 2N patients.

(4) Type 2M or 2U is a new entity defined by SSC ISTH and refers to qualitative variants with decreased platelet-dependent function (VWF:RCo), with the presence of large VWF multimers in a low-resolution agarose gel. Recent data show a relative decrease in large VWF multimers in a medium-resolution gel and the presence of ultralarge multimers on a low-resolution gel.

(5) Type 2A VWD refers to qualitative variants with absence of HMW multimers, normal or decreased RIPA and decreased VWF:RCo/Ag ratio. Using the lumping-splitting approach, 2A can be subdivided in the well-known variants of IIA, IIC, IIE and IID, as defined by Zimmerman et al. [6].

(6) Type 2B refers to qualitative variants with absence of HMW multimers and decreased VWF:RCo/Ag ratio and increased RIPA, as first described by Ruggeri et al. [5]

(7) Type 2N refers to qualitative variants with markedly decreased affinity for factor VIII, the presence of large VWF multimers and normal VWF:RCo/Ag ratio, as first discovered by Mazurier et al. [33]

enables the differentiation between patients with VWD type 3 and homozygous or double heterozygous recessive severe type 1, 2A subtype IIC (2C).

Multimer analysis is very important in the diagnosis of VWD type 2. Medium-resolution gels (1.4–2.0% agarose) allow to detect the presence or absence of all sizes of VWF multimers (including supranormal) together with triplet structure of the individual multimers. Low-resolution gels are better to evaluate the presence of differently sized multimers and detect ultralarge multimers but lack the detailing of the triplet structure of each VWF band. A hallmark of recessive VWD 2A subtype IIC (2C) is the increase in protomers (pronounced dimers), the absence of triplet structure and large VWF multimers not due to increased proteolysis but usually caused by a multimerization defect due to homozygous or double heterozygous mutation in the D1 and D2 domains (exons 11–16) of VWFpp (fig. 3, 5) [17, 25–31]. Dominant or recessive VWD type 2D caused by missense mutations in the CK domain is rare and characterized by the lack of HMW multimers and the presence of a characteristic intervening subband between individual oligomers (fig. 3) [15, 16].

VWD 2N is the result of gene mutations in FVIII binding D′–D3 domains (fig. 3) with reduced VWF:FVIII binding and FVIII:C levels and normal or near normal VWF:Ag, VWF:RCo and VWF:CB levels, and normal VWF:RCo/VWF:Ag ratio and BT (VWD type 1/N, fig. 3). Although some cases of VWD 2N have severe FVIII:C deficiency of 0.01–0.02 U/ml, the majority have FVIII:C levels >0.05 U/ml [32–35]. Consequently, recessive VWD type 2N is frequently misclassified as mild hemophilia. Intravenous DDAVP in VWD 2N and in mild hemophilia A due to a VWF binding defect of FVIII shows completely normal responses for VWF parameters consistent with type 1 VWD, but restricted responses of FVIII:C followed by a shortened half-life [33, 35]. The degree of restricted response of FVIII:C to DDAVP depends on the severity of the binding defect of FVIII to VWF [1, 32, 33]. In a large group of 144 unrelated patients with mild FVIII:C deficiency and normal VWF values, 15 were diagnosed as homozygous VWD 2N (FVIII:C 0.21 ± 0.14 U/ml, VWF:Ag 0.79 ± 0.43 U/ml) and 5 were diagnosed as heterozygous for the R854Q mutation (FVIII:C 0.33 ± 0.06, VWF:Ag 0.91 ± 0.18 U/dl) and VWD type 2N could be excluded in 124 (FVIII:C 0.30 ± 0.12, VWF:Ag 1.03.3 ± 0.45) [34].

All but a few mutants in the FVIII binding domain show a normal VWF multimeric pattern. Homozygous mutations C788R and C1225G and double heterozygous C788T/null mutations show a hybrid VWD phenotype of pronounced 2E in combination with a FVIII binding defect (2E/N; fig. 3) [36, 37]. Patients with recessive VWD 2N compound heterozygous for R854Q/R760C, R854Q/R763G and Y795C/null show a smeary pattern (sm) of VWF multimers with the presence of ultralarge multimers [38–40]. The sm pattern results from slower moving bands of large VWF protein with uncleaved VWFpp due to mutations around the VWFpp cleavage site (fig. 3). Asymptomatic carriers of R760C, R763G and Y795C show an sm pattern with ultralarge VWF multimers.
Diagnosis and Molecular Classification of VWD

**Fig. 5.** SSC-ISTH Classification of VWD type 2A and its subtypes IIA, IIC, IIE and IID [10–12].

**Table 2.** Response to DDAVP in patients with recessive VWD type 3, recessive severe VWD type 1, recessive VWD type 2, mild VWD type 1 with normal VWF multimers (MM), and dominant VWD type 1 and 2 with abnormal VWF multimers [1, 2, 49, 54, 57–62]

<table>
<thead>
<tr>
<th>VWD type</th>
<th>Mutation location</th>
<th>Response to DDAVP of FVIII:C, VWF and BT</th>
<th>FVIII:C</th>
<th>VWF:Ag</th>
<th>VWF:RCo</th>
<th>VWF:CB</th>
<th>BT correction</th>
</tr>
</thead>
<tbody>
<tr>
<td>Recessive 3</td>
<td>double null (n/n)/null/missense (m)</td>
<td>no</td>
<td>no</td>
<td>no</td>
<td>no</td>
<td>no</td>
<td></td>
</tr>
<tr>
<td>Recessive severe 1</td>
<td>compound n/m or m/m</td>
<td>partial</td>
<td>poor</td>
<td>no</td>
<td>no</td>
<td>no</td>
<td></td>
</tr>
<tr>
<td>Recessive severe 2C</td>
<td>D1, D2, CK double n/m or m/m</td>
<td>partial</td>
<td>partial</td>
<td>poor</td>
<td>poor</td>
<td>no</td>
<td></td>
</tr>
<tr>
<td>Recessive 2N</td>
<td>D'–D3</td>
<td>poor</td>
<td>short</td>
<td>good</td>
<td>good</td>
<td>good</td>
<td>normal BT</td>
</tr>
<tr>
<td>Mild 1/normal MM</td>
<td>variable</td>
<td>good</td>
<td>good (G)</td>
<td>good</td>
<td>good</td>
<td>good</td>
<td>yes</td>
</tr>
<tr>
<td>Dominant VWD/abnormal MM</td>
<td></td>
<td>good</td>
<td>transient</td>
<td>transient</td>
<td>transient</td>
<td>transient</td>
<td>transient</td>
</tr>
<tr>
<td>1/2E</td>
<td>D3</td>
<td>short/G</td>
<td>short/G</td>
<td>short/G</td>
<td>short/G</td>
<td>short/G</td>
<td>short/yes</td>
</tr>
<tr>
<td>1/Vicenza</td>
<td>D3</td>
<td>good</td>
<td>restricted</td>
<td>poor</td>
<td>restricted</td>
<td>transient</td>
<td></td>
</tr>
<tr>
<td>2M</td>
<td>A1</td>
<td>good</td>
<td>good</td>
<td>poor</td>
<td>restricted</td>
<td>transient</td>
<td></td>
</tr>
<tr>
<td>2B</td>
<td>A1</td>
<td>good</td>
<td>restricted</td>
<td>poor</td>
<td>poor</td>
<td>no</td>
<td></td>
</tr>
<tr>
<td>2A group I</td>
<td>A2</td>
<td>good</td>
<td>restricted</td>
<td>poor</td>
<td>short/G</td>
<td>yes</td>
<td></td>
</tr>
<tr>
<td>2A group II</td>
<td>A2</td>
<td>good</td>
<td>restricted/G</td>
<td>restricted/G</td>
<td>restricted/G</td>
<td>yes</td>
<td></td>
</tr>
<tr>
<td>1m, sm, smf</td>
<td>D4, B1–3, C1–2</td>
<td>partial</td>
<td>partial</td>
<td>poor</td>
<td>poor</td>
<td>no</td>
<td></td>
</tr>
<tr>
<td>2D</td>
<td>CK</td>
<td>partial</td>
<td>partial</td>
<td>poor</td>
<td>poor</td>
<td>no</td>
<td></td>
</tr>
</tbody>
</table>
\textbf{Autosomal Dominant VWD}

The grouping together of dominant IIA, recessive IIC, dominant IIE, and dominant or recessive IID as type 2A by the present SSC ISTH classification in figure 3 seems illogical and is often indistinct. The dominant VWD type 2 phenotypes IIA (2A) and IIB (2B) are clearly different from recessive VWD types IIC (2C), dominant or recessive IID (2D) and dominant IIE (2E) (fig. 3) [4, 7]. Careful analysis of reported cases with dominant VWD laboratory phenotype 1 due to mutations in the D3 domain (multimerization-secretion-clearance defect) are characterized by a type 2E multimeric pattern in medium-resolution gels (fig. 3) [41–46]. Applying a complete set of laboratory tools, including a sensitive method for the analysis of the VWF multimeric pattern within the setting of the European study on Molecular and Clinical Markers for the Diagnosis and Management of Type 1 VWD (MCMMDM-1VWD), Schneppenheim and Budde [7] produced consistent data on the relationship between VWF gene mutations and the proposed classification of dominant VWD types 1 and 2 in figures 3 and 5 [4, 7, 47–50]. Figure 3 shows the updated laboratory and molecular classification of VWD types 1 and 2 with abnormal multimers: 2A, 2B, 2C, 2D, 2E versus VWD type 1/2E, 2M or 2U, and type 1sm/2M. The integrated laboratory and molecular classification is mainly based on current knowledge regarding the structure and function relationship of the normal and mutated VWF gene and proteins [4, 7, 47–49]. Carriers of missense mutations (R763Q, R760C, Y795C) located in or around the VWFp cleavage site 753 have ultralarge VWF multimers with an sm pattern on top of VWD 2N when associated with R854Q or null allele [50–52], whereas ultralarge VWF multimers are also seen in VWD Vicenza R1205H/M740I, in contrast to VWD type 1/2E (fig. 3). The multimers of patients with dominant VWD type 1/2E due to mutations in the D3 domain show an aberrant triplet structure with lack of outer bands but with pronounced inner bands of the triplet together with a relative decrease in large multimers reflecting heterozygosity for multimerization defects. Interestingly, there is a relatively good response to DDAVP followed by rapid clearance of VWF:Ag and associated rapid clearance of VWF:RCO [49]. These abnormalities result in a laboratory phenotype VWD type 1 with a type 2E multimer pattern pathognomonic for this newly defined entity of VWD type 1/2E [47–49].

All variants of dominant type 2 VWD patients (except for 2N) show a defective VWF protein in multimeric analysis, decreased VWF:RCO/Ag and VWF:CB/Ag ratios and prolonged BT. High-resolution multimeric analysis of VWF in plasma demonstrates that proteolysis of VWF is increased in type 2A and 2B VWD with increased triplet structure of each visible band not present in type 2M and 2U (fig. 3). The most important findings in patients with VWD type 2A and 2B include prolonged PFA-100 closure times and BT, consistently low VWF:RCO/Ag and VWF:CB/Ag ratios, absence of high and – depending on severity – possibly also of intermediate VWF multimers, pronounced triplet structure of individual bands and increased VWF degradation products (fig. 3, 5). VWD 2B differs from 2A by the presence of normal VWF in platelets, and increased RIPA. RIPA is normal in mild VWD 2A, but decreased in moderate and severe VWD 2A. VWD miscellaneous (2M) or unclassifiable (2U) is distinct from 2A and 2B and typically characterized by low VWF:RCO and RIPA with large VWF multimers present but relatively decreased with less resolved triplet structure of each of the multimeric bands with or without an sm pattern in medium- or high-resolution gels (fig. 3) [1, 2, 10]. In VWD 2M or 2U, the response to DDAVP is poor for VWF:RCO and good for VWF:CB, VWF:Ag and FVIII:C. VWD type 2M or 2U refers to a loss-of-function mutation in the A1 domain leading to decreased VWF-GPIb-platelet dependent functional parameters (defective VWF:RCO and absence of RIPA) [1, 2].

\textbf{Recessive or Dominant VWD Type 1 and Mutations in D4, B1–B3 and C1–C2 Domains}

Data from the European MCMMDM-1VWD study show that the heterozygous mutations in the D4, B1–B3 and C1–C2 domains (L1774S, K1794E, C2304Y, R2313H, G2518S, Q2544X, C2693Y and P2722A) result in normal multimers and mild VWD type 1 with variable penetrance of bleeding manifestations. Heterozygous mutations in the D4, B1–B3, C1–C2 and CK domains (e.g. V1822G, L2207P, C2257S, C2302F, G2441C, R2464C, C2477Y, C2477S and Q2520P) have mild-moderate VWD type 1 with abnormal VWF multimers (usually sm pattern) [48, 49]. Nearly all the above mutations have increased FVIII:C/VWF:Ag ratios around or above 2 (indicated by an asterisk), indicating a secretion defect [48]. Several mutations (L2207P, C2257S, C2304Y, R2379, G2441C, R2464C, R2464C, C2469P and C2671Y) in the D4, B1–B3 and C1–C2 domains are characterized by a VWD type 1 phenotype and variable penetrance of bleeding symptoms and show an atypical sm pattern or an sm pattern with faster moving bands (smf) with the
presence of large multimers and no or less pronounced triplet structure of multimers (fig. 3) [47, 48]. Expression studies of mutant VWF show abnormal banding of VWF multimers as the cause of an sm pattern, which is more pronounced after DDAVP [47]. In the MCMDM-IVWD study, Budde et al. [47] observed that in 6 of 8 investigated mutations (L2207P, C2257S, C2304Y, C2362F, C2441Y, R2464C, C2477Y and C2477S) in exons 38, 40, 42 and 43 (D4, B1–B3 and C1 domains), the multimers showed an sm or smf pattern, with the presence of large VWF multimers and a laboratory phenotype of mild VWD type 1 with variable penetrance of bleeding manifestations (fig. 3). In 6 of these 8 mutations in the D4, B1–B3 and C1 domains, cysteine mutations were responsible. It may be that such cysteine mutations are causing the additional structural alterations explaining the sm pattern of VWF in heterozygotes (fig. 3) [47].

Role of the FVIII:C/VWF:Ag Ratio in the Classification of VWD 1 and 2

By definition, plasma FVIII:C and VWF:Ag is 1 U/ml [13]. Consequently, the FVIII:C/VWF:Ag ratio is ~1 in normal individuals both with blood group O and non-O [53]. The ratio of FVIII binding sites over VWF subunits on a molecular basis is 1:50 and independent of the size of VWF multimers indicating that many potential FVIII:C binding sites on VWF are free. As in quantitative VWD type 1, the VWF:Ag is 50% due to secretion or synthesis defects, the ratio of FVIII:C/VWF:Ag will increase to ~2, but remains 1 in case of rapid clearance of VWF:Ag and FVIII bound to VWF [53]. Carriers of recessive VWD type 3 heterozygous for the VWF null allele (decreased synthesis) have a ratio of 2.06 for FVIII:C/VWF:Ag, and this ratio appears to depend on the severity of the VWF:Ag deficiency with ratios of 3.2, 1.96 and 1.46 at VWF:Ag plasma levels <30, between 30 and 60, and >60 U/ml [53]. That an increased FVIII:C/VWF:Ag ratio in VWD type 1 and 2 refers to a VWF secretion defect can be documented by a restricted response of VWF to DDAVP compared to that of FVIII:C [13]. These findings and asymptomatic parents are typically seen in severe recessive VWD type 1 or 2 patients [54]. A normal FVIII:C/VWF:Ag ratio is consistent with normal secretion of mutant VWF protein in VWD type 1 (including Vicenza) and type 2 (2A group II and 2B) patients [2, 54]. A decreased FVIII:C/VWF:Ag ratio due to a FVIII binding defect in the VWF protein plus recessive heredity refers to homozygous or double heterozygous or heterozygous type 2N VWD. A decreased FVIII:C/VWF:Ag ratio is also seen in mild hemophilia A due to a VWF binding defect in FVIII [32–35, 53, 55].

Role of DDAVP Response Curves in the Classification of VWD

DDAVP is a synthetic analogue of the natural hormone vasopressin [56], but it has no pressor activity, in contrast to vasopressin. DDAVP stimulates the release of endogenous FVIII and VWF. The effect is virtually immediate, usually with 2- to 6-fold increases in the plasma concentrations of FVIII, VWF:Ag, VWF:RCo and VWF:CB [49, 54, 57, 58]. All patients with VWD type 1 and 2 (except 2B) should be given a test dose for diagnostic purposes to establish whether the response is sufficient or insufficient for clinical use [1, 2, 49, 54, 57, 58]. DDAVP can be used to treat major bleeding or to prevent bleeding in connection with surgery or other invasive procedures. if VWF:RCo and FVIII:C reach normal levels after infusion of DDAVP followed by near normal to normal half-lives. If response of VWF parameters is insufficient or the duration is short, a VWF/FVIII concentrate should be considered [49, 54, 57–60].

Patients with recessive type 3 do not respond to DDAVP. Severe recessive VWD type 1, IIC (2C) and IID (2D) respond poorly or not to DDAVP with regard to the VWF parameters but usually show an increase in FVIII:C to normal levels (table 2) [1, 2, 54].

Patients with mild VWD type 1 with normal VWF multimers and VWF values between 0.30 and 0.60 usually respond well to DDAVP (table 2). Interestingly, the response to DDAVP of FVIII:C is 2–3 times higher than that of VWF:Ag in carriers of a null allele or missense mutation (parents of VWD patients with recessive type 3 or severe type 1) [1, 42, 54].

A normal response of VWF parameters and restricted response of FVIII:C to DDAVP followed by shortened half-life of FVIII:C refers to VWD 2N with a FVIII binding defect in the VWF or mild hemophilia following a VWF binding defect in the FVIII protein [31, 33, 55, 58].

A short half-life of the VWF parameters and FVIII:C after DDAVP in type 1 Vicenza, 1/2E, 2M and 2U indicates rapid clearance of VWF antigen, which is not due to proteolysis, as demonstrated by the absence of triplet structures of VWF bands and of VWF degradation products (fig. 4) [54, 58, 61, 62].

Short half-lives of functional VWF parameters compared to near normal to normal half-life for VWF:Ag in
type 2A and 2B (table 2) [57, 58] indicates increased proteolysis, as demonstrated by the loss of large VWF multimers, the presence of VWF degradation products (fig. 5) and increased triplet structure of VWF multimer bands (fig. 3, 5).

Dominant VWD type 2M or 2U (fig. 3) due to loss of GPIb function mutation in the A1 domain show a poor response of VWF:RCo to DDAVP and good responses to DDAVP of VWF:CB, VWF:Ag and FVIII:C followed by a decreased half-life of VWF parameters (table 2; fig. 3) [1, 2, 54, 58].

Patients with dominant VWD type 2A group II show a good response of FVIII:C and VWF:Ag but a poor response of VWF:RCo to DDAVP with no correction of BT (table 2) [1, 2, 54, 58].

VWD type 2A group II have mild VWD characterized by near normal to prolonged values of BT, normal FVIII:C and VWF:Ag, and low VWF:RCo and VWF:CB. These mild VWD type 2A group II patients respond to DDAVP with complete correction of BT and functional VWF parameters to normal for only a few hours with a short half-life for VWF:RCo and VWF:CB (table 2) [1, 2, 58].

Role of the VWFpp/VWF:Ag Ratio in the Molecular Classification of VWD

VWFpp and mature VWF protein remain non-cova-

lently associated and are stored in Weibel-Palade bodies in endothelial cells for regulated release [63–67]. After

---

**Table 3.** Results of VWF:Ag and VWFpp levels in VWD type 1/2E or 1 SC with the mutations W1144G (D3 domain) and S2179F (D4 domain) and blood group O and in mild VWD type 1 due to mutations in the D1-D2-D’ domains and the D4-C1-C3-C1-C2 domains [67–70] compared to C1584 [23] and controls related to ABO blood group (means and ranges or ±SD)

<table>
<thead>
<tr>
<th>VWD type/mutation</th>
<th>Patients</th>
<th>VWF:Ag IU/dl</th>
<th>VWFpp IU/dl</th>
<th>VWFpp/Ag</th>
<th>T1/2 VWF:Ag after DDAVP, h</th>
</tr>
</thead>
<tbody>
<tr>
<td>USA/European studies [67, 68]</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Type 1 Vicenza: R1205H/M740I</td>
<td>3</td>
<td>6.8 (5.7–8.4)</td>
<td>72.8 (63–85)</td>
<td>10.9 (10.1–11.5)</td>
<td>&lt;2</td>
</tr>
<tr>
<td>Type 1/2E (IIE)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>C1130G/F/R</td>
<td>3</td>
<td>16 (8–13)</td>
<td>80 (52–107)</td>
<td>5.4 (4.6–6.5)</td>
<td>3–4</td>
</tr>
<tr>
<td>W1144G, blood group O</td>
<td>6</td>
<td>16 (13–22)</td>
<td>61 (55–69)</td>
<td>4.1 (3.1–5.1)</td>
<td>3–4</td>
</tr>
<tr>
<td>W1144G, blood group non-O</td>
<td>6</td>
<td>22 (14–32)</td>
<td>72 (68–80)</td>
<td>3.2 (2.1–5.0)</td>
<td>3–4</td>
</tr>
<tr>
<td>Type 1 SC: Type 1 SC: S2179F, blood group O</td>
<td>5</td>
<td>15 (9–24)</td>
<td>65 (56–77)</td>
<td>4.8 (3.2–6.4)</td>
<td>3–4</td>
</tr>
<tr>
<td>Mild type 1</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>D1, D2, D’ domains, G160W, N116I, M771I, R854Q/R854Q</td>
<td>6</td>
<td>46.1 (37–79)</td>
<td>64 (23–96)</td>
<td>1.13 (0.6–1.6)</td>
<td>normal</td>
</tr>
<tr>
<td>A3, D4, B-C domains, V1822G, R2063S/R2287W, C2304Y, C2477S, C2693Y, Int 13 1534-3C→A</td>
<td>6</td>
<td>58.4 (28–65)</td>
<td>77.6 (66–109)</td>
<td>1.85 (1.0–2.8)</td>
<td>near normal to normal</td>
</tr>
<tr>
<td>Blood group O</td>
<td>45</td>
<td>78 (43–136)</td>
<td>105 (55–154)</td>
<td>1.41 (1.04–2.4)</td>
<td>not measured</td>
</tr>
<tr>
<td>Blood group non-O</td>
<td>45</td>
<td>116 (79–164)</td>
<td>128 (84–178)</td>
<td>1.12 (0.84–1.78)</td>
<td>not measured</td>
</tr>
<tr>
<td>Italian study [70]</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Type 1 Vicenza: R1205H/M740I</td>
<td>14</td>
<td>12 ± 1.9</td>
<td>136 ± 22</td>
<td>13.0 ± 0.5</td>
<td>1.3 ± 0.2</td>
</tr>
<tr>
<td>Type 1/2E: C1130F</td>
<td>4</td>
<td>27 ± 10</td>
<td>105 ± 20</td>
<td>4.69 ± 0.67</td>
<td>4.1 ± 0.7</td>
</tr>
<tr>
<td>Mild type 1</td>
<td>18</td>
<td>40 ± 6</td>
<td>55 ± 7</td>
<td>1.56 ± 0.07</td>
<td>11.6 ± 1.4</td>
</tr>
<tr>
<td>Blood group O</td>
<td>17</td>
<td>79 ± 8.6</td>
<td>99 ± 8</td>
<td>1.6 ± 0.06</td>
<td>9.4 ± 1.0</td>
</tr>
<tr>
<td>Blood group non-O</td>
<td>13</td>
<td>113 ± 9</td>
<td>106 ± 6</td>
<td>1.2 ± 0.04</td>
<td>21 ± 4</td>
</tr>
<tr>
<td>UK studies by Davies et al. [69]</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>C1584 all</td>
<td>50</td>
<td>82 ± 35</td>
<td>94 ± 20</td>
<td>1.36 ± 0.40</td>
<td></td>
</tr>
<tr>
<td>C1584 group O</td>
<td>23</td>
<td>58 ± 14</td>
<td>94 ± 20</td>
<td>1.66 ± 0.33</td>
<td></td>
</tr>
<tr>
<td>C1584 group non-O</td>
<td>17</td>
<td>98 ± 34</td>
<td>109 ± 30</td>
<td>1.17 ± 0.25</td>
<td></td>
</tr>
<tr>
<td>Blood group O</td>
<td>50</td>
<td>97 ± 24</td>
<td>110 ± 31</td>
<td>1.16 ± 0.24</td>
<td></td>
</tr>
<tr>
<td>Blood group non-O</td>
<td>50</td>
<td>126 ± 30</td>
<td>110 ± 28</td>
<td>0.90 ± 0.21</td>
<td></td>
</tr>
</tbody>
</table>

1 n = 18: stop codon in 6, missense mutation in 8 (R115C, P2063F, C2362P, G2705) and mutation not found in 4.
release in plasma, VWFpp and the mature VWF multimers dissociate and circulate independently with a half-life of 2–3 h for VWFpp and 8–12 h for VWF:Ag. Concentrations of VWFpp and VWF:Ag in plasma are set as 1 U/dl in normal plasma. Consequently, the ratio of VWFpp to VWF:Ag in plasma is by definition equal to 1.0. The rationale behind the use of the VWF:pp/Ag ratio is that in the different variants of VWD type 1 and 2 the half-life of VWFpp is normal, whereas clearance of VWF:Ag may be shortened, resulting in aberrant ratios. In addition, VWD patients with a secretion defect but normal clearance and who thus show a restricted response of VWF to DDAVP followed by normal half-life of VWF:Ag are expected to have decreased values for VWFpp and VWF:Ag (reflecting the secretion defect) but with a normal ratio for VWFpp/VWF:Ag (reflecting normal clearance). VWD patients with a normal secretion but increased clearance of VWF:Ag (and associated functions VWF:RCo and FVIII:C) who show good responses to DDAVP followed by short half-lives of VWF:Ag are expected to have an increased VWFpp/VWF:Ag ratio (reflecting increased clearance). We have analyzed the published results in three studies on VWFpp/Ag ratios in relation to the level of VWF:Ag and the VWF:Ag survival times after DDAVP in VWD type 1/2E, type 1 Vicenza due to mutations in the D3 domain, VWD type ISC due to mutation in the D4 domain, mild VWD type 1 due to null mutations, mild VWD type 1 due to missense mutations in the D1-D2 or D4-B1-B3-C1-C2 domains and mild VWD type 1 related to the C1584 mutation with blood group O [67–70].

Two studies showed a dramatic increase in the VWFpp/Ag ratio (>10) in VWD type Vicenza cases with the R1205 H mutation in the D3 domain, and in mild VWD type 1 due to stop codon, nonsense mutation and in controls with blood group O and non-O [70] (cf. results in table 3).

The three studies uniformly showed significantly increased VWFpp/Ag ratios with mean values ranging from 3.2 to 4.69 in all cases of VWD type 1/2E with the mutation C1130, W1144G in the D3 domain and the S2179F mutation in the D4 domain indicating a pro-

---

Fig. 6. VWFpp and VWF:Ag related to VWF:Ag half-lives (SD of mean) after DDAVP in a recent Italian study in patients with VWD type Vicenza, type 1/2E (C1130F) and in mild VWD type 1 due to stop codon, nonsense mutation and in controls with blood group O and non-O [70] (cf. results in table 3).
nounced increased clearance of the VWF/FVIII complex. This could be independently documented by a good response to DDAVP followed by shortened half-lives for VWF of ~3–4 h (table 3). These observations clearly indicate that rapid clearance of VWF is the main cause of pronounced VWF deficiency in VWD type 1/2E (table 3).

There is a strong inverse correlation between rapid clearance of VWF:Ag after DDAVP and increased VWFpp/Ag ratios >10 in VWD Vicenza, and >2 in VWD type 1/2E and normal VWFpp/Ag ratios in mild VWD due to mutations in the D1, D2 and the D4-B-C domains (table 3, fig 4, 6).

There is no significant difference in the mean VWFpp levels between blood group O and non-O, but the VWFpp/Ag ratio differed significantly between O and non-O individuals in three studies (table 3). One study showed significantly shorter VWF:Ag half-lives after DDAVP in blood bank donors with blood group O versus non-O (table 3).

The absolute values of VWF:Ag and VWFpp in mild VWD type 1 are in the lower range of normal in two studies and significantly lower (55 ± 7 U/dl) than in controls in the Italian study (table 3) [70]. Mild VWD type 1 patients, who are mainly blood group O, have to be compared with controls belonging to blood group O. Both VWD type 1 and blood group O controls had comparable normal VWFpp/Ag ratios (1.1–1.8 vs. 1.4–1.6) and normal half-lives for VWF:Ag after DDAVP of ~10 h (table 3).

Patients with mild VWD type 1 due to missense mutations in the D1, D2 and D7 domains have low normal to decreased VWFpp levels and normal VWFpp/Ag ratios of just above 1 indicating a synthesis/secretion defect of VWF. Patients with mild VWD due to mutations in the D4-B1-C1-C2 domains have decreased values for VWFpp but normal to slightly increased VWFpp/Ag ratios indicative of a very mild clearance defect in some of them. Patients with the C1584 mutation and blood group O have decreased values for VWF:Ag but normal VWFpp values and slightly increased VWFpp/Ag ratios indicative of a mild clearance defect of VWF [69].

There is one report of a shortened VWF:Ag half-life after DDAVP and increased VWFpp/Ag ratios in 3 patients with VWD type 2M due to the II1416N mutation [71] (fig 4).

References


Diagnosis and Molecular Classification of VWD

Acta Haemost 2009;121:71–84

83


Laboratory Diagnosis and Molecular Basis of Mild von Willebrand Disease Type 1

Jan Jacques Michiels, Zwi Berneman, Alain Gadisseur, Marc van der Planken, Wilfried Schroyens, Huub H.D.M. van Vliet

A Hemostasis and Thrombosis Research Center, Antwerp University Hospital, Edegem, Belgium; B Department of Hemostasis and Thrombosis Research, Erasmus University Medical Center, and C Hemostasis and Thrombosis Science Center, Goodheart Institute, Rotterdam, The Netherlands

Abstract

Mild type 1 von Willebrand disease (VWD) is characterized by low to variable penetrance of bleeding, a high (increased) prevalence of blood group O, von Willebrand factor (VWF) values around and above 30% with normal ratios of VWF:ristocetin cofactor activity (RCO)/VWF:antigen (Ag), VWF:collagen binding (CB)/VWF:Ag and factor VIII (FVIII):coagulant activity (C)/VWF:Ag. Within this group of patients, the combination of the C1584 mutation and blood group O is rather frequent. Patients with mild VWD type 1 present good/normal responses of FVIII:C and VWF parameters to desmopressin (DDAVP). With the exclusion of dominant VWD type Vicenza, type 1/2E, recessive 2N and dominant 2M, missense mutations in patients with mild VWD type 1 with normal multimers are mainly located in the regulatory sequence region, the D1/D2 propeptide region, the D' VWF-FVIII binding site region and the D4, B1–B3 and C1–C2 domains but rarely in the D3, A1 or A2 domain. A new category of either dominant or recessive mild VWD type 1 due to mutations in the D4, B1–B3 and C1–C2 domains of the VWF gene consists of two groups: one group with mild VWD with normal VWF multimers and a second group with mild/moderate VWD with smearable multimer pattern.

Introduction

In routine daily practice, simple rules are applied to diagnose and grade bleeding in patients with congenital von Willebrand disease (VWD) [1, 2]. In patients with very mild von Willebrand factor (VWF) deficiency related to blood group O and/or VWD type 1, only one or two unclear minor bleeding symptoms occur and secondary bleeding following trauma and/or surgery is absent. In mild VWD, bleeding symptoms are mostly mucocutaneous, i.e. frequent episodes of epistaxis and/or prolonged or profuse menstruation or frequent hematoma, which usually do not require medical treatment or frequent hemosty, and these patients usually present rather good/normal responses of FVIII:coagulant activity (C) and VWF parameters to desmopressin (DDAVP). In classic VWD type 1, 2 or 3, either autosomal recessive or dominant, the patient has recurrent mucocutaneous bleeding episodes since...
early childhood, more than two bleedings after tooth extraction, trauma or surgery and bleeding requiring medical treatment and/or FVIII/VWF concentrate transfusion because of abnormal bleeding after surgery and/or trauma, or for severe menstrual blood loss in women. Prolonged bleeding for a few to several hours or even >24 h after a tooth extraction, minor trauma or surgery are not uncommon. A moderate/severe type of mucocutaneous bleeding, either recessive or dominant, since early childhood in addition to hemarthrosis, muscle bleeding and a need for prophylactic treatment with FVIII/VWF concentrate refers to a hemophilia bleeding type, which is usually seen in recessive VWD type 3 [1, 2].

According to established criteria, laboratory diagnosis and classification of VWD should be based on a complete set of laboratory measurements including bleeding time, PFA-100 closure time (PFC), FVIII:C, VWF:antigen (Ag), VWF:ristocetin cofactor activity (RCo), VWF:collagen binding (CB), ristocetin-induced platelet aggregation (RIPA) and the analysis of the VWF multimeric pattern using low-, medium- and high-resolution gels [3, 4]. The main categories of VWD can be distinguished: firstly recessive type 3 with pseudohemophilia and severe type 1 without hemophilic symptoms like hemarthrosis; secondly dominant type 1 and 2, and thirdly a large group of mild VWD with no or low penetrance of bleeding manifestations [1–5]. In general VWD type 1 is a quantitative VWF deficiency with equally decreased values of all VWF parameters (<0.70 U/ml), and a normal ratio for VWF:RCo/Ag and VWF:CB/Ag (>0.70) before and after DDAVP [3, 4]. VWD type 2 is a qualitative VWF deficiency with normal, near normal or decreased levels for FVIII:C and VWF:Ag and much lower values for VWF:RCo and VWF:CB with decreased ratios for VWF:RCo/Ag and VWF:CB/Ag (<0.70) [1–5]. VWF multimeric analysis using low- and medium-resolution gels can clearly distinguish VWD type 1 with the presence of normal VWF multimers from the various subtypes 2A (IIA, IIC, IIE and IIB) with loss of large VWF multimers. VWD type 2B has increased RIPA, while 2M has been defined by a decrease in RIPA and VWF:RCo with the presence of all but relatively decreased numbers of large VWF multimers not due to increased proteolysis [1, 2, 5].

Mild VWD Type 1 with Normal VWF Multimers

In 1999, Fressinaud et al. [6] evaluated the performance of the PFC in 60 patients with VWD classified according to the 1994 classification of the Scientific Standardization Committee (SSC) of the International Society on Thrombosis and Hemostasis (ISTH) proposed by Sadler [4]. This study included 36 patients with VWD type 1, 24 patients with VWD 2A, 2B, 2N and 3, and 14 patients with hemophilia (table 1). PFC Epi and ADP were normal in VWD 2N and in hemophilia patients. According to strict criteria proposed by Michiels et al. [5], the VWD type 1 patients could be reclassified as mild VWD type 1 in 15 cases (VWF:RCo 28–39 IU/dl), moderate VWD type 1 or 2 in 9 (VWF:RCo 15–27 IU/dl) and severe VWD type 1 or 2 in 12 cases (VWF:RCo 5–14 IU/dl). All patients with severe VWD type 1 or 2, 2A, 2B and 3 had a prolonged PFC >250 s as well as a prolonged bleeding time (BT) in the majority of the cases (table 1). BT varied from normal in a few to prolonged in the majority of patients with moderate/severe VWD type 1 or 2. Mild VWD type 1 patients had normal to marginally prolonged BT and prolonged PFC between the upper limit of normal (PFA-100 ADP >120 s and Epi >160 s) and 250 s (table 1).

These observations led to the conclusion that PFA-100 is clearly superior to BT regarding the detection of VWD. A normal PFC can exclude all VWD types except 2N. In a subsequent study, Fressinaud et al. [7] evaluated PFA-100 in the management of 23 mild/moderate VWD type 1 patients treated with DDAVP irrespective of the ABO blood group. Before DDAVP infusion, VWF:Ag ranged from 15 to 56 (mean 33 ± 9) IU/dl and VWF:RCo from 14 to 49 (29 ± 8) IU/dl. All VWD patients had prolonged PFC (ADP >120 s; Epi >160 s; fig. 1). Before DDAVP, PFC was >250 s in 13 patients and between the upper limit of normal to 250 s in 8 and 25 VWD patients (fig. 1). One hour after DDAVP, PFC (ADP and Epi) returned to normal values in all patients (fig. 1). There was no further follow-up apart from 3 cases showing only transient correction of PFC limited to a few hours (fig. 1). In our experience, such transient corrections of PFC are typically seen in patients with mild/moderate VWD type 1/2E, mild/moderate 2M, 2M Vicenza, mild 2A and in different variants of dominant VWD type due to a mild secretion and/or clearance defect.

In 2002, Michiels et al. [5] analyzed 275 patients classified as VWD irrespective of the blood group in the Academic Medical Center of Rotterdam, which has a catchment area of 2–2.5 million inhabitants. The first group of 128 (46.5%) patients had VWF:Ag and functional levels around 60 ± 20%, a very mild bleeding tendency, no family history, normal Ivy BT and completely normal responses of VWF and FVIII:C to DDAVP and as such could not be confirmed as suffering from VWD and were
### Table 1.
Laboratory parameters in patients with hemophilia, VWD type 1, 2A, 2B, 2N and type 3 classified according to the 1994 SSC ISTH classification [4] of VWD in the study by Fressinaud et al. [6] and reclassified more strictly according to the recommendations proposed by Michiels et al. [5] in 2002

<table>
<thead>
<tr>
<th>Patients VWD type (SSC ISTH) [4]</th>
<th>VWF:Ag</th>
<th>RCo</th>
<th>Ratio/ RCo/Ag</th>
<th>VWD type re-classified [5]</th>
<th>BT</th>
<th>PFC, s</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>ADP</td>
</tr>
<tr>
<td><strong>Type 1 mild</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>48</td>
<td>39</td>
<td>0.81</td>
<td>1 mild</td>
<td>10</td>
<td>136</td>
</tr>
<tr>
<td>2</td>
<td>40</td>
<td>39</td>
<td>0.98</td>
<td>1 mild</td>
<td>8</td>
<td>147</td>
</tr>
<tr>
<td>3</td>
<td>34</td>
<td>38</td>
<td>1.12</td>
<td>1 mild</td>
<td>6</td>
<td>154</td>
</tr>
<tr>
<td>4</td>
<td>62</td>
<td>38</td>
<td>0.61</td>
<td>1 mild</td>
<td>8.5</td>
<td>148</td>
</tr>
<tr>
<td>5</td>
<td>39</td>
<td>36</td>
<td>0.92</td>
<td>1 mild</td>
<td>18</td>
<td>193</td>
</tr>
<tr>
<td>6</td>
<td>45</td>
<td>35</td>
<td>0.77</td>
<td>1 mild</td>
<td>6</td>
<td>141</td>
</tr>
<tr>
<td>7</td>
<td>37</td>
<td>34</td>
<td>0.92</td>
<td>1 mild</td>
<td>6</td>
<td>144</td>
</tr>
<tr>
<td>8</td>
<td>35</td>
<td>34</td>
<td>0.97</td>
<td>1 mild</td>
<td>16</td>
<td>210</td>
</tr>
<tr>
<td>9</td>
<td>38</td>
<td>34</td>
<td>0.89</td>
<td>1 mild</td>
<td>5</td>
<td>145</td>
</tr>
<tr>
<td>10</td>
<td>45</td>
<td>34</td>
<td>0.76</td>
<td>1 mild</td>
<td>6</td>
<td>138</td>
</tr>
<tr>
<td>11</td>
<td>42</td>
<td>31</td>
<td>0.74</td>
<td>1 mild</td>
<td>5.5</td>
<td>127</td>
</tr>
<tr>
<td>12</td>
<td>38</td>
<td>30</td>
<td>0.79</td>
<td>1 mild</td>
<td>8</td>
<td>129</td>
</tr>
<tr>
<td>13</td>
<td>35</td>
<td>28</td>
<td>0.80</td>
<td>1 mild</td>
<td>18</td>
<td>197</td>
</tr>
<tr>
<td>14</td>
<td>29</td>
<td>28</td>
<td>0.97</td>
<td>1 mild</td>
<td>6</td>
<td>170</td>
</tr>
<tr>
<td>15</td>
<td>48</td>
<td>27</td>
<td>0.56</td>
<td>1 mild</td>
<td>3.5</td>
<td>141</td>
</tr>
<tr>
<td><strong>Type 1 moderate</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>16</td>
<td>59</td>
<td>27</td>
<td>0.56</td>
<td>1 or 2</td>
<td>&gt;20</td>
<td>&gt;250</td>
</tr>
<tr>
<td>17</td>
<td>31</td>
<td>26</td>
<td>0.84</td>
<td>1 moderate</td>
<td>7</td>
<td>&gt;250</td>
</tr>
<tr>
<td>18</td>
<td>38</td>
<td>26</td>
<td>0.68</td>
<td>1 moderate</td>
<td>17</td>
<td>&gt;250</td>
</tr>
<tr>
<td>19</td>
<td>28</td>
<td>22</td>
<td>0.79</td>
<td>1 moderate</td>
<td>15</td>
<td>&gt;250</td>
</tr>
<tr>
<td>20</td>
<td>23</td>
<td>22</td>
<td>0.96</td>
<td>1 moderate</td>
<td>12</td>
<td>&gt;250</td>
</tr>
<tr>
<td>21</td>
<td>39</td>
<td>22</td>
<td>0.56</td>
<td>2</td>
<td>17</td>
<td>181</td>
</tr>
<tr>
<td>22</td>
<td>30</td>
<td>22</td>
<td>0.73</td>
<td>1 moderate</td>
<td>15</td>
<td>181</td>
</tr>
<tr>
<td>23</td>
<td>38</td>
<td>16</td>
<td>0.42</td>
<td>2</td>
<td>8.5</td>
<td>197</td>
</tr>
<tr>
<td>24</td>
<td>24</td>
<td>15</td>
<td>0.62</td>
<td>1 moderate</td>
<td>6.5</td>
<td>&gt;250</td>
</tr>
<tr>
<td><strong>Type 1 severe</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>25</td>
<td>24</td>
<td>14</td>
<td>0.58</td>
<td>1 or 2 severe</td>
<td>&gt;20</td>
<td>&gt;250</td>
</tr>
<tr>
<td>26</td>
<td>44</td>
<td>13</td>
<td>0.33</td>
<td>2 severe</td>
<td>&gt;20</td>
<td>&gt;250</td>
</tr>
<tr>
<td>27</td>
<td>26</td>
<td>12</td>
<td>0.46</td>
<td>2 severe</td>
<td>&gt;20</td>
<td>&gt;250</td>
</tr>
<tr>
<td>28</td>
<td>44</td>
<td>13</td>
<td>0.30</td>
<td>2 severe</td>
<td>&gt;20</td>
<td>&gt;250</td>
</tr>
<tr>
<td>29</td>
<td>23</td>
<td>10</td>
<td>0.43</td>
<td>2 severe</td>
<td>12</td>
<td>&gt;250</td>
</tr>
<tr>
<td>30</td>
<td>12</td>
<td>10</td>
<td>0.83</td>
<td>1 severe</td>
<td>9</td>
<td>&gt;250</td>
</tr>
<tr>
<td>31</td>
<td>15</td>
<td>10</td>
<td>0.67</td>
<td>1 severe</td>
<td>&gt;20</td>
<td>&gt;250</td>
</tr>
<tr>
<td>32</td>
<td>27</td>
<td>10</td>
<td>0.37</td>
<td>2 severe</td>
<td>11</td>
<td>&gt;250</td>
</tr>
<tr>
<td>33</td>
<td>12</td>
<td>9</td>
<td>0.75</td>
<td>1 severe</td>
<td>4</td>
<td>&gt;250</td>
</tr>
<tr>
<td>34</td>
<td>29</td>
<td>9</td>
<td>0.31</td>
<td>2 severe</td>
<td>8.5</td>
<td>&gt;250</td>
</tr>
<tr>
<td>35</td>
<td>9</td>
<td>0</td>
<td>1.00</td>
<td>1 severe</td>
<td>&gt;20</td>
<td>&gt;250</td>
</tr>
<tr>
<td>36</td>
<td>9</td>
<td>5</td>
<td>0.55</td>
<td>1 or 2 severe</td>
<td>7.5</td>
<td>&gt;250</td>
</tr>
<tr>
<td><strong>Type 2A</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>75</td>
<td>43</td>
<td>0.57</td>
<td>2A mild</td>
<td>8.5</td>
<td>&gt;250</td>
</tr>
<tr>
<td>2</td>
<td>92</td>
<td>24</td>
<td>0.26</td>
<td>2A mild</td>
<td>8.5</td>
<td>&gt;250</td>
</tr>
<tr>
<td>3</td>
<td>85</td>
<td>23</td>
<td>0.27</td>
<td>2A mild</td>
<td>18</td>
<td>&gt;250</td>
</tr>
<tr>
<td>4</td>
<td>79</td>
<td>22</td>
<td>0.28</td>
<td>2A</td>
<td>&gt;20</td>
<td>&gt;250</td>
</tr>
<tr>
<td>5</td>
<td>69</td>
<td>22</td>
<td>0.32</td>
<td>2A</td>
<td>&gt;20</td>
<td>&gt;250</td>
</tr>
<tr>
<td>6</td>
<td>30</td>
<td>14</td>
<td>0.45</td>
<td>2A</td>
<td>&gt;20</td>
<td>&gt;250</td>
</tr>
<tr>
<td>7</td>
<td>72</td>
<td>14</td>
<td>0.20</td>
<td>2A</td>
<td>&gt;20</td>
<td>&gt;250</td>
</tr>
<tr>
<td>8</td>
<td>45</td>
<td>&lt;3</td>
<td>0.07</td>
<td>2A severe</td>
<td>&gt;20</td>
<td>&gt;250</td>
</tr>
<tr>
<td>9</td>
<td>92</td>
<td>&lt;3</td>
<td>0.03</td>
<td>2A severe</td>
<td>&gt;20</td>
<td>&gt;250</td>
</tr>
<tr>
<td>10</td>
<td>50</td>
<td>&lt;3</td>
<td>0.06</td>
<td>2A severe</td>
<td>&gt;20</td>
<td>&gt;250</td>
</tr>
</tbody>
</table>

**Type 1 mild**

**Type 1 moderate**

**Type 1 severe**

**Type 2A**

Diagnosis and Molecular Basis of Mild VWD Type 1
diagnosed as pseudo von Willebrand. The other 167 patients of 93 families were confirmed as VWD and classified as mild VWD type 1 in 65 families, severe dominant VWD type 1 in 10 families (including 1/2M in 3), type 2A, 2B and 2N in 10, 4 and 2 families, respectively, and recessive type 3 in 2 families, respectively [5].

Of the 65 families with mild VWD, we prospectively investigated the index case (proband) of 24 families with VWF levels between 30 and 60% at several occasions, diagnosed as mild VWD type 1 according to the ISTH SSC recommendations [5]. Following DDAVP, FVIII:C, VWF:RCo and VWF:CB levels reached completely normal values ≥150 or even >200%, with normal VWF:RCo/VWF:Ag ratio before and after DDAVP (fig. 2). This group of 24 probands could be further divided into a group of 14 probands with normal and a group of 10 probands with significantly shortened half-lives of VWF parameters, indicating increased clearance and/or proteolysis. Those patients with completely normal responses of FVIII:C, VWF:RCo and VWF:CB to DDAVP to values ≥200%, with normal half-lives and normal VWF levels up to 24 h after DDAVP were diagnosed as pseudo VWD (fig. 2, right) [5].

Franchini et al. [8] evaluated the PFA-100 for monitoring DDAVP therapy in 24 patients with mild VWD type 1. Baseline values of VWF:Ag and VWF:RCo were between 0.25 and 0.50 IU/dl irrespective of the ABO blood group (fig. 2). PFC (ADP and Epi) were prolonged in all patients (fig. 2). DDAVP induced a good but restricted response of VWF:Ag and VWF:RCo reaching normal values around 1.20 ± 0.25 IU/dl but not exceeding the values of 1.50 IU/dl except in 1–2 (fig. 3). PFC returned to normal values 1 h after DDAVP in all 24, thereby confirming the diagnosis of mild VWD type 1 (fig. 3).

van Vliet et al. [9] evaluated the responses of PFA, VWF and FVIII to DDAVP in routine clinical practice of 67 VWD type 1 patients with normal VWF:RCo/VWF:Ag ratio ≥0.70, normal VWF multimers and a history of mild/moderate bleeding irrespective of the ABO blood group [6]. In this study, VWF:Ag was measured by an enzyme-linked immunosorbent assay (ELISA) and VWF:CB by an ELISA using collagen type 1 [10–12]. VWF ratio (VWF:CB/VWF:Ag) was used as a surrogate measure of the multimer distribution [11, 12]. VWF:RCo was determined by measuring the rate of aggregation of fixed platelets induced by ristocetin and patient plasma (VWF) with the PAP-4 aggregometer (Biodata) [5]. PFC was assessed using PFA-100® (Dade-Behring, Marburg, Germany) [9]. PFC, VWF and FVIII levels before and after DDAVP were assessed in 67 VWD type 1 patients (fig. 4). Before treatment, FVIII-VWF parameters ranged from severe, moderate and mild VWF deficiency to normal

### Table 1 (continued)

<table>
<thead>
<tr>
<th>Patients VWD type (SSC ISTH) [4]</th>
<th>VWF:Ag</th>
<th>RCo</th>
<th>Ratio/ RCo/Ag</th>
<th>VWD type re-classified [5]</th>
<th>BT</th>
<th>PFC, s</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>ADP</td>
</tr>
<tr>
<td>Type 2B</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>208</td>
<td>88</td>
<td>0.42</td>
<td>2B mild</td>
<td>9</td>
<td>–</td>
</tr>
<tr>
<td>2</td>
<td>51</td>
<td>17</td>
<td>0.33</td>
<td>2B</td>
<td>&gt;20</td>
<td>&gt;250</td>
</tr>
<tr>
<td>3</td>
<td>61</td>
<td>15</td>
<td>0.25</td>
<td>2B</td>
<td>14</td>
<td>&gt;250</td>
</tr>
<tr>
<td>Type 3</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>4 patients</td>
<td>&lt;1</td>
<td>&lt;3</td>
<td>–</td>
<td>3</td>
<td>&gt;20</td>
<td>&gt;250</td>
</tr>
<tr>
<td>Type 2N</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>83</td>
<td>78</td>
<td>0.94</td>
<td>FVIII:BD</td>
<td>5.5</td>
<td>81</td>
</tr>
<tr>
<td>2</td>
<td>67</td>
<td>54</td>
<td>0.81</td>
<td>FVIII:BD</td>
<td>4</td>
<td>94</td>
</tr>
<tr>
<td>Hemophilia A</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>12 patients</td>
<td>&lt;1–27</td>
<td>57–200</td>
<td>54–164</td>
<td>0.80–1.00</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Hemophilia B</td>
<td>factor IX</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>1.5</td>
<td>74</td>
<td>58</td>
<td>0.78</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>2</td>
<td>3</td>
<td>190</td>
<td>186</td>
<td>0.89</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Normal values</td>
<td>56–207</td>
<td>58–209</td>
<td>&gt;0.60</td>
<td>&lt;8.5</td>
<td>&lt;120</td>
<td>&lt;160</td>
</tr>
</tbody>
</table>
values in 67 patients with diagnosed or suspected VWD type 1 (open symbols, fig. 4). In fact this patient cohort consisted of patients with mild/moderate VWD type 1 or pseudo VWD with no VWF deficiency (fig. 4). There are 2 patients with normal VWF values and increased PFC not due to VWD. Patients with VWF values between 0.50 and 0.80 IU/dl and normal PFC do not need to be treated for major surgery or trauma except for DDAVP 0.3 μg/kg once preoperatively for security reasons and have to be monitored by PFC and VWF parameters postoperatively. The PFC were normal or increased (>300 s) in the majority of patients and strongly prolonged (>300 s) in only a few cases of VWD type 1 (fig. 4). The VWD type 1 patients treated with DDAVP demonstrated a good dose-response curve with correction of PFC (closed symbols, fig. 4). PFC normalized (<150 s) in the majority of patients with VWD type 1. Normalization of PFC (<150 s) was reached at about 0.75 U/ml. The minimal PFC (about 80 s) or the maximal effect of von Willebrand factor was obtained at a level between 2.0 and 2.5 U/ml (fig. 4). The responses to DDAVP of VWF parameters were not significantly different from FVIII (Bonferroni’s multiple comparison test). Interestingly, the response of VWF:RCo to DDAVP showed two clusters (fig. 4; lower left). First, those with maximal values of VWF:RCo between 0.50 and 1.2 U/ml, consistent with a restricted response to DDAVP in VWD type 1, and second those with normal values of VWF:RCo >1.5–4.5 U/ml indicated complete normal responses to DDAVP in patients (individuals) with mild VWF deficiency before DDAVP. The results in figure 4 are in agreement with published data of Fressinaud et al. [6, 7] and Franchini et al. [8] and sub-
Fig. 2. Severe VWD type 1 with restricted response of VWF and normal response of FVIII to DDAVP indicative of a VWD type 1 secretion defect (left) versus completely normal responses of VWF parameters and FVIII:C levels to DDAVP in mild VWF-deficiency with normal FVIII/VWF:Ag and VWF:RCo/VWF:Ag ratios before and after DDAVP consistent with the diagnosis of pseudo VWD (right) [3].

Fig. 3. Means (black dots) ± SD and ranges outside SD of VWF:Ag, VWF:RCo and PFC before and 1 h after DDAVP (0.3 μg/kg) in 24 patients with VWD type 1 with normal multimers in the VWD type 1 study by Franchini et al. [8].
Diagnosis and Molecular Basis of Mild VWD Type 1

In 2003, Sadler [13] nicely extended the 2002 concept of Michiels et al. [2] in distinguishing severe, moderate, mild and pseudo VWD and calculated that most diagnoses of mild VWD type 1 are false-positive VWD. In 2002, we have labeled the category of false-positive VWD type 1 as pseudo VWD characterized by very mild VWF deficiency and completely normal responses to DDAVP of VWF parameters and FVIII:C (fig. 1A right) [5]. In the general population, 25% have one or two mild (clinically insignificant) bleeding symptoms and 2.5% of the general population have low plasma VWF; therefore it can be calculated that 0.25 × 0.025 = 0.6% individuals in the general population have a combination of low VWF and bleeding just by chance [10]. Patients with mild VWD type 1 with VWF:Ag, VWF:RCo and VWF:CB levels between 0.30 and 0.60 U/ml and normal VWF:RCo/Ag and VWF:CB/Ag ratios usually present with mild bleeding symptoms, no family history of bleeding and normal BT and normal PFC on repeated occasions [1, 3].

ABO blood group is a significant determinant of plasma VWF concentrations. In the study by Gill et al. [14], 456 normal individuals with blood group O had the lowest VWF:Ag levels (mean 0.75 U/ml, range 0.356–1.57) followed by blood group A (mean 1.06, range 0.48–2.33) and then blood group B and AB with completely normal VWF levels. About one third of individuals with blood group O, only a few with blood group A and hardly any

Fig. 4. Dose-response relation between PFC and FVIII-VWF in mild VWD type 1 patients before (□) and 1 h after (■) DDAVP infusion [9]; 0.80 IU/dl is the lower limit of normal for VWF:Ag and VWF:RCo (blood group non-O) and 150 s is the upper limit of normal for PFC.
with blood group B and AB have VWF levels <0.60 U/ml. Of 114 patients diagnosed as VWD type 1 in the population are asymptomatic or manifest mild bleeding, and have VWF levels at 50% of normal (true type 1 of VWD according to the law of Mendel) [15–21]. Such OC of a null allele or missense mutation may become more symptomatic when associated with blood group O or another modifier of the VWF level. Castaman and Eikenboom [22] demonstrated that ABO blood group significantly influences the VWF:Ag levels in OC of a null allele related to VWD type 3 or the missense mutation C2364F related to severe recessive VWD type 1 (table 2). From a genotypic point of view, obligatory carriers of a null allele in VWD type 3 are very similar to asymptomatic or mild VWD type 1 patients with a single missense allele.

We studied a consanguineous family with VWD type 3. The propositus was a boy with VWD type 3, who presented with mucocutaneous bleeding and recurrent hemarthrosis of an ankle. Laboratory analysis found FVIII:C <1% and an absence of VWF:Ag due to the homozygous nonsense splice site mutation IV7+1 G→A in intron 7 (0874+1G→A) [23]. Both parents were heterozygous for the nonsense mutation and completely asymptomatic with near normal to normal values for FVIII:C and VWF parameters, and normal ratios for FVIII:C/VWF:Ag (table 3). After DDAVP, the FVIII:C levels rose to much higher levels compared to VWF:Ag, VWF:RCo and VWF:CB levels. FVIII:C/VWF:Ag ratios were 1.3 before DDAVP but >3 after DDAVP, consistent with a carrier of a VWF null allele (table 2). The VWF:RCo/Ag and VWF:CB/Ag ratios were normal before and after DDAVP, consistent with true type 1 of VWD (table 3), demonstrating that an increased FVIII:C/VWF:Ag ratio after DDAVP is typically diagnostic for true VWF deficiency type 1 heterozygous for a null allele. This observation could also be confirmed by Lethagen et al. [24] in a carrier of a null allele (Q2470X/normal, fig. 5).

### Molecular Basis of Mild VWD Type 1 with Normal or Abnormal VWF Multimers

Parents of patients with recessive VWD type 3 are OC of a null allele and patients with severe VWD type 1 are carriers of a missense mutation (fig. 6) [1, 15–21]. Such heterozygous OC of a VWF null allele or a missense mutation have no history of bleeding or present with minor bleedings (one or two bleeding symptoms, mainly epistaxis, bruises and/or prolonged menstruations with no abnormal bleeding after tooth extraction, trauma or surgery). There is a wide range of values from 0.11 to 1.28

### Table 2. VWF:Ag levels in heterozygous carriers for a null allele related to pseudo hemophilia A-VWD type 3 and for the mutation C2364F related to severe recessive VWD type 1 [22]

<table>
<thead>
<tr>
<th>Carriers</th>
<th>Patients</th>
<th>VWF:Ag IU/dl</th>
<th>VWF:Ag range, IU/dl</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Null allele</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Blood group O</td>
<td>15</td>
<td>43.2 ± 10.8</td>
<td>30–66</td>
</tr>
<tr>
<td>Blood group non-O</td>
<td>15</td>
<td>61.3 ± 23.6</td>
<td>25–98</td>
</tr>
<tr>
<td><strong>C2364F</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Blood group O</td>
<td>8</td>
<td>35.2 ± 16.2</td>
<td>25–55</td>
</tr>
<tr>
<td>Blood group non-O</td>
<td>15</td>
<td>61.5 ± 26.6</td>
<td>30–140</td>
</tr>
</tbody>
</table>

Fig. 5. Good response of FVIII:C and restricted response of VWF:RCo to DDAVP (0.3 μg/kg) in a carrier of a null allele (Q2470X/normal, true type 1 of VWD) [19].

---


Michiels/Berneman/Gadisseur/van der Planken/Schroyens/van Vliet
U/ml for FVIII:C, from 0.94 to 12 for VWF:Ag, with ratios of FVIII:C/VWF:Ag from normal to >2 indicating the difficulty to distinguish carriers of VWF nonsense or missense mutations from VWF deficiency related to blood group O (fig. 6) [1, 16–19]. Carriers of recessive VWD type 3 heterozygous for the VWF null allele have a ratio of 2.06 for FVIII:C/VWF:Ag, and this ratio appeared to be dependent on the severity of the VWF:Ag deficiency with ratios of 3.2, 1.96 and 1.46 at VWF:Ag plasma levels <30, between 30 and 60 and >60 U/ml, respectively [1].

The 2006 database of the SSC of the ISTH reports 58 null alleles and 14 missense alleles involved in the etiology of type 3 VWD. The null alleles are located all over the

---

**Fig. 6.** Relationship of mild VWD type 1, blood group O versus non-O, carriers of nonsense and missense mutations in the VWF gene related to recessive severe VWD type 1 and 3.

**Table 3.** Response of FVIII:C and VWF parameters to DDAVP (0.3 μg/kg) in an OC of a null allele heterozygous for the nonsense splice site mutation IV7+1G→A in intron 7 (0874+1G→A) [23]

<table>
<thead>
<tr>
<th></th>
<th>Before DDAVP</th>
<th>After DDAVP</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>1 h</td>
</tr>
<tr>
<td>FVIII:C, IU/ml</td>
<td>0.84</td>
<td>5.0</td>
</tr>
<tr>
<td>VWF:Ag, IU/ml</td>
<td>0.64</td>
<td>1.3</td>
</tr>
<tr>
<td>VWF:RCo, IU/ml</td>
<td>0.67</td>
<td>1.8</td>
</tr>
<tr>
<td>FVIII:C/VWF:Ag ratio</td>
<td>1.3</td>
<td>3.8</td>
</tr>
<tr>
<td>VWF:RCo/A aggregates</td>
<td>1.05</td>
<td>1.38</td>
</tr>
</tbody>
</table>

---

**Diagnosis and Molecular Basis of Mild VWD Type 1**

Acta Haematol 2009;121:85–97
VWF gene in nearly all exons from 3 to 52 [15]. Missense mutations either homozygous (double heterozygous) or associated with a null allele as the cause of severe VWD either type 3 or severe type 1, are mainly located in the D1–D2 domains (D47H, S85P, Y87S, D141Y, D141N, C275S, W377C and I427N) and the D4, B1–B3, C1–C2 and cysteineknot domains (P2063S, C2174G, C2362F, N2546Y, C275S, W377C and I427N), but not in the D3, A1 and A2 domains [15]. There is one report of a severe type 1 VWD due to double heterozygosity of a missense mutation in the D3 domain, C1071F with a null allele [13]. Some so-called type 3 VWD patients who are compound heterozygous for a null allele and a missense mutation have detectable but very low VWF levels and measurable FVIII:C are incorrectly diagnosed as type 3 and should be reclassified as autosomal recessive severe VWD type 1 (fig. 6) [15–20]. The missense mutations in carriers of VWD 2C are located in the D1 and D2 domains [1, 15].

Heterozygous OC of a nonsense mutation related to VWD type 3 and heterozygous OC of a missense mutation related to severe recessive VWD type 1 in the population are asymptomatic or manifest mild bleeding, have VWF levels at 50% of normal. Following the laws of Mendel defining recessive disease, figure 6 shows the relationship between mild VWD type 1, blood group O versus non-O, heterozygous carriers of nonsense and missense mutations in the VWF gene related to recessive severe homozygous or double heterozygous VWD type 1 and type 3. Heterozygous carriers of a null allele or missense mutation do have lower VWF values and may become more symptomatic when associated with blood group O or another modifier of the VWF level (table 2; Castaman et al. [26] and Gallinaro et al. [25] in fig. 6). From a genotypic point of view, OC of VWD type 3 are very similar to asymptomatic or mild VWD type 1 patients with a single mutated allele. OC of type 3 with a null mutation or severe VWD type 1 with a missense mutation may have bleeding symptoms and meet the criteria of mild VWD type 1 in particular when associated with blood group O (fig. 6). Using the bleeding score assessment, Castaman et al. [26] compared the severity of bleeding symptoms in 70 OC of recessive VWD type 3, 42 OC of VWD type 1 and in 215 normal controls. OC of VWD type 3 with a null mutation had clearly less severe bleeding than patients diagnosed as VWD type 1. OC of VWD type 1 with a missense mutation were distinct from normal controls, presenting more epistaxis, cutaneous bleeding and usually do not significantly bleed after surgery.

The concept in figures 6 and 7 points to the wide heterogeneity of mild VWD type 1 as a heterozygous disorder with variable penetrance of bleeding manifestations but clearly distinct from the autosomal dominant VWD phenotypes VWD type 1 Vicenza, type 1 clearance defect with 2E multimeric pattern (I/2E) or 2M [27–29]. The DDAVP-induced response curves of FVIII:C, VWF:Ag, VWF:RCo and VWF:CB will clearly reveal typical diagnostic features to distinguish mild VWD type 1 with normal VWF function, clearance and multimers from those with abnormal multimers, clearance defects and/or VWF:RCo and RIPA defects in each of VWD type Vicenza, type 1C/2E, 2M, type 1 with smeary VWF multimers (1sm) or 1sm with faster moving band (1smf) [30–34].

Collins et al. [33] recently updated the mutations in the Canadian, European and UK VWD-1 studies (fig. 7). In the Canadian, European and UK VWD type 1 study the mutations 2436delC, 2685+2T→C, 3537+1G→A, 2686-1G→C3072delC, 3108+5G→Aand3379+1G→C, Q2544X, 7437+1G→A and 8412insTCCC are null alleles and associated with very mild VWD with low penetrance of bleeding [30–33]. The European MCMDM-I VWD study did contain typical examples of recessive or heterozygous VWD type 2N (heterozygous R816W, R854W and R854W/ R924Q, R854W/null) [30]. The mutations in exon 26, D3 domain, R1130G/F, W1144G, Y1146C and C1190R, in figure 7 usually present with a laboratory phenotype of dominant VWD 1 but have abnormal VWF multimers with typical features of VWD 2E [30]. In the study by Gadisseur et al. [27], this entity was classified as dominant VWD type 1/2E. Patients with dominant VWD type 1/2E due to missense mutations in the D3 domain are characterized by a multimerization defect and the secreted heterozygous mutant-normal VWF after DDAVP is rapidly cleared, a phenomenon very well known for many years and most prominent in VWD type 1 Vicenza, R1205H [34]. With the exclusion of VWD type Vicenza R1205H, VWD type 1/2E, 2N and 2M, in patients with mild VWD type 1 and normal multimers, missense mutations are mainly located in the regulatory sequence region (fig. 7), the D1–D2 propeptide region (fig. 8), the D’ VWF-FVIII binding site region K762E, M771I, P812fs, exon 21 skip, R924Q, R924W and C996E (fig. 6), and only a few in the D3 (S1024fs and I1094T), A1 (F1280fs, R1379C, P1413L and Q1475X) or A2 domain (R1583W and Y1584C; fig. 7) [30–33]. This large cohort of mild VWD with low to variable penetrance of bleeding has a (increased) prevalence of blood group O, VWF values >30% with normal ratios of VWF:RCo/VWF:Ag, VWF:CB/VWF:Ag and FVIII:C/VWF:Ag. Within this group of mild VWD type 1 with low penetrance of bleeding, the combination of C1584 and blood group O is rather frequent and typically...
shows a good/normal response to DDAVP [35, 36]. Interestingly, the mechanism of mild VWD type 1 of the C1584Y mutation is slightly increased in VWF proteolysis, which is typical for a mutation in the A2 domain. There is one case report on a homozygous Y1584C mutation characterized by moderate VWD type 1 with FVIII::C and VWF levels around 25% [37]. The response curves of FVIII:C and VWF parameters to DDAVP significantly contribute to a much better characterization of patients with various variants of VWD type 1 and 2 [6–9, 15, 16].

The VWD-1 studies discovered a new category of mild VWD type 1 due to mutations in the D4, B1–B3 and C1–C2 domains of the VWF gene with either normal or abnormal multimers [30, 32]. The group with normal VWF multimers (L1774S, K1794E, C2360Y, R2313H, G2518S, Q2544X, C2693Y and P2722A) has mild VWD type 1, either dominant or recessive with variable penetrance of bleeding manifestations (fig. 7) [30]. The group with abnormal multimers of heterozygous mutations in the D4, B1–B3, C1–C2 and cysteine knot domains (V1822G, L2207P, C2257S, C2304Y, C2362F, G2441C, R2464C, C2477Y, C2477S and Q2520P) has mild/moderate VWD type 1, and usually smeary pattern or smeary pattern with faster moving bands indicating abnormal VWF multimers discovered using low- and medium-resolution agarose gel [38–40]. As described by Gadisseur et al. [27], careful phenotyping of the missense mutations in the D4, B1–B3 and C1–C2 domains is warranted and has been performed for only a few in the European MCMDM-VWD study. Additional expression studies of mutant VWF will help to predict normal or abnormal banding of mutant VWF multimers as the cause of a normal or smeary pattern in heterozygous cases [31, 38–41]. Different types of smeary patterns of VWF multimers documented by Budde et al. [38, 40] are more pronounced after DDAVP for mutations around the cleavage site of VWF propeptide and mature VWF, 763, in some cases of VWD 2M located in the A1 domain and in the mutations R2464C, C2362F, C2304Y, C2441C, C2477Y, C2477S located in the C1 domain of the VWF gene [36–41].
References


Diagnosis and Molecular Basis of Mild VWD Type 1


C1584: Effect on von Willebrand Factor Proteolysis and von Willebrand Factor Antigen Levels

James Anthony Davies  Peter William Collins  Lee Sarah Hathaway  Derrick John Bowen
Department of Haematology, School of Medicine, Cardiff University, Cardiff, UK

Key Words
ADAMTS13 • Blood group O • C1584 • Proteolysis • Y/C1584

‘Discovery’ of C1584

The C1584 variant of von Willebrand factor (VWF) first gained attention as a result of a search for a founder VWF gene haplotype in type 1 von Willebrand disease (VWD) [1]. The study revealed that the ‘G’ allele of the single nucleotide polymorphism 4751A/G was enriched among a cohort of unrelated Canadian type 1 VWD patients compared with controls. 4751A/G encodes the amino acid variation tyrosine/cysteine at residue 1584 (Y/C1584) in VWF. The 4571A allele encodes Y1584 and the 4751G allele encodes C1584, thus C1584 was enriched among the Canadian type 1 VWD cohort. The study further demonstrated that C1584 caused marked intracellular retention when expressed in the homozygous form in cell culture and this offered a rationale for the decreased VWF level in type 1 VWD [1]. However, statistically non-significant intracellular retention was observed when C1584 was expressed in heterozygous form [1].

Shortly afterwards, C1584 gained even greater relevance when it was demonstrated that heterozygous Y/C1584 VWF showed increased susceptibility to proteolysis by the metalloprotease ADAMTS13 (a disintegrin and metalloprotease with thrombospondin repeats) [2]. This discovery arose from studies investigating the possibility that ADAMTS13 proteolysis of VWF may form part of the catabolic pathway for VWF and hence may be impli-
Table 1. Mean VWF level according to Y/C1584 and ABO blood group phenotypes in 5,052 blood donors from South Wales (UK)

<table>
<thead>
<tr>
<th>Blood group</th>
<th>Y/C1584 individuals</th>
<th>Y/C1584 individuals</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>n</td>
<td>VWF:Ag IU/dl^{-1}</td>
<td>n</td>
</tr>
<tr>
<td>All</td>
<td>5,002</td>
<td>111 ± 37</td>
<td>50</td>
</tr>
<tr>
<td>O</td>
<td>2,307</td>
<td>95 ± 29</td>
<td>23</td>
</tr>
<tr>
<td>A</td>
<td>2,072</td>
<td>124 ± 37</td>
<td>17</td>
</tr>
<tr>
<td>B</td>
<td>444</td>
<td>131 ± 37</td>
<td>8</td>
</tr>
<tr>
<td>AB</td>
<td>179</td>
<td>137 ± 43</td>
<td>2</td>
</tr>
</tbody>
</table>

= Not done due to insufficient individuals in one of the groups being compared.

C1584: Effect on VWF Proteolysis and VWF:Ag

With 4751G in VWF and hence C1584 in VWF. Thus the proteolysis findings coincided directly with the findings for the founder haplotype in the Canadian type 1 VWD study described above [1] and offered a mechanism for the enrichment of 4751G in type 1 VWD.

Subsequent studies have (1) corroborated the enrichment of C1584 in type 1 VWD [6]; (2) shown that C1584 is necessary for enhanced VWF proteolysis and not simply linked to a causative change elsewhere in VWF [7], and (3) demonstrated that C1584 is not, on its own, causative of type 1 VWD: in families in which it is present, the variant is found in both affected and unaffected individuals [6].

Properties of C1584

To determine more accurately the effect of C1584 on VWF level, function and proteolysis, a cohort of individuals possessing the variant was required that was sufficiently large to allow statistical analysis. Preliminary estimates of frequency suggested that 1 in 100 individuals were heterozygous Y/C1584 [1, 2], and power calculations indicated that a cohort of 50 heterozygotes would permit statistical analysis of relevant phenotypic parameters. A cohort of 5,052 blood donors was recruited and genotyped for C1584, this yielded 50 Y/C1584 heterozygous individuals and 5,002 Y/Y1584 homozygous individuals; no C/C1584 homozygous individuals were detected [8]. The findings indicated a rate of heterozygosity of 1% for Y/C1584 in the population. The following paragraphs summarise the data obtained from the large blood donor cohort study [8].

The mean VWF:Ag level for Y/C1584 heterozygotes was significantly lower than for Y/Y1584 homozygotes (table 1). Within each ABO blood group, VWF:Ag was lower for Y/C1584 heterozygotes compared with Y/Y1584 homozygotes and this was statistically significant in blood groups O and A, for which there were sufficient Y/C1584 heterozygotes for valid comparisons to be made (table 1).

Heterozygosity for Y/C1584 had a greater effect on VWF:Ag than blood group O. This was illustrated by two comparisons: (1) for all Y/C1584 heterozygotes collectively, the mean VWF:Ag was lower than for all blood group O donors collectively (82 ± 35 vs. 95 ± 29 IU/dl^{-1}, p < 0.001), and (2) for group O Y/C1584 heterozygotes the mean VWF:Ag was 58 ± 14 IU/dl^{-1} whereas for all group O Y/Y1584 homozygotes the mean VWF:Ag was 95 ± 29 IU/dl^{-1} (p < 0.001).
Approximately 1.5% of the entire blood donor cohort had a VWF:Ag ≤50 IU/dl⁻¹. In contrast, 2.4% of blood group O donors and 18% of Y/C1584 heterozygous donors had VWF:Ag ≤50 IU/dl⁻¹. Most strikingly, approximately 35% of donors who were blood group O and Y/C1584 heterozygous had VWF:Ag ≤50 IU/dl⁻¹. The data demonstrated an enrichment for Y/C1584 heterozygosity among donors with a low VWF:Ag level and this enrichment was 7.5-fold greater than for blood group O. Furthermore, the data suggested a synergistic interaction between Y/C1584 heterozygosity and blood group O in decreasing the VWF level; the enrichment for both together was almost equal to the product of each alone (35 vs. 18 and 2.4%).

Enhanced in vitro proteolysis by ADAMTS13 was observed for VWF from all 50 Y/C1584 heterozygous plasma samples. Proteolysis was measured by the percent loss in VWF collagen binding activity (VWF:CB) – which is related to VWF multimer length – after a constant incubation period and was approximately 50% higher for Y/C1584 VWF compared with Y/Y1584 VWF. Evidence for altered proteolysis in vivo was obtained by the demonstration of a subtle but highly reproducible difference in the triplet structure of plasma VWF from Y/C1584 heterozygotes compared with Y/Y1584 homozygotes; there was a relative decrease in the upper satellite band of each multimer triplet in heterozygous compared with homozygous VWF. This may appear contrary to expectation (proteolysis gives rise to the triplet structure therefore enhanced proteolysis would be expected to yield relative increases in the satellite bands), however it is consistent with previously published VWF multimer profiles in type 2A VWD in which VWF shows markedly increased susceptibility to proteolysis: a relative decrease in the upper satellite band is observed in some cases, giving the multimer profile the appearance of doublets rather than triplets [9]. There are currently no data to indicate whether this subtle change in triplet structure has any physiological or functional relevance.

C1584 was associated with a decrease in phenotypic measures of VWF function. Comparison of the mean VWF:CB and mean ristocetin cofactor activity (VWF:RCO) for the 50 Y/C1584 heterozygotes and 50 random ABO-matched Y/Y1584 homozygotes demonstrated a highly significant decrease for both parameters for heterozygous VWF (table 2). The ratios of VWF:CB to VWF:Ag and VWF:RCO to VWF:Ag were likewise both significantly lower for Y/C1584 VWF compared with Y/Y1584 VWF (table 2). These data could be explained by a direct effect of cysteine at residue 1584 on the ability of VWF to bind both collagen and platelet glycoprotein 1b, or by an alteration in the composition of plasma VWF multimers in heterozygotes (potentially a minor relative decrease in high-molecular-weight forms) arising from enhanced proteolysis.

### C1584 and VWF Clearance

The possibility that C1584 may influence VWF clearance has recently been explored by examination of the ratio of VWF propeptide to VWF:Ag, and the ratio of factor VIII coagulant activity to VWF:Ag [10]. Both ratios have been used previously to provide information on the steady state kinetics of VWF [11, 12]. The ratios were determined for the 50 Y/C1584 heterozygous plasma samples and the 50 random ABO-matched Y/Y1584 homozygous plasma samples. The data indicated that clearance of heterozygous Y/C1584 VWF was marginally higher than that for Y/YVWF and additionally supported an interaction between blood group O and C1584 in enhancing VWF clearance [10]. The data further suggested that secretion was not significantly decreased among heterozygotes, a finding that was consistent with the previous expression studies noted above which showed a statistically non-significant intracellular retention when C1584 was expressed in heterozygous form [1].

### Physiological and Clinical Relevance of C1584

The data from the cohort of 50 individuals heterozygous for C1584 indicate that, overall, the variant is associated with a decreased VWF level (especially in combination with blood group O), with decreased VWF function, with slightly enhanced VWF proteolysis and with a mar-

---

### Table 2. VWF functional activity and Y/C1584 phenotype

<table>
<thead>
<tr>
<th>Measure of functional activity</th>
<th>Y/Y1584 individuals (n = 50)</th>
<th>Y/C1584 individuals (n = 50)</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>VWF:RCO, %</td>
<td>110 ± 43</td>
<td>73 ± 40</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>VWF:CB, %</td>
<td>124 ± 41</td>
<td>83 ± 36</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>VWF:RCO/VWF:Ag ratio</td>
<td>1.0 ± 0.2</td>
<td>0.9 ± 0.2</td>
<td>0.01</td>
</tr>
<tr>
<td>VWF:CB/VWF:Ag ratio</td>
<td>1.16 ± 0.29</td>
<td>1.04 ± 0.20</td>
<td>0.02</td>
</tr>
</tbody>
</table>

1 ABO matched.

---

Davies/Collins/Hathaway/Bowen
Originally increased rate of VWF clearance. Physiologically, these properties could compromise clot formation and this in turn could lead to clinically relevant bleeding.

However, it is evident that for a given heterozygous individual, VWF level, function and clearance may be normal – C1584 shows variable penetrance, its absolute effect is modified by additional factors which are predominantly unknown (ABO blood group is certainly one of them).

Enhanced proteolysis of VWF by ADAMTS13 was a consistent finding for all 50 Y/C1584 heterozygotes. This property may be highly relevant at a time of haemostatic challenge [5]. During normal clot formation, VWF multimers recruit platelets to the site of vessel damage, and are, at the same time, proteolysed by circulating ADAMTS13. In the extreme scenario where all VWF multimers are proteolysed to the shortest length before any platelets bind, there would be little scope for a soft clot to form. The rate of VWF proteolysis can therefore have a profound effect upon the kinetics and success of clot formation. The enhanced proteolysis associated with C1584 is not as dramatic as the extreme scenario illustrated above, however it may be sufficient to have an adverse effect on haemostasis. Depending upon the extent of this within an individual, clinically relevant bleeding may result [5].

**Conclusion**

The C1584 variant of VWF has the potential to exert a variety of negative effects that may predispose towards bleeding. It is evident that within an individual the effect of C1584 is attenuated or exacerbated by modifiers and these result in a variable phenotype between individuals. What these modifiers are, and how many there may be, is currently unknown, however the variable penetrance of C1584 is testimony to their existence. Future research focussing on C1584 could investigate its efficiency in primary haemostasis in more physiological settings – how do the in vitro proteolysis findings translate in terms of clot formation? Additionally, future research could be aimed at identifying the modifiers that confer variable penetrance on C1584. Such research may be fruitful in providing insight into the penetrance of VWD in general. Genome-wide association studies for loci involved in the control of the VWF level represent a powerful way to approach this. A better understanding of the variables influencing the VWF level and function may facilitate diagnosis and counselling in type 1 VWD families in which (and individuals in whom) C1584 is present.

**References**


Factor VIII-von Willebrand Factor Binding Defects in Autosomal Recessive von Willebrand Disease Type Normandy and in Mild Hemophilia A

New Insights into Factor VIII-von Willebrand Factor Interactions

Marc Jacquemin
Center for Molecular and Vascular Biology, University of Leuven, Leuven, Belgium

Abstract
This concise review is focused on genetic, molecular and clinical aspects of von Willebrand disease (VWD) type 2N and of mild hemophilia A due to mutations impairing FVIII-von Willebrand factor (VWF) interactions. Missense mutations in the VWF gene impairing the binding to FVIII do not impair the structure of VWF multimers nor the ability of VWF to aggregate platelets but causes an accelerated clearance of FVIII. Missense mutations in the FVIII gene impairing the binding to VWF are a common cause of mild/moderate hemophilia A. The implications of these observations for the treatment of patients with coagulation factor concentrates and desmopressin are discussed.

Copyright © 2009 S. Karger AG, Basel

Introduction

One of the unique structural aspects of von Willebrand factor (VWF) is that it can form a complex with factor VIII (FVIII). In plasma, VWF transports and stabilizes FVIII. VWF is also important for FVIII secretion and possibly contributes to intracellular trafficking and storage of FVIII.

The importance of VWF for FVIII stability was first demonstrated by the observation that severe von Willebrand disease (VWD) with no circulating VWF results in secondary FVIII deficiency [1]. Later, a missense mutation impairing the capacity of VWF to bind to FVIII was identified in a patient originating from Normandy in France. This mutation did not impair the structure of VWF multimers nor the ability of VWF to aggregate platelets but causes an accelerated clearance of FVIII. This type of VWD was called ‘Normandy’ or type 2N. Conversely, FVIII mutations impairing FVIII binding to VWF were identified as a common cause of mild/moderate hemophilia A [4-8].

FVIII-VWF Interaction

VWF binds FVIII via the amino-terminal part of the molecule, the D’ and D3 domain, whereas FVIII contacts VWF through the carboxy-terminal A3, C1 and C2 domains forming the light chain. In vitro studies established that VWF protects FVIII by different mechanisms. VWF may facilitate the binding of FVIII heavy and light...
chains, increase secretion and protect FVIII from degradation or clearance. In addition, VWF also regulates FVIII activation by factor Xa and thrombin.

FVIII-VWF interaction is also important for the FVIII increase following 1-deamino-8-D-arginine vasopressin (DDAVP) administration, i.e. the treatment of choice for patients with mild/moderate hemophilia A. The mechanisms responsible for the FVIII increase following DDAVP administration remain to be elucidated.

The kinetics of the response to DDAVP suggests that the FVIII increase in plasma is not due to accelerated synthesis but rather to FVIII release from a storage pool. This FVIII storage is believed to be dependent on the interaction of FVIII with VWF because FVIII levels remain unchanged following DDAVP administration in patients with severe VWD [9, 10]. The lung endothelial cell was recently identified as a co-storage site for both FVIII and VWF [11, 12].

**VWD Type 2N**

VWD 2N is inherited in a recessive manner. The disease is caused by mutations located at the amino-terminus of mature VWF in the FVIII binding site [3, 13–16]. Patients are either homozygous for type 2N mutations or compound heterozygous carrying two type 2N mutations. Compound heterozygosity for a VWD 2N mutation and a quantitative defect on the second allele are also frequent [17, 18].

The diagnosis of VWD 2N is based on the measurement of the ability of plasma VWF to bind exogenous FVIII. This test should be performed in all patients with a FVIII deficiency not linked to hemophilia A.

Homozygous mutations result in a clinical phenotype indistinguishable from mild/moderate hemophilia A by conventional laboratory tests. Compound heterozygosity with a quantitative VWF defect may appear as VWD type I. A normal multimer distribution is observed in most patients. However, some mutations eliminating or introducing additional cysteines influence the multimer structure, resulting in loss of large multimers or expression of aberrant high-molecular-weight multimers. Such mutations result in both impaired FVIII binding and a low ratio of VWF ristocetin cofactor activity to VWF antigen [2, 3, 13–16] (fig. 1).

In most patients, bleeding episodes are similar to those in patients with mild/moderate hemophilia A, with bleeding occurring after trauma or surgery. In some patients, a defect in primary hemostasis is also observed. Patients can frequently be treated with DDAVP, which induces a rise in FVIII despite the defect in VWF binding. Alternatively, patients should receive VWF concentrates able to stabilize endogenous FVIII that they synthesize at normal levels. VWD 2N patients should not be treated with FVIII products not containing VWF.

---

**Fig. 1.** Mutations reducing FVIII binding to VWF result in mild/moderate hemophilia A and type 2N VWD.
Mild/Moderate Hemophilia A due to Mutations Impairing FVIII Binding to VWF

The first mutation reducing FVIII binding to VWF was shown to affect a single residue, Tyr1680 in the FVIII A3 domain. This substitution prevents sulfation which is critical for FVIII binding to VWF and significantly reduces the affinity of the mutated FVIII for VWF [4]. Although the role of C2 in FVIII binding to VWF had been well established, mutations in the C2 domain leading to altered FVIII binding to VWF were identified only much later [5–8]. Several mutations in the C1 domain resulting in mild/moderate hemophilia A also reduce binding to VWF [4–8]. Thus, mutations located in the FVIII light chain impairing FVIII binding to VWF now appear to be a common cause of mild/moderate hemophilia A. Many of those mutations affect surface-exposed residues delineating potential binding sites for VWF.

DDAVP administration is the treatment of choice for patients with mild/moderate hemophilia A. However, certain patients do not respond to DDAVP administration. It has to be noted that patients with VWD type 2 respond to DDAVP in a clinically useful manner [19] (fig. 2), and similar observations were recently made in patients with hemophilia A and mutations reducing FVIII binding to VWF [20, 21]. The mechanisms sustaining those paradoxically good responses to DDAVP are currently being investigated.

References


9 Cattaneo M, Simoni L, Gringeri A, Mannucci PM: Patients with severe von Willebrand disease are insensitive to the releasing effect of DDAVP: evidence that the DDAVP-induced increase in plasma factor VIII is not secondary to the increase in plasma von Willebrand factor. Br J Haematol 1994;86:333–337.


16 University of Sheffield: ISTH SSC VWF Database: http://www.vwf.group.shef.ac.uk/index.html


Autosomal Recessive von Willebrand Disease Type 1 or 2 due to Homozygous or Compound Heterozygous Mutations in the von Willebrand Factor Gene

A Single Center Experience on Molecular Heterogeneity and Laboratory Features in 12 Families

G. Castaman    S. Giacomelli    F. Rodeghiero

Department of Hematology and Hemophilia and Thrombosis Center, San Bortolo Hospital, Vicenza, Italy

Introduction

von Willebrand disease (VWD) is an inherited bleeding disorder caused by a deficiency in or abnormal function of von Willebrand factor (VWF), a multimeric protein which promotes platelet adhesion to the subendothelium and acts as a carrier for factor VIII (FVIII) in the circulation [1]. VWD can be grouped into two major categories, which are characterized by quantitative (type 1 and 3) or qualitative (type 2) VWF defects. Partial quantitative deficiency in VWF in plasma and/or platelets identifies type 1 VWD, whereas type 3 VWD is characterized by the virtual absence or trace amounts of VWF in plasma and platelets. Type 1 is easily distinguished from type 3 by its milder VWF deficiency (usually in the range of 20–40 U/dl), the autosomal dominant inheritance pattern and the presence of milder bleeding symptoms [2]. However, in recent years, it has become clear that the heterogeneity of clinical and laboratory phenotypes within some families with apparent type 1 was indeed explained by compound heterozygosity for VWF gene mutations. For example, the co-inheritance of the heterozygous R854Q VWF mutation, which alone usually does not cause bleeding, with het-
A large heterogeneity of the molecular bases of autosomal recessive VWD has been demonstrated [4]. The probands may be homozygotes for null alleles (partial or total gene deletion, stop codons, splice site or frameshift mutations, usually typical for type 3 VWD) or compound heterozygotes for missense mutations and null alleles, especially when VWF is measurable. In an initial group of patients from the Veneto region with recessive VWD, five families showed compound heterozygosity for the C2362F mutation and null mutations (R2535X, splice site mutation at position 11/24 in the consensus sequence of intron 13, 7375insC in exon 42), a single propositus only was a true homozygote for C2362F, one was compound heterozygous for Y1584C/4699delG resulting in V1485fs and the last for C2671Y and gene deletion [5]. Subsequently, we identified two additional families with the splice site mutation in intron 13 and C2362F [6], a family with a novel nonsense mutation (229 C→T in exon 4 predicting Q77X), a novel null mutation (2908delC in exon 22) in compound heterozygosity for C2362F [7] and a family with compound heterozygosity for R365X/S1731T (table 1). All these probands had measurable VWF levels, while FVIII coagulant activity (FVIII:C) was moderately reduced. The increased FVIII:C/VWF:antigen (Ag) ratio indicates a defect in the synthesis and/or secretion of mutant VWF [8].

In general, the age at diagnosis was not different from that of patients homozygous for null alleles (type 3 VWD). In these families, a total of 62 subjects were heterozygotes for a single C2362F or null mutation and were mostly asymptomatic [5–7], as usually reported for the obligatory heterozygotes of type 3 VWD [9]. Interestingly, even though the range of measurements is wide and also influenced by blood group, most of these subjects have an increased FVIII:C/VWF:Ag ratio, as observed in carriers for null alleles.

Evidence indicates a small increase in VWF:Ag with age and lessening of the severity of spontaneous bleeding episodes, apart from menorrhagia which was mostly controlled by combined estroprogestinic treatment or the administration of FVIII/VWF concentrate. Multimeric analysis shows heterogeneous patterns, ranging from absence of high-molecular-weight multimers to the presence of all multimer forms with the lack of triplet structure, with faster running central band, smeary structure and indistinct satellite bands (fig. 1). The heterozygous subjects for C2362F showed minor abnormality only, with slightly faster central band and milder smeary pat-

<table>
<thead>
<tr>
<th>Family</th>
<th>Age at diagnosis (years)</th>
<th>VIII:C (IU/dl)</th>
<th>VWF:Ag (IU/dl)</th>
<th>VWF:RCo (IU/dl)</th>
<th>Bleeding time (min)</th>
<th>Blood group</th>
<th>VWF mutation</th>
</tr>
</thead>
<tbody>
<tr>
<td>T</td>
<td>3</td>
<td>20–31</td>
<td>4–6</td>
<td>&lt;3–6</td>
<td>&gt;30</td>
<td>A</td>
<td>Q77X/1534-3 C→A splice site intron 13</td>
</tr>
<tr>
<td>C</td>
<td>2</td>
<td>7–11</td>
<td>4–7</td>
<td>&lt;3</td>
<td>&gt;15</td>
<td>O</td>
<td>R365X/S1731T</td>
</tr>
<tr>
<td>S</td>
<td>4</td>
<td>25–37</td>
<td>10–12</td>
<td>&lt;3</td>
<td>15–&gt;20</td>
<td>O</td>
<td>4699delG/Y1584C</td>
</tr>
<tr>
<td>M</td>
<td>7</td>
<td>19–44</td>
<td>2–5</td>
<td>&lt;3</td>
<td>&gt;30</td>
<td>A</td>
<td>C2362F/C2362F</td>
</tr>
<tr>
<td>B</td>
<td>1</td>
<td>8–21</td>
<td>0.5–2</td>
<td>&lt;3</td>
<td>&gt;30</td>
<td>O</td>
<td>C2362F/7375insC</td>
</tr>
<tr>
<td>V</td>
<td>1</td>
<td>10–21</td>
<td>1–5</td>
<td>&lt;3</td>
<td>&gt;30</td>
<td>O</td>
<td>C2362F/1534-3 C→A splice site intron 13</td>
</tr>
<tr>
<td>DZ</td>
<td>3</td>
<td>18–24</td>
<td>5–7</td>
<td>&lt;3–6</td>
<td>&gt;15</td>
<td>B</td>
<td>C2362F/1534-3 C→A splice site intron 13</td>
</tr>
<tr>
<td>DZ</td>
<td>1</td>
<td>18–19</td>
<td>6–7</td>
<td>&lt;3–6</td>
<td>&gt;15</td>
<td>A</td>
<td>C2362F/1534-3 C→A splice site intron 13</td>
</tr>
<tr>
<td>F</td>
<td>1</td>
<td>25</td>
<td>8</td>
<td>6</td>
<td>&gt;15</td>
<td>O</td>
<td>C2362F/1534-3 C→A splice site intron 13</td>
</tr>
<tr>
<td>Z</td>
<td>1</td>
<td>8–21</td>
<td>1–4</td>
<td>&gt;3</td>
<td>&gt;30</td>
<td>A</td>
<td>C2362F/R2535X</td>
</tr>
<tr>
<td>P</td>
<td>1</td>
<td>14–21</td>
<td>3–8</td>
<td>&lt;3</td>
<td>&gt;15</td>
<td>B</td>
<td>C2362F/2908delC</td>
</tr>
<tr>
<td>A</td>
<td>9</td>
<td>10–19</td>
<td>1.8–3</td>
<td>&lt;3</td>
<td>&gt;30</td>
<td>O</td>
<td>C2671Y/gene deletion</td>
</tr>
<tr>
<td>I</td>
<td>4</td>
<td>18–33</td>
<td>5–6</td>
<td>&lt;3</td>
<td>20</td>
<td>O</td>
<td>C2671Y/W2193R</td>
</tr>
<tr>
<td>Normal range</td>
<td>–</td>
<td>52–173</td>
<td>47–165</td>
<td>51–188</td>
<td>&lt;7.5</td>
<td>–</td>
<td>–</td>
</tr>
</tbody>
</table>
tern, while carriers for the heterozygous ‘true’ null allele showed a normal multimeric pattern (fig. 1).

There have been some reports describing compound heterozygosity and homozygosity for other mutated cysteine residues (2739, 2754, 2804 and 2806) in type 3 VWD, all located in the carboxy-terminal cysteine knot domain of VWF involved in dimerization (reviewed by Eikenboom [4]). The C2362F mutation induces the loss of cysteine in a VWF region to which no function has been assigned, but it is located outside the cysteine knot domain, thus excluding a possible role in the interchain disulfide binding involved in dimerization. C2362F is minimally secreted, thus mimicking a null allele [10]. Patients homozygous or hemizygous for the C2362F mutation present high-molecular-weight multimers, but a smeary pattern, with faster moving central band and loss of triplet structure. Increased proteolysis of the mutant C2362F VWF has been reported [11], but this cannot be explained by increased susceptibility to proteolytic degradation by ADAMTS13 [10]. The multimeric pattern of heterozygotes for the C2362F mutation was intermediate, with faster mobility compared to normal plasma, but slower in comparison to compound heterozygotes or homozygotes [10].

At variance with observations with other cysteine mutations outside the cysteine knot domain (e.g. C1130F and C1149R) [12, 13], heterozygosity for C2362F does not induce a major decrease in plasma VWF. In heterozygotes for C2362F, plasma VWF is only slightly reduced, similarly to results for heterozygotes for type 3 VWD carrying null alleles [14]. This is in keeping with the demonstration that C2362F is minimally secreted and largely retained intracellularly [10].

Another frequent mutation in the Veneto region is the 1534-3 C→A mutation in the consensus sequence of the acceptor splicing site of intron 13. This mutation appears to be peculiar to type 1 VWD since homozygotes have no major multimeric abnormality and the mutation does not abolish the normal processing of mRNA [15]. In fact, in addition to skipping of exon 14 and the activation of a cryptic splice site in intron 13, both showing a premature stop codon in VWF propeptide, the correct recognition of the mutated splice site does occur, yielding measurable VWF levels [15]. To further support this view, homozygotes have greater VWF levels compared to patients with compound heterozygosity for C2362F [15].

**Therapeutic Implications**

Patients with compound heterozygosity or homozygosity for the C2362F mutation have a significant increase in FVIII:C after desmopressin (DDAVP), while the VWF changes are minimal [16]. Typically, FVIII:C can attain levels after infusion ≥ 50 U/dl, while VWF:Ag and VWF

---

**Fig. 1.** Multimeric pattern of plasma VWF. Lane 1 = Compound heterozygote for C2362F/splice site intron 13, diluted 1:10; lanes 2, 4, 6 = plasma from heterozygous C2362F subjects, diluted 1:10; lane 3 = normal plasma, diluted 1:20; lane 5 = plasma from C2362F/2908delC, diluted 1:5; lane 7 = plasma from a heterozygous 2908delC subject, diluted 1:10. Multimeric patterns of lanes 1 and 5 were obtained after longer gel exposure. Arrows indicate differences in the migration of multimers.
ristocetin cofactor activity reach values around 10 U/dl. In these patients, the compound was successfully used in some instances to treat or prevent bleeding. An even stronger effect was observed in the patient hemizygous for the splice site mutation in intron 13, resulting in a very prolonged FVIII:C survival. This pattern was observed also in a true homozygote [15]. In the latter case, FVIII:C was around 150 U/dl after infusion, while VWF amounted to twice the levels reached in hemizygous subjects. Interestingly, a significant increase in FVIII:C occurs during pregnancy, with levels clearly >50 U/dl prepartum.

No changes at all were observed after DDAVP in the patient with C2671Y/gene deletion, and the abnormal multimeric pattern (lack of high- and intermediate-molecular-weight multimers) persisted (fig. 2).

In conclusion, while it is well known that type 3 patients do not at all respond to DDAVP since their storage compartments do not synthesize VWF, recessive patients with severely reduced basal levels can have a significant FVIII:C rise after DDAVP treatment. In our experience, several minor bleeding episodes have been treated or prevented with DDAVP in these patients, thus reducing the costs of treatment inherent to the usage of FVIII/VWF concentrates. Therefore, a DDAVP trial is also warranted in patients with recessive inheritance and measurable FVIII/VWF parameters.

**Epidemiological Considerations**

The prevalence of null VWF alleles in the general population is unknown but may be higher than previously believed. A theoretical prevalence of 25 of 1,000,000 homozygotes is anticipated according to the Hardy-Weinberg equation on the basis of 1% symptomatic VWD heterozygotes in the population, but the observed genotypic heterogeneity of VWD would make the Hardy-Weinberg equation inapplicable [17]. This is also supported by the fact that type 3 VWD patients are not the offspring of symptomatic type 1 VWD [9,18]. The prevalence of cases with type 3 recessive VWD has been estimated to be between 0.11 and 0.55 out of 1,000,000 in Europe and 3.1 and 3.2 out of 1,000,000 in Sweden and Israel (reviewed by Eikenboom [4]). However, our observations suggest that these figures could be underestimated, since many patients have a true recessive disorder, which does not completely abolish VWF synthesis.

**References**


Laboratory and Molecular Characteristics of Recessive von Willebrand Disease Type 2C (2A Subtype IIC) of Variable Severity due to Homozygous or Double Heterozygous Mutations in the D1 and D2 Domains

Jan Jacques Michiels\textsuperscript{a, b} Alain Gadisseur\textsuperscript{a} Marc van der Planken\textsuperscript{a} Wilfried Schroyens\textsuperscript{a} Zwi Berneman\textsuperscript{a}

\textsuperscript{a}Hemostasis and Thrombosis Research Center, Antwerp University Hospital, Edegem, Belgium; \textsuperscript{b}Hemostasis and Thrombosis Science Center, Goodheart Institute, Rotterdam, The Netherlands

Abstract

The detection of even tiny amounts of von Willebrand factor (VWF):antigen after desmopressin treatment or in hidden sites like platelets allows the differentiation between patients with recessive von Willebrand disease (VWD) type 3, severe type 1, and 2C (2A subtype IIC). Recessive VWD 2C of various severity displays a characteristic multimeric pattern with pronounced dimer band, absence of triplet structure and lack of large multimers not due to increased proteolysis. Recessive VWD type 2C (2A subtype IIC) is caused by homozygosity or double heterozygosity of missense mutations in the D1 and D2 domains of the VWF propeptide (pp) that catalyzes the multimerization in the D3 domain at the N terminus of mature VWF. In expression studies of recombinant mutant VWF, secretion of VWF mainly consisted of dimers which failed to form intermediate- and high-molecular-weight multimers consistent with the clinical diagnosis of VWD 2C (2A subtype IIC). Carriers of a heterozygous missense mutation in the VWFpp region (D1–D2 domain) of the VWF gene may present mild VWD type 1 and show a typical multimeric pattern with a heavy predominance of VWF dimers.

Key Words

D1/D2 domain · Double heterozygous mutations · Subtype IIC · Type 2A · von Willebrand disease 2C

Introduction

In 1982, Ruggeri et al. \cite{1} first described a Swedish family with recessive von Willebrand disease (VWD) IIC (2C). The propositus was a 39-year-old male who had moderate bleeding symptoms since early childhood and no family history of a bleeding diathesis. Parents, daughter and son were asymptomatic (fig. 1, table 1) \cite{1}. Analysis of von Willebrand factor (VWF) multimers showed the absence of large multimers, the absence of the two bands adjacent to the central band in each triplet, and the fastest moving band (dimer) was markedly increased (fig. 1b: left lane, c: right lane) \cite{1}. The predominant band had a slower migration rate than in normal controls and VWD IIA with pronounced proteolytic triplet structure (fig. 1b: IIA).

According to Hoyer et al. \cite{2}, in 1983, recessive VWD type IIC (2A subtype IIC) was defined by the absence of large VWF multimers not due to increased proteolysis and the presence of bold protomers (dimer). Both asymptomatic heterozygous carriers and symptomatic homozygous or double heterozygous patients with recessive VWD IIC show a heavy predominance of VWF dimers (fig. 2). The clinical and laboratory findings of this family with recessive VWD type 2C has been reported previously \cite{3}.
Recently, Rosenberg et al. [4] studied a family with clinical symptoms of dominant mild type 1 of VWD in two affected members in two generations of one family (mother and daughter). A heavy predominance of VWF dimers was observed in their plasma, equaling approximately 50% of the total VWF antigen (Ag), similar to the heterozygous state of the IIC (2C) family described by Hoyer et al. [2] (fig. 2). Genomic analysis in both the mother and daughter revealed a heterozygous mutation Tyr87Ser in the D1 domain of the VWF gene. Expression studies showed that this mutation resulted in the synthesis of dimeric VWF with almost complete loss of N-terminal multimerization indicating that the Tyr87Ser VWF propeptide (pp) defect directly affected VWF multimerization [4]. Therefore, in the literature, evidence is accumulating that the multimeric pattern of a heavy predominance of VWF dimers (fig. 1, 2) appears to be characteristic or even diagnostic for a heterozygous missense mutation in the pp region (D1–D2 domain) of the VWF gene.

Table 1. Clinical expression and laboratory data of the Swedish family with VWD type 2C [11]

<table>
<thead>
<tr>
<th>bleeding symptoms</th>
<th>Ivy BT min</th>
<th>FVIII:C U/dl</th>
<th>FVIII:Ag U/dl</th>
<th>FVIII:RCo U/dl</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal</td>
<td>–</td>
<td>&lt;9</td>
<td>&gt;50</td>
<td>&gt;50</td>
</tr>
<tr>
<td>Propositus, male</td>
<td>moderate</td>
<td>&gt;30</td>
<td>67</td>
<td>50</td>
</tr>
<tr>
<td>Daughter</td>
<td>none</td>
<td>9</td>
<td>95</td>
<td>95</td>
</tr>
<tr>
<td>Son</td>
<td>none</td>
<td>6</td>
<td>120</td>
<td>120</td>
</tr>
</tbody>
</table>

Hoyer et al. [2] (fig. 2). Genomic analysis in both the mother and daughter revealed a heterozygous mutation Tyr87Ser in the D1 domain of the VWF gene. Expression studies showed that this mutation resulted in the synthesis of dimeric VWF with almost complete loss of N-terminal multimerization indicating that the Tyr87Ser VWF propeptide (pp) defect directly affected VWF multimerization [4]. Therefore, in the literature, evidence is accumulating that the multimeric pattern of a heavy predominance of VWF dimers (fig. 1, 2) appears to be characteristic or even diagnostic for a heterozygous missense mutation in the pp region (D1–D2 domain) of the VWF gene.

A few missense mutations related to autosomal recessive severe VWD have been identified in the VWF prosequence D1 and D2 domains [5–8]. There are two reports on double heterozygous missense/null mutation D141Y/null and C275S/null associated with VWD type 3.
with documented hemarthrosis in one of them (table 2) [5]. Expression studies of the missense mutation D141Y and C275S showed a severe secretion defect mainly in dimers while higher-molecular-weight bands like tetramers and hexamers were barely detectable, consistent with the 2C phenotype. Homozygotes for the missense mutations for R273W [6] and W377C [7] in the pp D1 domain have been described to be associated with autosomal recessive severe type 1 VWD phenotype (table 2). The homozygous missense mutation C570S in the D2 domain has been described as the cause of recessive severe type 1 but in fact also showed a laboratory phenotype of type 2C (2A subtype IIC) VWF multimers [9]. In retrospect, the multimeric pattern of the VWD patients homozygous for R273W and C570S clearly showed the absence of high-molecular-weight multimers and a pronounced dimeric band consistent with type 2C (2A subtype IIC) VWD [6, 10]. In addition, expression studies of recombinant R273W, W377C and C570S showed secretion of VWF mainly consisting of dimers which failed to form intermediate- and high-molecular-weight multimers consistent with the clinical diagnosis of VWD 2C (2A subtype IIC) [6–8]. These findings indicate that mutations in the D1 and D2 VWFpp region completely abolish multimerization of VWF. Heterozygous asymptomatic carriers of such missense mutations in the D1 or D2 domain are asymptomatic or may present with mild type 1 of VWD with borderline values of VWF parameters (<0.50 U/dl) and typically show a pronounced VWF dimer band (fig. 2). Consequently, cases of severe recessive VWD type 3 or 1 due to homozygous mutations in the D1 and D2 domains (table 2) have to be reclassified as typical examples of severe recessive VWD 2C (2A subtype IIC).

Autosomal recessive VWD type 2C caused by homozygosity for a missense mutation or double heterozygosity of a null allele and missense mutation in the D1 and D2 domains (exons 11–16, table 3) of the VWFpp is rare. All cases reported in the literature diagnosed as VWD type IIC are classified as VWD type 2A in the VWF mutation database (homozygous: N528S, G550R and C623W; heterozygous: N528S, G550R and C623W; missense mutations in the D1 and D2 domains of the VWF gene related to VWD 2A subtype IIC (2C) according to Ruggeri et al. [1] and Hoyer et al. [2] (fig. 1, 2)

### Table 2. Reports of autosomal recessive severe type 1 of VWD caused by homozygous missense or double heterozygous missense/null mutations in the D1 or D2 domain

<table>
<thead>
<tr>
<th>Mutation</th>
<th>Gender/years</th>
<th>BT (min)</th>
<th>FVIII:C (U/dl)</th>
<th>VWF:Ag (U/dl)</th>
<th>VWF:RCo (U/dl)</th>
<th>VWF:VWD type</th>
<th>Domain VWF type</th>
</tr>
</thead>
<tbody>
<tr>
<td>D141Y/null [5]</td>
<td>F/63</td>
<td>&gt;30</td>
<td>0.03</td>
<td>&lt;1</td>
<td>&lt;1</td>
<td>D1</td>
<td>type 3</td>
</tr>
<tr>
<td>C275S/null [5]</td>
<td>F/26</td>
<td>&gt;30</td>
<td>0.03</td>
<td>&lt;1</td>
<td>&lt;1</td>
<td>D1</td>
<td>type 3</td>
</tr>
<tr>
<td>R273W/R273W [6]</td>
<td>boy</td>
<td>15</td>
<td>0.20</td>
<td>0.06</td>
<td>0.06</td>
<td>D1</td>
<td>severe 1/2C</td>
</tr>
<tr>
<td>R273W/R273W [6]</td>
<td>boy</td>
<td>15</td>
<td>0.33</td>
<td>0.09</td>
<td>0.04</td>
<td>D1</td>
<td>severe 1/2C</td>
</tr>
<tr>
<td>W377C/W377C [7]</td>
<td>child</td>
<td>&gt;20</td>
<td>0.02</td>
<td>0.03</td>
<td>0.03</td>
<td>D1</td>
<td>severe 1/no data</td>
</tr>
<tr>
<td>C570S/C570S [8]</td>
<td>boy</td>
<td>1</td>
<td>0.12</td>
<td>0.05</td>
<td>0.05</td>
<td>D2</td>
<td>severe 1/2C</td>
</tr>
</tbody>
</table>

### Table 3. Missense mutations in the D1 and D2 domains of the VWF gene related to VWD 2A subtype IIC (2C) according to Ruggeri et al. [1] and Hoyer et al. [2] (fig. 1, 2)

<table>
<thead>
<tr>
<th>VWD type SSC/ISTH</th>
<th>VWD type reclassified</th>
<th>Exon</th>
<th>Mutation</th>
<th>Publication</th>
</tr>
</thead>
<tbody>
<tr>
<td>2A IIC 2C</td>
<td>11</td>
<td>1212ins6 (AATCCC)</td>
<td>F404insNP</td>
<td>yes</td>
</tr>
<tr>
<td>2A IIC 2C</td>
<td>12</td>
<td>1309–1326del</td>
<td>D437–R442del</td>
<td>no</td>
</tr>
<tr>
<td>2A IIC 2C</td>
<td>12</td>
<td>1309–1326del</td>
<td>D437–R442del</td>
<td>no</td>
</tr>
<tr>
<td>2A IIC 2C</td>
<td>14</td>
<td>1583A→G</td>
<td>N528S</td>
<td>yes</td>
</tr>
<tr>
<td>2A IIC 2C</td>
<td>14</td>
<td>1648G→A</td>
<td>G550R</td>
<td>yes</td>
</tr>
<tr>
<td>2A IIC 2C</td>
<td>14</td>
<td>1709G→C</td>
<td>C570S</td>
<td>yes</td>
</tr>
<tr>
<td>2A IIC 2C</td>
<td>15</td>
<td>1869C→G</td>
<td>C623W</td>
<td>yes</td>
</tr>
<tr>
<td>2A IIC 2C</td>
<td>15</td>
<td>1872InsGCG</td>
<td>InsG625</td>
<td>yes</td>
</tr>
</tbody>
</table>
compound heterozygous: D437-R422del, F405insNP/null allele and A625InsG/nul26pdelCT; table 3) [10]. The laboratory phenotype of recessive VWD type 2C appears to be variable.

**Laboratory and Molecular Characteristics of Recessive VWD Type 2C (2A Subtype IIC)**

The molecular defect in the Swedish family with mild VWD type 2C (table 1) [11] was studied in 1998 in a 53-year-old patient [11]. A six-nucleotide insert, AATCCC, was found in exon 11 of the VWF gene, predicting the insertion of the amino acids asparagine and proline between phenylalanine 404 and threonine 405 of the VWFpp. The proband in figure 1 is double heterozygous for F404insNP/null [11]. The same mutation was found in the patient’s two asymptomatic children (fig. 1). Analyses of VWF multimers of the patient and one of the children heterozygous for F404insNP disclose the typical 2C pattern with a typical predominance of VWF dimers (fig. 1c) [11]. Multimeric analysis of mutant recombinant VWF released to the medium showed only the fastest moving band (dimers), a trace of the next oligomer and lack of triplet structure.

The propositus of the well-documented French family with recessive VWD 2C (case III-4, fig. 3) was a 19-year old female with a pronounced bleeding tendency since early childhood ecchymoses: epistaxis, severe gastrointestinal bleeding and recurrent life-threatening hemorrhagic ruptures of ovarian follicles for which cryoprecipitate or factor VIII (FVIII)/VWF concentrate was needed [12, 13]. The heterozygous family members studied had no bleeding history but showed a typical predominance of VWF dimers (fig. 3). The propositus III-4 (solid symbol) is double heterozygous for the paternal null allele with a translational stop codon at position 711 and the maternal allele harboring a 3-bp insertion resulting in an additional Gly at position 625 (A625InsG/null). The laboratory phenotype of the proband with Ivy bleeding time (BT) >20 min, FVIII:coagulant activity (C) 24%, VWF:Ag 13%, VWF:ristocetin cofactor activity (RCO) <0.03,

![Pedigree and VWF multimeric patterns of the French family with autosomal recessive VWD type 2C double heterozygous for A625InsG/null](image-url)
absence of ristocetin-induced platelet aggregation (RIPA) and VWF multimers, and pronounced dimer band is consistent with severe recessive VWD type 1/2C (case III-4, fig. 3) [12, 13]. Analysis of VWF multimers in direct comparison to the Swedish 2C family showed the absence of large multimers and no triplet structure. Even most of the bands (number 2–5) were dramatically decreased in the Swedish family, and the relative concentration of the fastest moving band (number 1) was increased. Analysis of VWF multimers of the mother (II-2) and twin sister (III-3) heterozygous for the A625InsG mutation showed a relatively increased intensity of the fastest-moving dimer band but no significant differences in the triplet structure of each band. Intravenous desmopressin (DDAVP; 0.4 µg/kg) induced a good response of FVIII and no response of both VWF:Ag and VWF:RCO without correction of a strongly prolonged Ivy BT (fig. 4). Thirty minutes after DDAVP, faint bands of large multimers appeared, which rapidly disappeared together with the appearance of a much more pronounced dimer band indicating a multimerization defect in mutant VWF as the cause of the lack of large VWF multimers (fig. 4).

The propositus of the German family with rather mild recessive VWD type 2C was the only severely affected member in a three-generation family with a pronounced bleeding history since early childhood (fig. 5, table 4) [14]. Reported spontaneous bleeding symptoms were frequent epistaxis, easy bruising and heavy menorrhagia that finally required hysterectomy. The propositus had normal VWF:Ag and decreased VWF:RCO and VWF:

**Table 4. Laboratory data of the German family with mild recessive VWD type 2C homozygous for the missense mutation G550R** [13].

<table>
<thead>
<tr>
<th>ID</th>
<th>BT min</th>
<th>VWF:Ag IU/ml</th>
<th>RCOC IU/ml</th>
<th>VWF:CB IU/ml</th>
<th>FVIII:C IU/ml</th>
</tr>
</thead>
<tbody>
<tr>
<td>I-1</td>
<td>3.5</td>
<td>2.09</td>
<td>1.45</td>
<td>1.44</td>
<td>&gt;1.00</td>
</tr>
<tr>
<td>II-1</td>
<td>10.0</td>
<td>1.59</td>
<td>0.25</td>
<td>0.29</td>
<td>&gt;1.00</td>
</tr>
<tr>
<td>III-1</td>
<td>2.0</td>
<td>1.34</td>
<td>0.80</td>
<td>0.79</td>
<td>&gt;1.00</td>
</tr>
<tr>
<td>III-2</td>
<td>6.0</td>
<td>0.86</td>
<td>0.66</td>
<td>0.57</td>
<td>&gt;1.00</td>
</tr>
<tr>
<td>Normal range</td>
<td>&lt;6.0</td>
<td>0.6–1.6</td>
<td>0.6–1.6</td>
<td>0.6–1.6</td>
<td>0.7–1.4</td>
</tr>
</tbody>
</table>

**Fig. 4.** Poor response of VWF:Ag and VWF:RCO and good response of FVIII:Ag to DDAVP in recessive VWD type 2C case III-4 double heterozygous for A625InsG/null in the French family [11]. Please note the strong increase in VWF dimers and lack of multimerization.

**Fig. 5.** Pedigree and VWF multimeric analysis in the German family with mild recessive VWD type 2C homozygous for the missense mutation G550R [13].

VWD 2C (2A Subtype IIC) of Variable Severity
CB, with very low ratios of VWFRCo/VWF:Ag (0.16) and VWF:CB/VWF:Ag (0.18), consistent with mild VWD type 2. The parents and both of the patient’s children were phenotypically normal (table 4). The propositus was homozygous for the missense mutation G550R, whereas the mother and her two children were heterozygous. SDS agarose gel electrophoresis of VWF showed the loss of large multimers, the lack of satellite band (no triplets) and a pronounced protomer in the propositus, consistent with mild VWD 2C homozygous for G550R (fig. 5, middle lane) [14]. The electrophoretic VWF multimeric pattern of the heterozygous family members appeared normal except for the presence of a pronounced dimer band and a lower intensity of satellite band (fig. 5) [14].

The pedigree and laboratory phenotype of the Spanish family with autosomal recessive VWD 2C is shown in figure 6 and table 5 [9, 15]. The propositi (III-1 and III-3) were a 14-year-old girl and a 16-year-old boy with a tendency to mild bleeding since early childhood characterized by easy bruising, prolonged bleeding from skin cuts and epistaxis. Their brother (III-2; aged 15) had no bleeding symptoms though he also had laboratory evidence of mild VWD with a prolonged BT and decreased RIPA (table 5). Low-resolution SDS-agarose gel (1%) electrophoresis revealed the absence of large multimers in the two affected propositi consistent with severe VWD 1 (table 5). The asymptomatic brother (III-2) showed a decrease in all multimers except for the smallest band 1 (fig. 6). Using high-resolution SDS-agarose gel (2%), a typical type 2C VWF multimeric pattern was detected in the two symptomatic propositi with the loss of large multimers, no triplets and increased band 1, but with no evidence of a faint intervening band (fig. 6). The smallest multimeric band 1 was relatively increased and the triplet structure of each band was less pronounced in the asymptomatic father (II-2), grandfather (I-2) and brother (III-2) indicat-
ing heterozygosity for a missense mutation. All VWF parameters and VWF multimeric patterns in low- and high-resolution gels were normal in I-1, I-4, II-3 and II-4. Consequently, the asymptomatic case (II-3) is predicted to carry a null allele. These observations strongly suggest the interplay of at least two genetic defects (double heterozygosity) of the two affected propositi with recessive VWD 2C. Intravenous DDAVP (0.4 μg/kg) in the two affected propositi with severe recessive VWD 2C induced a good response of FVIII and no response of both VWF:Ag and VWF:RCo without correction of prolonged BT (fig. 7) [15]. Mutation analysis has not been performed in this Spanish family with recessive VWD type 2C [Batlle, pers. commun.].

Table 5. Laboratory data of the Spanish family with autosomal recessive VWD type IIC [15]

<table>
<thead>
<tr>
<th>Mutation</th>
<th>Age years</th>
<th>Gender</th>
<th>BT min</th>
<th>FVIII:C</th>
<th>VWF:Ag</th>
<th>VWF:RCo</th>
<th>VWF:RCo/Ag</th>
<th>RIPA</th>
<th>Multimeric pattern</th>
</tr>
</thead>
<tbody>
<tr>
<td>Unknown</td>
<td>I-2</td>
<td>M</td>
<td>N</td>
<td>1.60</td>
<td>1.60</td>
<td>0.88</td>
<td>0.55</td>
<td>nt</td>
<td>abnormal</td>
</tr>
<tr>
<td></td>
<td>I-3</td>
<td>F</td>
<td>N</td>
<td>1.30</td>
<td>1.26</td>
<td>0.50</td>
<td>0.40</td>
<td>nt</td>
<td>abnormal</td>
</tr>
<tr>
<td></td>
<td>II-2</td>
<td>F</td>
<td>N</td>
<td>1.30</td>
<td>1.26</td>
<td>0.50</td>
<td>0.40</td>
<td>nt</td>
<td>normal</td>
</tr>
<tr>
<td></td>
<td>II-3</td>
<td>F</td>
<td>N</td>
<td>N</td>
<td>N</td>
<td>N</td>
<td>N</td>
<td>nt</td>
<td>normal</td>
</tr>
<tr>
<td></td>
<td>II-4</td>
<td>M</td>
<td>N</td>
<td>N</td>
<td>N</td>
<td>N</td>
<td>N</td>
<td>nt</td>
<td>normal</td>
</tr>
<tr>
<td>III-1</td>
<td>16</td>
<td>M</td>
<td>&gt;20</td>
<td>0.09</td>
<td>0.13</td>
<td>&lt;0.01</td>
<td>&lt;0.10</td>
<td>0</td>
<td>2C, severe 1</td>
</tr>
<tr>
<td>III-2</td>
<td>15</td>
<td>M</td>
<td>↑</td>
<td>1.26</td>
<td>0.80</td>
<td>0.42</td>
<td>0.53</td>
<td>↓</td>
<td>abnormal</td>
</tr>
<tr>
<td>III-3</td>
<td>14</td>
<td>F</td>
<td>&gt;20</td>
<td>0.20</td>
<td>0.15</td>
<td>&lt;0.01</td>
<td>&lt;0.10</td>
<td>0</td>
<td>2C, severe 1</td>
</tr>
</tbody>
</table>

Fig. 7. Poor response of VWF:Ag (enzyme immunoassay, EIA, and immunoradiometric assay, IRMA) and VWF:RCo, and good response of FVIII:C to DDAVP and no correction of prolonged BT in both cases with recessive VWD type 2C (Spanish family) and unknown mutation [14].
Conclusions

Recessive VWD type 2C (2A subtype IIC) is caused by homozygosity or double heterozygosity of missense mutations in the D1 and D2 domains of VWFpp that catalyzes the multimerization in the D3 domain at the N-terminus of mature VWF.

Expression studies of recombinant mutant VWF demonstrated the secretion of dimers which failed to form intermediate- and high-molecular-weight multimers consistent with the clinical diagnosis of VWD 2C (2A subtype IIC).

The laboratory phenotype in patients with recessive VWD type 2C (2A subtype IIC) varies widely from severe (with very low levels of FVIII:C and VWF:Ag, unmeasurable VWF:RCo, absent RIPA and strongly prolonged BT as documented in five kindreds [6–8, 12, 15]) to rather mild VWD type 2C (with normal values for FVIII:C and VWF:Ag, low levels for VWF:RCo and VWF:CB and prolonged BT as reported in two kindreds [1, 11, 14]). The combination of a null mutation and a missense mutation in the D1 domain may mimic severe VWD type 3 [5].

Carriers of a heterozygous missense mutation in the VWFpp region (D1–D2 domains) of the VWF gene usually present with mild VWD type 1 and show a typical multimeric pattern with a heavy predominance of VWF dimers [2–4, 12, 14, 15].

References

10 University of Sheffield: ISTH SSC VWF Database. http://www.vwf.group.shef.ac.uk/index.html
Recessive von Willebrand Disease Type 2 Normandy: Variable Expression of Mild Hemophilia and VWD Type 1

Jan Jacques Michiels\textsuperscript{a, b}, Alain Gadisseur\textsuperscript{a}, Inge Vangenegten\textsuperscript{a}, Wilfried Schroyens\textsuperscript{a}, Zwi Berneman\textsuperscript{a}

\textsuperscript{a}Hemostasis and Thrombosis Research Center, Antwerp University Hospital, Edegem, Belgium; \textsuperscript{b}Hemostasis and Thrombosis Science Center, Goodheart Institute, Rotterdam, The Netherlands

Abstract

Missense mutations in the von Willebrand factor (VWF) gene impairing the binding to factor VIII (FVIII) do not impair the structure of VWF multimers nor the ability of VWF to aggregate platelets but causes an accelerated clearance of FVIII. Recessive VWD type Normandy (N) encompasses all patients with a deficiency in FVIII:coagulant activity (C) caused by a markedly decreased affinity of VWF for FVIII:C due to a FVIII binding defect in VWF but with normal or near normal VWF: antigen (Ag), VWF:ristocetin cofactor activity (RCO) and VWF: collagen binding (CB) levels, normal VWF:RCO/VWF:Ag ratio, normal VWF multimeric pattern and normal VWF-dependent platelet functions including ristocetin-induced platelet aggregation and bleeding time (BT) consistent with VWD type 1. The response to 1-deamino-8-D-arginine vasopressin (DDAVP) of VWF parameters is usually normal, but the degree of restricted response curves to DDAVP of FVIII:C depends on the severity of the FVIII binding defect to the mutated VWF. The homozygous mutations R816W and R854W are typically associated with severe and mild VWD 1/N, respectively. Homozygous or heterozygous/null mutations of C788, D879N or C1225G do not only dramatically decrease FVIII binding, but also induce a multimerization and secretion defect with a decrease in the large VWF multimers, lack of triplet structure and prolonged BT consistent with severe VWD 2E/N. The missense mutations Y795C and R763G either heterozygous or as a component of recessive VWD (double heterozygous) are responsible for the FVIII binding defect (VWD 1/N) and abnormal banding of VWF multimers leading to the presence of a smeary pattern with the presence of ultralarge VWF multimers.

Copyright © 2009 S. Karger AG, Basel

Introduction

In most patients with von Willebrand disease (VWD) type 2N, bleeding episodes are similar to those in patients with mild/moderate hemophilia A, with bleeding occurring after trauma or surgery. Clinicians have to be aware that patients with VWD type Normandy present with normal bleeding time (BT) and equally decreased von Willebrand factor (VWF):antigen (Ag) and VWF:ristocetin cofactor activity (RCO) simulating recessive type 1 VWD, but factor VIII (FVIII):coagulant activity (C) is much lower compared to VWF:Ag due to a FVIII binding defect in the D’–D3 domain of the VWF protein [1]. Numerous VWF gene mutations in the FVIII binding domain have been characterized in patients with VWD N,
which are localized in exons 18–24 of the VWF gene (table 1) [2]. VWD N encompasses all patients with FVIII:C deficiency caused by a markedly decreased affinity of VWF for FVIII:C due to a FVIII binding defect of VWF. Diagnostic differentiation between mild hemophilia and VWD 2N is based on the measurement of the ability of plasma VWF to bind exogenous FVIII: FVIII:VWF:RCo ratio, normal VWF multimeric pattern and normal VWF-dependent platelet functions including ristocetin-induced platelet aggregation and bleeding time (BT). Although some cases have severe FVIII:C deficiency of 1–2 U/dl, the majority of patients with VWD 2N have FVIII:C levels >5 U/dl [1]. Consequently, recessive VWD type Normandy may be misclassified as mild hemophilia. The FVIII to VWF:Ag ratios are not significantly different in homozygotes or heterozygotes for the R854Q mutation compared to those who are compound heterozygous with R854Q/type 3 or R854Q/other type 2N mutations. The ratio of FVIII to VWF:Ag is significantly lower in 2N patients not harboring the R854Q mutation. The FVIII to VWF:Ag ratio is <0.2 in patients homozygous for T791, R816W or C1060R [1].

The homozygous R816W mutation is associated with severe recessive VWD type Normandy due to a severe FVIII binding defect of VWF, whereas patients homozygous for R854W present with mild recessive VWD type Normandy (table 2). Intravenous 1-deamino-8-D-arginine vasopressin (DDAVP) treatment in VWD N induces completely normal responses for VWF parameters consistent with type 1 VWD, but restricted responses of FVIII:C followed by shortened half-lives [6]. The degree of the restricted response of FVIII:C to DDAVP depends on the severity of the FVIII binding defect of mutated VWF [1, 6]. Mazurier et al. [6] evaluated the biological effect of DDAVP in 3 patients with severe type N homozygous for R816W and 4 patients with mild type VWD N homozygous for R854W (fig. 1).

The laboratory phenotype of VWD type Normandy is characterized by reduced FVIII:C levels and normal or near normal VWF:Ag, VWF:RCo and VWF:collagen binding (CB) levels, normal VWF:RCo/VWF:Ag ratio, normal VWF multimeric pattern and normal VWF-dependent platelet functions including ristocetin-induced platelet aggregation and bleeding time (BT). Intravenous 1-deamino-8-D-arginine vasopressin (DDAVP) treatment in VWD N induces completely normal responses for VWF parameters consistent with type 1 VWD, but restricted responses of FVIII:C followed by shortened half-lives [6]. The degree of the restricted response of FVIII:C to DDAVP depends on the severity of the FVIII binding defect of mutated VWF [1, 6].

### Table 1. Missense mutation involved in VWD 2N due to mutations in the D’–D3 domain [2]

<table>
<thead>
<tr>
<th>Exon mutation</th>
<th>Mutation</th>
</tr>
</thead>
<tbody>
<tr>
<td>18 2344C→T</td>
<td>R782W</td>
</tr>
<tr>
<td>18 2354G→A</td>
<td>G785E</td>
</tr>
<tr>
<td>18 2359G→A</td>
<td>E787K</td>
</tr>
<tr>
<td>18 2362T→C</td>
<td>C788R</td>
</tr>
<tr>
<td>18 2363G→A</td>
<td>C788Y</td>
</tr>
<tr>
<td>18 2372C→T</td>
<td>T791M</td>
</tr>
<tr>
<td>18 2384A→G</td>
<td>Y795C</td>
</tr>
<tr>
<td>18 2398A→G</td>
<td>M800V</td>
</tr>
<tr>
<td>18 2411G→T</td>
<td>C804F</td>
</tr>
<tr>
<td>18 2435C→T</td>
<td>P812L</td>
</tr>
<tr>
<td>19 2446C→T</td>
<td>R816W</td>
</tr>
<tr>
<td>19 2447G→A</td>
<td>R816Q</td>
</tr>
<tr>
<td>19 2451T→A</td>
<td>H817Q</td>
</tr>
<tr>
<td>20 2561G→A</td>
<td>R854Q</td>
</tr>
<tr>
<td>20 2573G→T</td>
<td>C858F</td>
</tr>
<tr>
<td>20 2635G→A</td>
<td>D879N</td>
</tr>
<tr>
<td>21 2771G→A</td>
<td>R924Q</td>
</tr>
<tr>
<td>24 3159G→T</td>
<td>Q1053H</td>
</tr>
<tr>
<td>24 3178T→C</td>
<td>C1060R</td>
</tr>
</tbody>
</table>

The laboratory phenotype of VWD type Normandy is characterized by reduced FVIII:C levels and normal or near normal VWF:Ag, VWF:RCo and VWF:collagen binding (CB) levels, normal VWF:RCo/VWF:Ag ratio, normal VWF multimeric pattern and normal VWF-dependent platelet functions including ristocetin-induced platelet aggregation and bleeding time (BT). Although some cases have severe FVIII:C deficiency of 1–2 U/dl, the majority of patients with VWD 2N have FVIII:C levels >5 U/dl [1]. Consequently, recessive VWD type Normandy may be misclassified as mild hemophilia. The FVIII to VWF:Ag ratios are not significantly different in homozygotes or heterozygotes for the R854Q mutation compared to those who are compound heterozygous with R854Q/type 3 or R854Q/other type 2N mutations. The ratio of FVIII to VWF:Ag is significantly lower in 2N patients not harboring the R854Q mutation. The FVIII to VWF:Ag ratio is <0.2 in patients homozygous for T791, R816W or C1060R [1].

The homozygous R816W mutation is associated with severe recessive VWD type Normandy due to a severe FVIII binding defect of VWF, whereas patients homozygous for R854W present with mild recessive VWD type Normandy (table 2). Intravenous 1-deamino-8-D-arginine vasopressin (DDAVP) treatment in VWD N induces completely normal responses for VWF parameters consistent with type 1 VWD, but restricted responses of FVIII:C followed by shortened half-lives [6]. The degree of the restricted response of FVIII:C to DDAVP depends on the severity of the FVIII binding defect of mutated VWF [1, 6]. Mazurier et al. [6] evaluated the biological effect of DDAVP in 3 patients with severe type N homozygous for R816W and 4 patients with mild type VWD N homozygous for R854W (fig. 1).
VWD 2N: Variable Expression of Mild Hemophilia and VWD 1

![Figure 1](image-url)  
*Fig. 1.* Response of FVIII to DDAVP in VWD N homozygous for R816W is poor (a, b) [6]. The response of FVIII to DDAVP is better than normal but followed by very short half-lives of FVIII in VWD 1/N homozygous for R854W (c, d) [6]. The responses of VWF parameters to DDAVP are normal, followed by normal half-lives in VWD 1/N homozygous for R816W and R854W.

**Table 2.** Variable phenotypes of VWD N is related to the degree of FVIII binding defect of mutated VWF [6]

<table>
<thead>
<tr>
<th>Age (years)/sex</th>
<th>Bleeding manifestations</th>
<th>FVIII:C IU/dl</th>
<th>FVIII:Ag IU/dl</th>
<th>VWF:Ag IU/dl</th>
<th>VWF:RCo IU/dl</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Homozygous R816W [6]: 3 cases with VWD 1/N and mild hemophilia A</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>20/M</td>
<td>ecchymoses, hematomas, hemorrhis</td>
<td>6.5</td>
<td>7.5</td>
<td>118</td>
<td>110</td>
</tr>
<tr>
<td>10/F</td>
<td>intramuscular hematoma, excessive bleeding after injury</td>
<td>5</td>
<td>6</td>
<td>108</td>
<td>80</td>
</tr>
<tr>
<td>2–7/F</td>
<td>ecchymoses, hematomas</td>
<td>4</td>
<td>6</td>
<td>104</td>
<td>100</td>
</tr>
<tr>
<td><strong>Homozygous R854W [6]: 4 cases with VWD 1/N and mild FVIII deficiency</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>29/F</td>
<td>asymptomatic, no bleeding after appendectomy</td>
<td>38</td>
<td>23</td>
<td>105</td>
<td>–</td>
</tr>
<tr>
<td>48/F</td>
<td>no spontaneous bleeding, bleeding after tooth extractions</td>
<td>22</td>
<td>42</td>
<td>83</td>
<td>79</td>
</tr>
<tr>
<td>25/F</td>
<td>ecchymoses, epistaxis, bleeding after delivery</td>
<td>23</td>
<td>33</td>
<td>110</td>
<td>110</td>
</tr>
<tr>
<td>42/F</td>
<td>moderate bleeding symptoms, transfused for delivery</td>
<td>36</td>
<td>29</td>
<td>115</td>
<td>100</td>
</tr>
</tbody>
</table>

VWD 1 phenotype (fig. 1). A double heterozygous R854Q/R924Q mutation results in a mild FVIII:BD/VWD 1 phenotype, even milder than a heterozygous R816W mutation (table 3). In double heterozygous R854W/Y1584C mutations, the VWD phenotype is mild type 1 (with a near normal FVIII:C/VWF:Ag ratio) but more prominent than in heterozygous Y1584C showing borderline values for FVIII:C and VWF:Ag, and normal ratios of VWF:RCo/VWF:Ag, being more pronounced when associated with blood group O. In double heterozygous R954Q/R1205H, the laboratory phenotype of a mild FVIII:BD changes into pronounced VWD type 1 Vicenza with
good response but rapid clearance of FVIII:C and VWF parameters after DDAVP consistent with VWD type 1 Vicenza with a rapid clearance defect [7].

The steady state ratio of plasma VWF propeptide (pp) to VWF:Ag can be used to identify VWD type 1 patients with a normal or reduced VWF:Ag survival [8, 9]. This applies for type 1 Vicenza, which has a very short VWF survival after DDAVP and a very high VWFpp/Ag ratio. In mild VWD type 1 due to mutations in the D1–D2 domains, FVIII binding, and D4, B1–B3 and C1-C2 domains, the survival of VWF:Ag is usually normal, which is associated with a normal VWFpp/Ag ratio [8, 9].

### Recessive Homozygous or Double Heterozygous VWD 2E/N

The D’ and D3 domains correspond to amino acid residues 769–865 and 866–1242, respectively. Mutations in 1053, 1060, 1078 and 1225 are located in the D3 domain outside the D’ FVIII binding site. Usually VWD 2N does not affect the VWF multimer structure and BT. There appears to be an overlapping phenotypic expression of VWD N and a multimeric secretion defect in some VWD N mutations C788Y, C788R and C1225G [10]. Homozygous C788R and C1225G mutations in two unrelated consanguineous families do not only dramatically decrease FVIII binding, but also induce impaired secretion and multimerization with a decrease in the large VWF multimers and lack of triplet structure, as documented in expression studies, consistent with severe VWD 2E/N with prolonged BT (table 4, fig. 2) [11].

Jorieux et al. [10] studied three patients from two unrelated families (P1 and P2, family 1, and P3, family 2 with a combined FVIII binding defect and VWF multimerization impairment; table 5, fig. 3). Patient 1 was double heterozygous for C788T/null allele and showed a hybrid picture of VWD 2E/N with a FVIII binding defect of VWF, a prolonged BT and very low levels of VWF due to impaired secretion of multimers, as demonstrated in expression studies (table 5, fig. 3). Patient 2 was double heterozygous for C788T/R854W and presented with VWD 1/N with normal BT and normal VWF multimeric pattern (table 5, fig. 3) [10]. Expression studies of recombinant (r) VWF C788T revealed a secretion defect with lack of large multimers and a smeary pattern, whereas rVWF C854W demonstrated normal secretion with normal VWF multimers. In contrast, rVWF C858F showed a pronounced secretion defect with lack of intermediate and large VWF multimers (multimerization defect). Heterozygous expression of rVWF C858F with wild type (WT) or R854W showed a type 2E-like multimeric pattern with all multimers being present but a relative decrease in large VWF multimers in 1.5% SDS-agarose resolution gels (fig. 3) [10].

Jorieux et al. [12] reported another case diagnosed as VWD type IIE caused by a double heterozygous D879N/null (R1659X) mutation (table 6). COS-7 cell expression of rVWF D879N (D3 domain) was significantly decreased together with a significant decrease in large VWF multimers consistent with VWD type IIE (impaired multimerization and secretion, data not shown). The FVIII binding ability of patient plasma and of rVWF D879N was dramatically decreased consistent with VWD 2N. The response of both VWF and FVIII to DDAVP is predicted to be very restricted or even poor, as can be derived from in vitro expression studies and the FVIII binding defect of mutated VWF. In conclusion, the

### Table 3. VWF mutations in the FVIII binding domain classified as VWD type 1 in the MCMDM-IVWD study [7]

<table>
<thead>
<tr>
<th>Mutation</th>
<th>FVIII:C</th>
<th>VWF:Ag</th>
<th>RCo</th>
<th>CB</th>
<th>RCo/Ag ratio</th>
<th>FVIII:C/VWF:Ag</th>
<th>VWFpp VWF:Ag</th>
<th>VWD type 2008</th>
</tr>
</thead>
<tbody>
<tr>
<td>Heterozygous</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>M771I</td>
<td>20</td>
<td>38</td>
<td>59</td>
<td>77</td>
<td>1.55</td>
<td>0.52</td>
<td>1.1</td>
<td>1 FVIII:BD</td>
</tr>
<tr>
<td>R816W</td>
<td>14</td>
<td>49</td>
<td>52</td>
<td>57</td>
<td>1.06</td>
<td>0.28</td>
<td>–</td>
<td>1 FVIII:BD</td>
</tr>
<tr>
<td>R854Q</td>
<td>28</td>
<td>37</td>
<td>32</td>
<td>43</td>
<td>1.15</td>
<td>0.75</td>
<td>–</td>
<td>1 FVIII:BD</td>
</tr>
<tr>
<td>Double heterozygous</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>R854Q/1109+2T+C (null)</td>
<td>9</td>
<td>59</td>
<td>44</td>
<td>38</td>
<td>0.75</td>
<td>0.15</td>
<td>1.6</td>
<td>1 FVIII:BD</td>
</tr>
<tr>
<td>R854Q/R924Q</td>
<td>15</td>
<td>25</td>
<td>38</td>
<td>38</td>
<td>1.52</td>
<td>0.60</td>
<td>–</td>
<td>1 FVIII:BD</td>
</tr>
<tr>
<td>R854W/Y1584C</td>
<td>40</td>
<td>47</td>
<td>38</td>
<td>59</td>
<td>0.85</td>
<td>0.85</td>
<td>1.4</td>
<td>1 FVIII:BD</td>
</tr>
<tr>
<td>R954Q/R1205H</td>
<td>18</td>
<td>11</td>
<td>7</td>
<td>6</td>
<td>0.64</td>
<td>1.6</td>
<td>increased</td>
<td>1-Vicenza</td>
</tr>
</tbody>
</table>
Fig. 2. Autosomal recessive VWD 2E/N homozygous for C788R or C1225G show the absence of intermediate and large VWF multimers in high-resolution gels (a: 2%; b: 3%), whereas the VWF multimeric pattern in heterozygous carriers of C788R and C1225G is normal [11].

Table 4. Recessive VWD 2N/2E with FVIII binding defect and prolonged BT due to homozygous mutations C788R and C1225G in the D' and D3 domain, respectively [11]

<table>
<thead>
<tr>
<th>Mutation</th>
<th>FVIII:C</th>
<th>VWF:Ag</th>
<th>VWF:RCo</th>
<th>FVIII:C/ VWF:Ag</th>
<th>VWF:RCo/ VWF:Ag</th>
<th>VWD type multimers</th>
<th>BT</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>fig. 2a</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>C788R/C788R</td>
<td>15</td>
<td>18</td>
<td>10</td>
<td>0.83</td>
<td>0.55</td>
<td>2E/N</td>
<td>prolonged</td>
</tr>
<tr>
<td>C788R father</td>
<td>47</td>
<td>36</td>
<td>52</td>
<td>1.3</td>
<td>1.4</td>
<td>mild 1</td>
<td>normal</td>
</tr>
<tr>
<td>C788R mother</td>
<td>69</td>
<td>76</td>
<td>78</td>
<td>0.90</td>
<td>1.0</td>
<td>normal</td>
<td>normal</td>
</tr>
<tr>
<td>C788R brother</td>
<td>44</td>
<td>57</td>
<td>84</td>
<td>0.77</td>
<td>1.5</td>
<td>mild 1</td>
<td>normal</td>
</tr>
<tr>
<td><strong>fig. 2b</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>C1225G/C1225G</td>
<td>24</td>
<td>07</td>
<td>10</td>
<td>3.4</td>
<td>0.55</td>
<td>2E/N</td>
<td>prolonged</td>
</tr>
<tr>
<td>C1225G father</td>
<td>78</td>
<td>82</td>
<td>74</td>
<td>0.95</td>
<td>0.90</td>
<td>normal</td>
<td>normal</td>
</tr>
<tr>
<td>C1225G mother</td>
<td>60</td>
<td>72</td>
<td>54</td>
<td>0.83</td>
<td>0.75</td>
<td>normal</td>
<td>normal</td>
</tr>
<tr>
<td>C1225G sister</td>
<td>nt</td>
<td>54</td>
<td>35</td>
<td>–</td>
<td>0.65</td>
<td>mild 1</td>
<td>normal</td>
</tr>
</tbody>
</table>
C788T/null, C788R/C788R, D879N/null and R1225G/R12225G genotypes clearly show that such mutations in the D’–D3 domain can cause a hybrid phenotype of rather severe VWD 2E/N with FVIII binding defect and prolonged BT.

Recessive VWD Normandy with a Smeary Pattern of VWF Multimers

The Y795C/null (R1566X) mutation showed a typical VWD 2N with low FVIII due to severe FVIII binding defect, decreased FVIII:C (7–13%), and normal values of
VWF:Ag (91%), VWF:RCo (51%) and VWF:CB (85%) with an aberrant multimer pattern both in vivo and in vitro (fig. 4) [13]. Expression studies indicated a relative decrease in large multimers, lack of a defined triplet structure, a smeary pattern and the presence of supranormal multimers (fig. 4) [13]. The same smeary pattern of VWF multimers with the presence of ultralarge multimers and the lack of a defined triplet structure was seen in the asymptomatic father, who was heterozygous for the Y795C mutation (fig. 4) [13].

Casonato et al. [14] reported an interesting case of 2N compound heterozygous for R854W/R760C with a...
The hybrid rVWF R763G/R854Q displayed an abnormal multimeric pattern with broad bands (smeary pattern) and the presence of ultralarge VWF multimers also present in the asymptomatic carriers of the R763G mutation (fig. 6).

As shown in figures 4 and 6 and table 8, the missense mutations Y795C and R763G either heterozygous or as a component of recessive VWD type 1/N (double heterozygous) are responsible for the FVIII binding defect and abnormal banding of VWF multimers leading to the presence of a smeary pattern and ultralarge VWF multimers [13, 15]. The mutant G763 discloses slower moving bands. In contrast, rVWF R763G displays a mild FVIII binding defect and an abnormal multimeric pattern due to slower migration of the various multimers (table 7, fig. 5). Expression studies of recombinant R760C mutation showed a decreased VWF secretion and lack of intermediate and large VWF multimers indicating a multimerization defect (table 7, fig. 5) [14].

Another case of VWD 2N compound heterozygous for R854Q/R763G showed a smeary pattern of VWF multimers with the presence of ultralarge VWF multimers (table 8, fig. 6) [15]. Expression studies of rVWF disclosing abnormal moving bands of mutant R763G VWF and a smeary pattern of VWF heterozygous for R763G or double heterozygous for R763G/R854W [15].

Table 8. Laboratory features of a family with VWD 1/N double heterozygous for R763G and R854Q and heterozygous carriers of R763G or R854Q [15]

<table>
<thead>
<tr>
<th>Member</th>
<th>Sex</th>
<th>VWF:Ag IU/dl</th>
<th>VWF:RCo IU/dl</th>
<th>FVIII:Ag IU/dl</th>
<th>FVIII:C IU/dl</th>
<th>VWF:FVIII:BD</th>
<th>VWF mutation</th>
</tr>
</thead>
<tbody>
<tr>
<td>I-1</td>
<td>M</td>
<td>76</td>
<td>40–115</td>
<td>10</td>
<td>17</td>
<td>markedly decreased</td>
<td>R854Q/R763G</td>
</tr>
<tr>
<td>II-1</td>
<td>F</td>
<td>88–125</td>
<td>–</td>
<td>10</td>
<td>10–23</td>
<td>moderately decreased</td>
<td>R763G</td>
</tr>
<tr>
<td>II-2</td>
<td>M</td>
<td>15–22</td>
<td>–</td>
<td>16</td>
<td>–</td>
<td>moderately decreased</td>
<td>R763G</td>
</tr>
<tr>
<td>II-3</td>
<td>M</td>
<td>33</td>
<td>–</td>
<td>37</td>
<td>–</td>
<td>moderately decreased</td>
<td>R763G</td>
</tr>
<tr>
<td>II-4</td>
<td>F</td>
<td>96</td>
<td>–</td>
<td>120</td>
<td>–</td>
<td>moderately decreased</td>
<td>R854Q</td>
</tr>
<tr>
<td>Normal</td>
<td>–</td>
<td>50–150</td>
<td>50–150</td>
<td>50–150</td>
<td>50–150</td>
<td>normal</td>
<td>–</td>
</tr>
</tbody>
</table>

Fig. 6. Left: VWF multimers in a family with VWD1/N (table 8) showing a smeary pattern and ultralarge multimers in the proband double heterozygous for R854W/R763G (I-1) and in heterozygous carriers of R763G (II-1, II-2, II-3) [15]. Right: VWF multimers of recombinant VWF disclosing abnormal moving bands of mutant R763G VWF and a smeary pattern of VWF heterozygous for R763G or double heterozygous for R763G/R854W [15].
References

1 Mazurier C, Goudemand J, Hilbert L, Caron C, Fressinaud E, Meyer D: Type 2N von Wi-
   llebrand disease: clinical manifestations, pathophysiology, laboratory diagnosis and
   molecular biology. Best Pract Res Clin Hae-
   matol 2001;14:337–347.

2 University of Sheffield: ISTH SSC VWF Da-
   tabase: http://www.vwf.group.shef.ac.uk/in-
   dex.html.

3 Liu ML, Shen BW, Nakaya S, Pratt KP, Fuji-
   kawa K, Davie EW, Stoddard BL, Thompson
   AR: Hemophilic factor VIII C1- and C2-
   domain missense mutations and their mod-
   eling to the 1.5-angstrom human C2-do-
   main crystal structure. Blood 2000;96:
   979–987.

4 D’Oiron R, Lavergne JM, Lavend’homme R,
   Benhida A, Bordet JC, Negrier C, Peerlinck
   K, Vermeylen J, Saint-Remy JM, Jacquemin
   M: Deletion of alanine 2201 in the FVIII C2
   domain results in mild hemophilia A by im-
   pairing FVIII binding to VWF and phospho-
   lipids and destroys a major FVIII antigenic
   determinant involved in inhibitor develop-

5 Caron C, Mazurier C, Goudemand J: Large
   experience with factor VIII binding assay of
   plasma von Willebrand factor using com-
   mercial reagents. Br J Haematol 2002;117:
   716–718.

6 Mazurier C, Gaucher C, Jorieux S, Goude-
   mand M: Biological effect of desmopressin
   in eight patients with type 2N (‘Normandy’)
   von Willebrand disease. Collaborative

7 Goodeve A, Eikenboom J, Castaman G, Rode-
   ghiero F, Federici AB, Battle J, Meyer D, Ma-
   zurier C, Goudemand J, Schneppenheim R,
   Buddle U, Ingerslev J, Habart D, Vorlova Z,
   Hohnberg L, Lethagen S, Pasi J, Hill F, Hash-
   emi Soteh M, Baronciani L, Halden C, Guil-
   liatt A, Lester W, Peakes I: Phenotype and gen-
   otype of a cohort of families historically di-
   agnosed with type I von Willebrand disease
   in the European study, Molecular and Clini-
   cal Markers for the Diagnosis and Manage-
   ment of Type I von Willebrand disease (MC-

8 Haberichter SL, Balistreri M, Christopher-
   son P, Morateck P, Gavazova S, Bellissimo
   DB, Manco-Johnson MI, Gill JC, Montgomery
   RR: Assay of von Willebrand factor (VWF)
   propeptide to identify patients with type I von
   Willebrand disease with decreased VWF sur-

9 Sztukowska M, Gallinaro L, Cattini MG,
   Pontara E, Sartorello F, Padrini R, Pagan R,
   Casonato A: von Willebrand factor propeptide
   makes it easy to identify the shorter von
   Willebrand factor survival in patients with type
   I and type Vicenza von Willebrand disease. Br J

10 Jorieux S, Fressinaud E, Goudemand J, Gau-
    cher C, Meyer D, Mazurier C: Conforma-
    tional changes in the D’ domain of von
    Willebrand factor induced by CYS 25 and CYS
    95 mutations lead to factor VIII binding defect
    and multimeric impairment. Blood 2000;95:
    3130–3145.

11 Allen S, Abuzaadah AM, Blagg JL, Hinks J,
   Nesbitt IM, Goodeve AC, Gursel T, Ingerslev
   J, Peake IR, Daly ME: Two novel type 2N von
   Willebrand disease-causing mutations that
   result in defective factor VIII binding, mul-
   timerization, and secretion of von Wille-

12 Jorieux S, Gaucher C, Goudemand J, Mazu-
    rier C: A novel mutation in the D3 domain of
    von Willebrand factor markedly decreases
    its ability to bind factor VIII and affects its
    4670.

13 Schneppenheim R, Lenk H, Obser T, Olden-
    burg J, Oyen F, Schneppenheim S, Schweab
    R, Will K, Buddle U: Recombinant expression
    of mutations causing von Willebrand disease
    type Normandy: characterization of a com-
    bined defect of factor VIII binding and mul-
    timerization. Thromb Haemost 2004;92:36–
    41.

14 Casonato A, Sartorello F, Cattini MG, Pon-
    tera E, Soldera C, Bertomoro A, Girolami A:
    An Arg760Cys mutation in the consensus se-
    quence of the von Willebrand factor propep-
    tide cleavage site is responsible for a new von
    Willebrand disease variant. Blood 2003;101:
    151–156.

15 Hilbert L, Nurden P, Caron C, Nurden AT,
   Goudemand J, Myer D, Fressinaud E, Ma-
   zurier C: Type 2N von Willebrand disease
   due to compound heterozygosity for R854Q
   and a novel R763G mutation at the cleavage
   site of von Willebrand factor propeptide.
Laboratory Diagnosis of von Willebrand Disease Type 1/2E (2A Subtype IIE), Type 1 Vicenza and Mild Type 1 Caused by Mutations in the D3, D4, B1–B3 and C1–C2 Domains of the von Willebrand Factor Gene

Role of von Willebrand Factor Multimers and the von Willebrand Factor Propeptide/Antigen Ratio

Alain Gadisseur, Zwi Berneman, Wilfried Schroyens, Jan Jacques Michiels

Hemostasis and Thrombosis Research Center, Antwerp University Hospital, Edegem, Belgium; Hemostasis and Thrombosis Science Center, Goodheart Institute, Rotterdam, The Netherlands

Key Words
Genotypes · Phenotypes · Propeptides · Type 1 Vicenza · von Willebrand disease

Abstract
Autosomal dominant von Willebrand disease (VWD) type 1/2E is a quantitative/qualitative defect in the von Willebrand factor (VWF) caused by heterozygous cysteine and non-cysteine mutations in the D3 domain of the VWF gene and results in a secretion-multimerization-clearance defect in mutant VWF with the loss of large VWF multimers not due to proteolysis. The multimers of patients with dominant VWD type 1/2E due to mutations in the D3 domain show an aberrant triplet structure with lack of outer bands but with pronounced inner bands of the triplet structure combined with a relative decrease in large multimers reflecting heterozygosity for multimerization defects. There is a good response to desmopressin (DDAVP) followed by rapid clearance of VWF:antigen (Ag), factor VIII coagulant activity (FVIII: C) and VWF:ristocetin cofactor activity (RCo) as the main cause of VWD type 1 or 2 with typical 2E multimeric pattern (VWD type 1/2E). Cysteine mutations in the D3 domains (C1130, C1149 and C1190) show pronounced features of VWD 1/2E with the relative loss of large and relative increase in small VWF multimers with abnormal triplet structure in heterozygotes. Such abnormalities are less pronounced in patients with a milder form of VWD type 1 due to non-cysteine mutations W1144G, T1156M and W1120S in the D3 domain. VWD type 1 Vicenza is caused by the R1205H mutation in the D3 domain and characterized by equally low levels of FVIII: C, VWF:Ag and VWF:RCo. The response to DDAVP in VWD Vicenza is good for FVIII:C, VWF:Ag and VWF:RCo, which is followed by a rapid clearance in less than a few hours of FVIII: C and VWF parameters. The ratios for FVIII:C/VWF:Ag, VWF: RCo/Ag and VWF:CB/Ag remain normal before and after DDAVP indicating that VWD Vicenza clearly differs from VWD type 1 or 2 with typical 2E multimeric pattern (VWD type 1/2E). Cysteine mutations in exons 38, 40, 42 and 43 (D4, B1–B3 and C1–C2 domains), show smearable patterns (either smf or sm), with the presence of large VWF multimers and a laboratory phenotype of mild VWD type 1 with variable penetrance of bleeding manifestations. Recent studies showed that the ratio of...
VWF propeptide (pp) to VWF:Ag can be used to predict a shorter than normal half-life for VWF:Ag. There is a strong inverse correlation between rapid clearance of VWF:Ag after DDAVP and increased VWFpp/Ag ratios >10 in VWD type 1 Vicenza, and >2 in VWD type 1/2E but normal or slightly increased (1–2) VWFpp/Ag ratios in mild-type VWD due to nonsense or missense mutations in the D1, D2, D4, B and C domains.

**Introduction**

Zimmerman et al. [1] first described one kindred with autosomal dominant von Willebrand disease (VWD) type IIE, which was featured by prolonged bleeding time (BT), normal factor VIII (FVIII):coagulant activity (C) and moderately decreased von Willebrand factor (VWF) parameters, but normal VWF:ristocetin cofactor activity (RCo)/antigen (Ag) ratio. The lack of large VWF multimers in VWD type IIE is not due to increased proteolysis and the pattern of the individual multimers shows only one clearly identifiable band (fig. 1, lane IIE) [1]. Schneppenheim et al. [2] and Schneppenheim and Budde [3] have produced good evidence that VWD type 2E (IIE) is associated with a cluster of mutations in the D3 domain of the VWF gene. In the 2006 database of the Scientific Standardization Committee (SSC) of the International Society on Thrombosis and Hemostasis (ISTH), VWF mutations in the D3 domain, which are required for multimerization, have been classified as unclassifiable (U) or type 1 of VWD but not as 2E or IIE, indicating that the SSC ISTH classification does not recognize this entity correctly (table 1) [4]. Consequently, we propose to label VWD 2A subtype IIE as VWD type 1/2E. In this report, we present an updated review of the literature on dominant VWD types 1 and 2 caused by mutations in the D3 domain as well as the D4, B1–B3 and C1–C2 domains with the exception of VWD 2A, 2B and 2M caused by mutations in the A1 and A2 domains.

**Dominant VWD Type 1/2E (IIE) due to Mutations in the D3 Domain**

Critical appraisal of the literature reveals that patients with VWF mutations in the D3 domain have laboratory features of dominant VWD type 1 with a type 2E VWF multimeric pattern in low- and medium-resolution gels (table 2). The first case of VWD IIE (2E) described by Zimmerman et al. [1] was a 12-year-old boy with mild bleeding, prolonged BT (>20 min, normal <8.5 min), VWF:Ag 0.26, VWF:RCo 0.30 (VWF:RCo/Ag ratio 1.15) and FVIII:C 1.00 U/ml (ratio FVIII:C/VWF:Ag 3.8; table 2) [1]. The mother showed similar laboratory abnormalities: VWF:Ag 0.22, VWF:RCo 0.11 (VWF:RCo/Ag ratio 0.5) and FVIII:C 1.00 U/ml (ratio FVIII:C/VWF:Ag 4.5; table 2). Despite the VWD type 1 phenotype, there was loss of large VWF multimers not due to increased proteolysis. In addition, the pattern of individual VWF multimers in the son and mother was aberrant in that there was only one clear identifiable band without a triplet structure (fig. 1, lane IIE).

Eikenboom et al. [5] described 5 cases in two families of classical dominant type 1 VWD with high penetration of moderate bleeding symptoms caused by a heterozygous missense mutation C1130F in one and C1149R in another family. These two heterozygous missense mutations in the D3 domain interfere with the normal VWF subunits coded by the normal VWF leading to a secretion-multimerization defect in the VWF by endothelial cells [5]. C1130F and C1149G mutations cause a dominant type 1 of VWD with low VWF levels (<0.20 U/ml), the presence of all sizes of VWF multimers and an increased FVIII:C/VWF:Ag ratio of 2.0–2.3 consistent with a VWF secretion defect (table 2) [5]. Castaman et al. [6] reported the presence of the C1130F mutation in three unrelated families with typical features of pronounced dominant type 1 of VWD (VWF values <15 U/dl and increased

---

**Table 1. SSC ISTH database of VWF missense mutations in the D3 domain [4]**

<table>
<thead>
<tr>
<th>VWD type</th>
<th>Exon mutation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Unclassified</td>
<td>25 3301T→C</td>
</tr>
<tr>
<td>U</td>
<td>25 3303C→G</td>
</tr>
<tr>
<td>U</td>
<td>25 3320A→G</td>
</tr>
<tr>
<td>U</td>
<td>25 3359G→C</td>
</tr>
<tr>
<td>I</td>
<td>26 3389G→T</td>
</tr>
<tr>
<td>I</td>
<td>26 3445T→C</td>
</tr>
<tr>
<td>I</td>
<td>26 3467C→T</td>
</tr>
<tr>
<td>I</td>
<td>26 3470G→T</td>
</tr>
<tr>
<td>U</td>
<td>26 3511G→C</td>
</tr>
<tr>
<td>U</td>
<td>26 3586T→C</td>
</tr>
<tr>
<td>U</td>
<td>27 3614G→T</td>
</tr>
<tr>
<td>U</td>
<td>27 3614G→A</td>
</tr>
<tr>
<td>2M/1</td>
<td>27 3614G→A</td>
</tr>
</tbody>
</table>
FVIII:C/VWF:Ag ratios; table 2). In vitro experiments (expression studies) indicate that the C1130F and C1149R mutations cause intracellular retention of VWF dimers and a multimerization defect (inability to form intracellular multimers). Secretion of a mixture of mutant VWF dimers/normal VWF protein in vivo results in a VWF multimeric pattern with the absence of large multimers and no triplet structure of individual bands indicative of a multimerization defect consistent with VWD type 1/2E (table 2) [5, 7, 8]. Recently, we found two mutations (W1120S and C1190R) in the D3 domain associated with dominant VWD type 1/2E (table 3; expression studies and manuscript in preparation). We observed that VWD type 1/2E due to the heterozygous mutations W1120S and C1190S in the D3 domain shows good responses of FVIII:C and VWF parameters to desmopressin (DDAVP) followed by short half-lives of VWF parameters indicating a minor secretion but pronounced rapid clearance defect of VWF:Ag and associated functions (FVIII:C and VWF:RCo) [10].

Casana et al. [12] reported the association of dominant mild type 1 of VWD with increased FVIII:C/VWF:Ag ratios in seven members of one family with heterozygosity for T1156M in the D3 domain of the VWF gene indicating a secretion defect (table 2). Unfortunately, this study provided no data on VWF multimers and no DDAVP response curves of VWF parameters and FVIII.

Fig. 1. Classification of VWD type 2 based on multimeric analysis of mutated VWF/VWD with a type IIE multimeric pattern according to Schneppenheim et al. [2] and Schneppenheim and Budde [3] and the updated SSC ISTH classification appears to be a hybrid of VWD phenotype 1 with a type 2E multimeric pattern showing a relative decrease in large multimers and individual bands without a triplet structure due to a missense mutation in the D3 multimerization domain. VWD Normandy (2N) and collagen binding defect (CBD) usually have normal VWF multimers. a Zimmermann et al. [1]. b Schneppenheim and Budde [3].
C. In vitro experiments indicate that the T1156M mutation causes intracellular retention of VWF and secretion of VWF monomers and dimers consistent with a multimerization defect with the absence of large multimers [13]. Heterozygosity for T1156M in two studies is consistent with dominant mild type 1 with normal VWF multimers [12, 13]. Expression studies of recombinant VWF T1156M showed a secretion-multimerization defect [13].

Haberichter et al. [14] reported two families with dominant VWD type 1 caused by a missense mutation in the D3 multimerization domain W1144G (table 2) with complete penetrance of moderate bleeding manifestations including bruising, menorrhagia and multiple instances of prolonged bleeding after injury or tooth extraction. DDAVP resulted in restricted but significantly increased FVIII and VWF levels followed by very short half-lives of both VWF and FVIII:C of about 2–3 h consistent with rapid clearance and consequently to be diagnosed as VWD type 1 secretion-clearance (VWD I-SC) defect.

VWF multimers show a relative decrease in large multimers.

Table 2. Laboratory characteristics of dominant type 1 SC with a type 2E multimeric pattern due to mutations in the D3 domain (VWD type 1/2E)

<table>
<thead>
<tr>
<th>Mutation</th>
<th>FVIII:C %</th>
<th>VWF:Ag %</th>
<th>VWF:RCo/VWF:Ag</th>
<th>VWF:RCo/VWF:Ag</th>
<th>VWD type</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Unknown</td>
<td>100</td>
<td>26</td>
<td>30</td>
<td>3.8</td>
<td>1/2E</td>
<td>Zimmerman et al. [1]</td>
</tr>
<tr>
<td></td>
<td>100</td>
<td>22</td>
<td>11</td>
<td>4.5</td>
<td>0.5</td>
<td>2E</td>
</tr>
<tr>
<td>C1130F</td>
<td>10</td>
<td>&lt;20</td>
<td></td>
<td>2.1</td>
<td>1/2E</td>
<td>Eikenboom et al. [5]</td>
</tr>
<tr>
<td>1 family</td>
<td>22</td>
<td>11</td>
<td>&lt;20</td>
<td>2.0</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td></td>
<td>35</td>
<td>15</td>
<td>&lt;20</td>
<td>2.3</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>C1149G: Father</td>
<td>21</td>
<td>10</td>
<td>&lt;20</td>
<td>2.1</td>
<td>±1</td>
<td>1/2E</td>
</tr>
<tr>
<td>Son</td>
<td>22</td>
<td>11</td>
<td>&lt;20</td>
<td>2.3</td>
<td>±1</td>
<td>1/2E</td>
</tr>
<tr>
<td>C1130F: n = 12, 5 families</td>
<td>10–35</td>
<td>5–20</td>
<td>&lt;10</td>
<td>1.8</td>
<td>0.8</td>
<td>1/2E</td>
</tr>
<tr>
<td>C1130R: 3 families</td>
<td>13–15</td>
<td>12–22</td>
<td>7–13</td>
<td>±1</td>
<td>0.58–0.59</td>
<td>2E</td>
</tr>
<tr>
<td>C1130G</td>
<td>8</td>
<td>10</td>
<td>10</td>
<td>0.8</td>
<td>1.0</td>
<td>1/2E</td>
</tr>
<tr>
<td>T1156M: n = 7, 1 family</td>
<td>59–80</td>
<td>28–39</td>
<td>17–29</td>
<td>1.9–2.3</td>
<td>0.6–0.8</td>
<td>mild type 1</td>
</tr>
<tr>
<td>W1144G: n = 13, 2 families</td>
<td>16–82</td>
<td>5–48</td>
<td>8–43</td>
<td>1.5–7001.7</td>
<td>0.66–2.1</td>
<td>1/2E</td>
</tr>
<tr>
<td>W1149G: Case report</td>
<td>24</td>
<td>31</td>
<td>12</td>
<td>0.77</td>
<td>0.39</td>
<td>2E</td>
</tr>
<tr>
<td>G1180C: Before DDAVP</td>
<td>15</td>
<td>13</td>
<td>12</td>
<td>1.2</td>
<td>0.9</td>
<td>1/2E</td>
</tr>
<tr>
<td>After DDAVP</td>
<td>1.54</td>
<td>1.02</td>
<td>59</td>
<td>1.5</td>
<td>0.6</td>
<td></td>
</tr>
<tr>
<td>T1156M/null</td>
<td>0.36</td>
<td>0.07</td>
<td>&lt;0.13</td>
<td>5.0</td>
<td>–</td>
<td>severe 1/2E</td>
</tr>
</tbody>
</table>

1 2538G→A transition which results in G1180R.

Table 3. Laboratory characteristics of dominant VWD in Antwerp due to the W1120S and C1190R mutations in the D3 multimerization domain typically show VWD type 1/2E and 2E, respectively

<table>
<thead>
<tr>
<th>Mutation</th>
<th>FVIII:C %</th>
<th>VWF:Ag %</th>
<th>VWF:RCo/VWF:Ag</th>
<th>VWF:RCo/VWF:Ag</th>
<th>VWD type</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>W1120S</td>
<td>72</td>
<td>34</td>
<td>21/19</td>
<td>0.74</td>
<td>&gt;300/normal</td>
<td>VWD 1/2E</td>
</tr>
<tr>
<td>C1190R</td>
<td>56</td>
<td>41</td>
<td>14/23</td>
<td>0.34</td>
<td>&gt;300/zero</td>
<td>VWD 2E</td>
</tr>
</tbody>
</table>
mers and satellite bands were nearly absent consistent with a type 2E pattern (1/2E) [14].

James et al. [15] published a very interesting case of dominant VWD phenotype 1 with the lack of large VWF multimers and absence of triplets (2E) with the G1180C mutation in the D3 domain (table 2). Expression studies showed that secretion of the G1180C mutant protein was reduced by 66 and 93% for the heterozygous and homozygous G1180C mutant transfections, respectively, indicating intracellular retention (secretion defect) with the loss of high-molecular-weight VWF multimers similar to that described for the C1130F and C1149G mutants of the D3 domain [7].

The mutations C1157F and C1196R in the D3 multimerization domain published in the SSC ISTH database have been labeled as VWD unclassifiable (table 1). Baldord et al. [16] and Gaucher et al. [17] identified the Y1107C mutation in the D3 domain in the described type 2-Bern, which showed a multimeric pattern consistent with autosomal dominant type VWD 2E. Interestingly, compound heterozygosity for a missense mutation (C1071F) in the D3 domain with a null allele (W642X) was associated with severe VWD labeled as type 2E and not 2M of VWD [10, 18, 24]. Congenital VWD type 1/2E; table 2) with severe VWD labeled as type 2E but very likely type 1 [10, 18, 24].

VWD Type 1 Vicenza

VWD type 1 Vicenza is caused by the R1205H mutation and featured by equally low levels of FVIII:C, VWF:Ag and VWF:RCo, and the presence of unusually large vWF multimers in plasma [18–26]. The response to DDAVP in VWD Vicenza is good for FVIII:C, VWF:Ag and VWF:RCo, which is followed by unexplained very short half-lives of less than a few hours for FVIII:C and all VWF parameters, indicating a rapid clearance defect as the cause of a hemophilia-like phenotype of VWD (CVWD Rotterdam) [18, 20, 24]. The FVIII:C/VWF:Ag, VWF:RCo/Ag and VWF:CB/Ag ratios remain within the normal range before and after DDAVP, indicating that VWD Vicenza and CVWD Rotterdam belong to type 1 and not 2M of VWD [10, 18, 24]. Congenital VWD type 1 Vicenza due to a single mutation (R1205H) in the D3 domain clearly differs from 2M and 2U due to a mutation in the A1 domain with loss of VWF:RCo function [24–26]. The absence of increased triplet structure seen in type 2A and type 2B (fig. 1) indicates that the mechanism of rapid clearance of the FVIII-VWF complex in VWD Vicenza and CVWD Rotterdam is completely different and not caused by proteolysis [24]. Ultralarge high-molecular-weight VWF:Ag multimers (even in 1% agarose gels) were not seen in any of the seven UK kindreds with VWD Vicenza R1205H, and the triplet structure was reported to be normal [6]. A possible explanation for the presence of ultralarge VWF:Ag multimers has been proposed by assuming a very short half-life of the R1205H VWF molecule: there is a relatively higher proportion of newly secreted ultralarge VWF:Ag multimers with less opportunity for proteolysis by ADAMTS13, similar to the multimer distribution directly following DDAVP treatment [13, 14]. An alternative explanation may be that due to a multimerization defect mutant and normal VWF multimers form unstable multimers, which are rapidly cleared after secretion from endothelial cells into plasma. Casonato et al. [18] demonstrated the presence of ultralarge VWF:Ag multimers in four kindreds who additionally presented the M740I mutation, but not in the individuals with the R1205H mutation alone. In the study by Castaman et al. [23] affected individuals with the R1205H mutation appeared to have ultralarge multimers in the presence or absence of the additional M740I mutation. Expression studies of R1205H and M740I are warranted to demonstrate which of these two mutations is responsible for the ultralarge VWF multimers.

Mild VWD Type 1 (1 m, 1 sm, 2 smf) due to Missense Mutations in the D4, B1–B3, C1–C2 Domains

Ciavarella et al. [27] described in 1985 five individuals from two unrelated families with autosomal dominant mild bleeding history of recurrent epistaxis, menorrhagia and bleeding following tooth extraction. Replacement therapy has never been required. Although the VWF:Ag levels were in the lower range of normal, the values for VWF:RCo were slightly decreased and BT prolonged. Using high-resolution gels, the VWF multimeric structure showed the presence of large multimers and single bands without a triplet structure. Following DDAVP treatment, a new discrete band between the normal dimer and tetramer bands appeared together with a smeary pattern of intermediate and high VWF multimers [27].

Haberichter et al. [28] reported two unrelated families with the heterozygous S2179F mutation in the D4 domain
In an affected member, small, intermediate and large multimers were present, with a significant decrease in intensity of VWF satellite bands (similar to 2E with the presence of large multimers and lack of triplet structure), being comparable to the original observations by Ciavarella et al. [27] and as documented recently by Budde et al. [11] in the MCMDM-1VWD Study for mutations in the D4, B1–B3 and C1–C3 domains. Further studies showed that affected family members heterozygous for S2179F are characterized by pronounced dominant VWD type 1, increased FVIII:C/VWF:Ag ratio (secretion defect), restricted response of VWF to DDAVP with transient appearance of a smeary VWF pattern followed by rapid clearance of FVIII-VWF parameters consistent with VWD type 1 SC (table 4, fig. 2) [28]. Homozygotes for S2179F/S2179F or double heterozygotes for S2179F with a null allele (S2179F/null) are predicted to present severe VWD type 1.

The majority of heterozygous mutations in D1, D2, D’, D4, B1–B3, C1–C2 and in the connecting region between C2 and cysteine knot (CK) domains are associated with mild VWD with no or mild penetrance of bleeding manifestations [9–11]. Data from the European MCMDM-1VWD Study show that heterozygous mutations in the D4, B1–B3, C1–C2 and CK domains L1774S*, K1794E*, C2304Y*, R2313H, G2518S*, Q2544X*, C2693Y* and P2722A have mild VWD type 1 and normal multimers [9]. Heterozygous mutations in the D4, B1–B3, C1–C2 and CK domains V1822G*, L2207P*, C2257S*, C2304Y*, C2362F*, G2441C*, R2464C*, C2477Y*, C2477S* and Q2520P* have mild-moderate VWD type 1, with abnormal VWF multimers (usually smeary pattern) [9].

In asymptomatic or mild VWD type 1 heterozygous for C2364F, large multimers are present but with an abnormal pattern, with slightly faster (f) central band and mild smeary pattern (sm) consistent with asymptomatic VWD type 1.

---

**Table 4. Dominant VWD type 1 SC caused by S2179F mutation in the D4 domain**

<table>
<thead>
<tr>
<th>Mutation</th>
<th>FVIII:C Ag, %</th>
<th>VWF:RCo %</th>
<th>VWF: VWF:Ag, %</th>
<th>FVIII:C/VWF:Ag ratio</th>
<th>VWF:RCo/ ratio</th>
<th>VWD type</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>S2179F</td>
<td>19–35</td>
<td>7–18</td>
<td>6–14</td>
<td>1.5–3.0</td>
<td>0.6–1.2</td>
<td>type 1SC</td>
<td>Haberichter et al. [28]</td>
</tr>
<tr>
<td>n=6, 2 families</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>fig. 2</td>
<td></td>
</tr>
<tr>
<td>S2179F</td>
<td>26</td>
<td>6</td>
<td>6</td>
<td>4.3</td>
<td>1.0</td>
<td>1</td>
<td>Goodve et al. [9]</td>
</tr>
</tbody>
</table>

---

**Fig. 2.** Restricted response of VWF parameters and FVIII:C to DDAVP followed by rapid clearance of VWF parameters in dominant VWD type 1 SC caused by a S2179F mutation in the D4 domain (case A-III) versus normal responses of VWF parameters and FVIII:C to values >1.5 IU/ml in a case with mild VWD type 1 (Haberichter et al. [28]).
Role of VWF Multimers in the Diagnosis of VWD Type 1/2E, 2M and Mild Type 1 with Smeary VWF Multimers

The multimers of patients with dominant VWD type 1/2E (IIE) due to mutations in the D3 domain show an aberrant triplet structure with lack of outer bands but with pronounced inner bands of the triplet together with a relative decrease in large multimers reflecting heterozygosity for a multimerization defect [2, 3, 11]. Interestingly, there is a very good response to DDAVP followed by rapid clearance of VWF:Ag and associated rapid clearance of FVIII:C and VWF:RCo with increased VWF propeptide (pp)/Ag ratio as the main cause of the phenotype with a type 2E multimeric pattern. This picture of laboratory features is pathognomonic for the new entity of VWD type 1/2E (IIE) [10, 28]. The rapid clearance at high shear of the VWF:Ag/FVIII complex after DDAVP may be related to loosely connected unstable large multimers as a main feature of a multimerization defect. It seems to us that cysteine mutations in the D3 domain (C1130, C1149 and C1190) show the relative loss of large and relative increase in small VWF multimers with abnormal triplet structure in heterozygotes. Such abnormalities seem to be less pronounced in the non-cysteine mutations W1144G, T1156M and S1120 in the D3 domain in patients having a milder variant of VWD type 1/2E (IIE).

Patients with VWD 2M due to loss of function in the A1 domain have decreased ristocetin-induced platelet aggregation in the presence of a normal or near normal VWF multimeric pattern in a low-resolution agarose gel, a poor response to DDAVP of VWF:RCo, good responses to DDAVP of both VWF:CB and VWF:Ag and FVIII:C and a relative decrease in the large VWF multimers with less resolved triplet structure of each of the multimeric bands in medium- or high-resolution gels (fig. 3). VWD type 2M and 2U share common laboratory features due to a selective loss of RCo function mutation in the A1 domain not due to increased proteolysis [31].

In the MCMDM-1VWD Study, Budde et al. [11] observed that six of nine mutations (L2207P, C2257S, C2304Y, C2362F, C2441Y, C2441 C, R2464C, C2477Y and C2477S) in exons 38, 40, 42 and 43 (D4, B1–B3 and C1 domains) showed a smeary pattern (either smf or sm), with the presence of large VWF multimers and a laboratory phenotype of mild VWD type 1 with variable penetrance of bleeding manifestations. In six of these nine mutations, cysteine mutations in the D4, B1–B3 and C1 domains were responsible, possibly inducing the additional structural alterations leading to the smeary pattern of VWF in heterozygotes (fig. 3) [11]. C2364F is a true recessive mutation since heterozygous subjects are asymptomatic or present with very mild VWD type 1 in particular when associated with blood group O [29]. Patients heterozygous for C2362F mutation present large multimers, but a smeary pattern, with faster moving central band and loss of triplet structure [11, 29]. Expression studies demonstrated that mutant C2362F VWF is synthesized but minimally secreted and largely retained intracellularly [8].
Role of the VWFpp/Ag Ratio in the Differential Diagnosis of VWD Type 1/2E, Type 1 Vicenza and Mild 1 with Variable Penetration of Bleeding Symptoms

VWFpp and VWF:Ag are non-covalently associated and stored in Weibel-Palade bodies in endothelial cells for controlled release [30, 32, 33]. After their release in plasma, VWFpp and mature VWF multimers dissociate and circulate independently with a half-life of 2–3 h for VWFpp and 8–12 h for VWF:Ag. Plasma concentrations of VWFpp and VWF:Ag are expressed in units per milliliter of normal plasma, consequently, the ratio of VWFpp to VWF:Ag in plasma is set to 1.0. The rationale behind the use of the VWFpp/VWF:Ag ratio is that in all variants of VWD type 1 and 2 the half life of VWFpp is normal, whereas the clearance of VWF:Ag may be very short, shortened or normal. VWD patients with a secretion defect but normal clearance do show a restricted response of VWF to DDAVP followed by normal half-lives of VWF:Ag and decreased values for VWFpp and VWF:Ag with a normal VWFpp/VWF:Ag ratio. Those VWD
patients with normal secretion but increased clearance of VWF:Ag (and VWF:RCo and FVIII:C) do show good responses to DDAVP followed by short half-lives of VWF:Ag leading to an increased VWFpp/VWF:Ag ratio. The response to DDAVP in VWD Vicenza is very good for FVIII:C, VWF:Ag and VWF:RCo, followed by very short half-lives of less than a few hours for FVIII:C and all VWF parameters, indicating a rapid clearance defect of VWF:Ag as the cause of pronounced type 1 deficiency in VWD Vicenza [18]. Patients with VWD type 1/2E due to C1130G/F or R and W1144G mutations in the D3 multimerization domain and the S2179F mutation in the D4 domain have shortened VWF:Ag half-lives after DDAVP, indicating rapid clearance as the main mechanism for a laboratory phenotype 1 [10]. Patients with mild VWD type 1 usually show normal responses to DDAVP of VWF parameters followed by normal half-lives and are therefore candidates for DDAVP in the prevention and treatment of bleedings [24]. Mild type 1 of VWD is related to nonsense or missense mutations in the D1, D2, D‘/H11541, D4, B1–B3 and C1–C2 domains (table 5) [9].

Table 5. Result of VWF:Ag and VWFpp levels in VWD type 1/2E or 1 SC with the mutations W1144G (D3 domain) and S2179F (D4 domain) and blood group O and in mild VWD type 1 due to mutations in the D1-D2-D‘ domain and the D4-C1-C3-C1-C2 domain [14, 28, 36] compared to C1584 [34, 35] and controls related to ABO blood group

<table>
<thead>
<tr>
<th>VWD type, mutation</th>
<th>Patients</th>
<th>VWF:Ag mean (range)</th>
<th>VWFpp mean (range)</th>
<th>VWFpp/Ag mean (range)</th>
<th>T1/2 VWF:Ag after DDAVP h</th>
</tr>
</thead>
<tbody>
<tr>
<td>USA/European studies [14, 28]</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Type 1 Vicenza: R1205H/M740I</td>
<td>3</td>
<td>6.8 (5.7–8.4)</td>
<td>72.8 (63–85)</td>
<td>10.9 (10.1–11.5)</td>
<td>&lt;2 h</td>
</tr>
<tr>
<td>Type 1/2E (IIIE)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>C1130G/F/R</td>
<td>3</td>
<td>16 (8–13)</td>
<td>80 (52–107)</td>
<td>5.4 (4.6–6.5)</td>
<td>3–4 h</td>
</tr>
<tr>
<td>W1144G, blood group O</td>
<td>6</td>
<td>16 (13–22)</td>
<td>61 (55–69)</td>
<td>4.1 (3.1–5.1)</td>
<td>3–4 h</td>
</tr>
<tr>
<td>W1144G, blood group non-O</td>
<td>6</td>
<td>22 (14–32)</td>
<td>72 (68–80)</td>
<td>3.2 (2.1–5.0)</td>
<td>3–4 h</td>
</tr>
<tr>
<td>Type 1 SC: S2179F, blood group O</td>
<td>5</td>
<td>15 (9–24)</td>
<td>65 (56–77)</td>
<td>4.8 (3.2–6.4)</td>
<td>3–4 h</td>
</tr>
<tr>
<td>Mild type 1</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>D1, D2, D‘ domain, G160W, N116I, M771I, R854Q/R854Q, A3, D4, B–C domains, V1822G, R2063S/R2287W, C2304Y, C2477S, C2693Y, Int 13 1534-3C→A</td>
<td>6</td>
<td>46.1 (37–79)</td>
<td>64 (23–96)</td>
<td>1.13 (0.6–1.6)</td>
<td>normal</td>
</tr>
<tr>
<td>Blood group O</td>
<td>45</td>
<td>78 (43–136)</td>
<td>105 (55–154)</td>
<td>1.41 (1.04–2.4)</td>
<td>not measured</td>
</tr>
<tr>
<td>Blood group non-O</td>
<td>45</td>
<td>116 (79–164)</td>
<td>128 (84–178)</td>
<td>1.12 (0.84–1.78)</td>
<td>not measured</td>
</tr>
<tr>
<td>Italian study [36]</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Type 1 Vicenza: R1205H/M740I</td>
<td>14</td>
<td>12 ± 1.9</td>
<td>136 ± 22</td>
<td>13.0 ± 0.5</td>
<td>1.3 ± 0.2 h</td>
</tr>
<tr>
<td>Type 1/2E: C1130F</td>
<td>4</td>
<td>27 ± 10</td>
<td>105 ± 20</td>
<td>4.69 ± 0.67</td>
<td>4.1 ± 0.7 h</td>
</tr>
<tr>
<td>Mild type 1</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Blood group O</td>
<td>17</td>
<td>79 ± 8.6</td>
<td>99 ± 8</td>
<td>1.6 ± 0.06</td>
<td>9.4 ± 1.0 h</td>
</tr>
<tr>
<td>Blood group non-O</td>
<td>13</td>
<td>113 ± 9</td>
<td>106 ± 6</td>
<td>1.2 ± 0.04</td>
<td>21 ± 4 h</td>
</tr>
<tr>
<td>UK studies [35]</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>C1584 all</td>
<td>50</td>
<td>82 ± 35</td>
<td>94 ± 20</td>
<td>1.36 ± 0.40</td>
<td>–</td>
</tr>
<tr>
<td>C1584 group O</td>
<td>23</td>
<td>58 ± 14</td>
<td>94 ± 20</td>
<td>1.66 ± 0.33</td>
<td>–</td>
</tr>
<tr>
<td>C1584 group non-O</td>
<td>17</td>
<td>98 ± 34</td>
<td>109 ± 30</td>
<td>1.17 ± 0.25</td>
<td>–</td>
</tr>
<tr>
<td>Blood group O</td>
<td>50</td>
<td>97 ± 24</td>
<td>110 ± 31</td>
<td>1.16 ± 0.24</td>
<td>–</td>
</tr>
<tr>
<td>Blood group non-O</td>
<td>50</td>
<td>126 ± 30</td>
<td>110 ± 28</td>
<td>0.90 ± 0.21</td>
<td>–</td>
</tr>
</tbody>
</table>

1 n = 18: stop codon 6, missense mutation 8 (R115C, P2063F, C2362P, G2705) and mutation not found 4.
These observations clearly indicate that rapid clearance of VWF:Ag is the main cause of the pronounced VWF deficiency in VWD type 1/2E [SC defect (table 5)].

There is a strong inverse correlation between rapid clearance of VWF:Ag after DDAVP and increased VWFpp/VWF:Ag ratios (1.1–1.8 vs. 1.4–1.6) and normal half-lives for VWF:Ag after DDAVP of around 10 h (table 5).

The three studies uniformly disclosed significantly increased VWFpp/VWF:Ag ratios with mean values ranging from 3.2 to 4.69 in all cases of VWD type 1/2E with the mutation C1130, W1144G in the D3 domain and the S2179F mutation in the D4 domain indicating that a markedly increased clearance (C) of the VWF:Ag/FVIII: C complex [14, 28, 36]. This could be independently documented by a good response to DDAVP followed by shortened half-lives for VWF:Ag of about 3–4 h (table 5) [10, 11, 14, 28, 36]. These observations clearly indicate that rapid clearance of VWF:Ag is the main cause of the pronounced VWF deficiency in VWD type 1/2E [SC defect (table 5)].

There is a strong inverse correlation between rapid clearance of VWF:Ag after DDAVP and increased VWFpp/VWF:Ag ratios >10 in VWD Vicenza, and >2 in VWD type 1/2E. In contrast, VWFpp/VWF:Ag ratios are normal or only slightly increased (1–2) in mild type VWD patients due to mutations in the D1-D2 and the D4-B-C domains (table 5) [10, 11, 14, 28, 33]. Patients with mild VWD type 1 due to missense mutations in the D1, D2 and D' domains have low normal to decreased VWFpp levels and normal VWFpp/VWF:Ag ratios of just above 1 indicating a synthesis/secretion defect of VWF [9–11, 28]. Patients with mild VWD due to mutations in the D4-B1-B3-C1-C2 domains have decreased values for VWFpp but normal to slightly increased VWFpp/VWF:Ag ratios indicative of a synthesis/secretion defect and a very mild clearance defect in some of them [28]. Patients with the C1584 mutation and blood group O have decreased values for VWF:Ag but normal values for VWFpp and slightly increased VWFpp/VWF:Ag ratios indicative of a mild clearance defect in VWF [36].

### References

8. Tjernberg P, Vos HL, Spaargaren-van Riel CC, Luken BM, Voorberg J, Bertina RM,


Gadirousse/Berneman/Schroyens/Michiels
Autosomal Dominant von Willebrand Disease Type 2M

Cedric Hermans a, Javier Batlle b

a Hemostasis and Thrombosis Center, Hemophilia Clinic, St. Luc University Hospital, Brussels, Belgium; b Departamento de Medicina, Universidad de Santiago de Compostela, Santiago de Compostela, and Servicio de Hematología y Hemoterapia, Complejo Hospitalario Universitario Juan Canalejo, La Coruña, Spain

Key Words
Desmopressin response • Multimeric patterns • Phenotype • Platelet adhesion • Type 2M • von Willebrand disease

Abstract
von Willebrand disease (VWD) type 2M is a distinct entity and clearly differs from type 1. The genotype-phenotype correlation for cases with ristocetin cofactor activity (RCo)/antigen (Ag) ratios <0.60 is clear, whereas the von Willebrand factor (VWF):collagen binding (CB)/VWF:Ag ratio is normal in VWD 2M. Typical laboratory features of VWD type 2 M are decreased ristocetin-induced platelet aggregation in the presence of a normal or near normal VWF multimeric pattern on a low-resolution agarose gel, a poor response to desmopressin (DDAVP) of VWF:RCo, and a good response of both VWF:CB and VWF:Ag to DDAVP. The phenotypic definition of VWD type 2M may need to be more stringent and should be the subject of an international standardization initiative.

von Willebrand disease (VWD) is a common, congenital bleeding disorder caused by a deficiency in or dysfunction of von Willebrand factor (VWF). VWF is a large, multimeric plasma glycoprotein that plays an important role in hemostasis, both as a mediator of platelet adhesion to the vascular subendothelium and as a carrier of factor VIII (FVIII) stabilizing its activity [1]. There are three main subtypes, classically characterized by excessive mucocutaneous bleeding, a positive family history and abnormal VWF laboratory results. VWD type 1 is a partial deficiency in qualitatively normal VWF, type 2 is caused by functionally abnormal VWF, and type 3 VWD is characterized by virtual absence of the VWF protein. Type 2 VWD refers to loss of function [VWF:ristocetin cofactor activity (RCo)] attributed to the absence of high-molecular-weight multimers (HMWM), and type 2M (M for ‘multimer’ or ‘miscellaneous’) to loss of function not caused by the loss of HMWM in low-resolution agarose gels.

Due to the complexity of VWF and VWD, various approaches to classify VWD have arisen in the past, and, consequently, a patient with the same defect may still be grouped into different VWD types depending on the nomenclature employed. The revised classification proposed in 1994 by the Scientific Standardization Committee (SSC) of the International Society on Thrombosis and Hemostasis (ISTH) was an extremely important step forward to unify criteria in this heterogeneous and complex area (fig. 1) [1]. Recently, it has been updated with very limited, but wise, modifications [2], considering possible further subdivisions of VWD type 1 or type 2A in the future.
According to the updated classification, type 2A includes variants with decreased platelet adhesion caused by a selective deficiency in HMWM. Type 2M includes variants with markedly defective platelet adhesion despite a relatively normal size distribution of VWF multimers. However, controversy exists regarding the classification of some particular mutations. VWD Vicenza caused by the heterozygous mutation Arg1205His was initially considered as type 2M, but now, despite some controversy, this variant is classified as VWD type 1 [2], since an increased clearance of VWF may be sufficient to explain the ultralarge multimer distribution of VWD Vicenza by shifting the plasma VWF multimer distribution towards that secreted initially from endothelial cells.

A very good example of controversy concerns the R1374C VWF mutation [3, 4]. Its phenotype shows several definite characteristics, which we have corroborated in 23 members of three unrelated families: (1) a decreased VWF:RCo/VWF:antigen (Ag) ratio, with the VWF:collagen binding (CB)/VWF:Ag ratio usually within the normal range, indicating a dysfunctional (‘variant’) VWF protein; (2) presence of all-size multimers although with a lower relative proportion of HMWM, a certain degree of smearing in the low-resolution VWF pattern, and medium-resolution gels show a normal satellite pattern, suggesting normal proteolytic VWF processing, and (3) regular association to a decreased VWF plasma level, which may explain previous diagnoses as type 1 VWD.

Several investigators have considered that R1374C VWD would fit better in the type 2M group because of the presence of HMWM, which are sometimes partly larger than those seen in normal plasma [3, 5, 6]. Other investigators preferred to classify it as type 2A due to the lower relative proportion of HMWM [7]. It has also been categorized as severe type 1 VWD [8], as well as type 1B [9], and 1B variant of 2A [10]. Finally, in the ISTH registry of VWD [11], it has been included as unclassified VWD. Thus, this mutation constitutes a true ‘Tower of Babel’ as far as its nomenclature is concerned (fig. 2).
To some extent, this controversy may be related to an unnecessary separation between type 2A and 2M, instead of grouping both as a single subtype ‘2A’ that includes all variants with a decreased platelet-dependent function, regardless of the multimeric distribution. According to the recently updated VWD classification published by the ISTH Subcommittee on VWF, VWD type 2M, which is the focus of this communication, includes qualitative variants with decreased VWF-dependent platelet adhesion without a selective deficiency in HMWM [2]. The assembly and secretion of large-molecular-weight multimers is approximately normal and the functional defect is caused by mutations within the A1 domain of the VWF gene, the region that encodes the binding site for platelet glycoprotein Ibα [3], disrupting binding of VWF to platelets or subendothelium. For VWD 2M, a total of 16 mutations have been identified in exon 28 and 1 mutation in exon 27 [4]. Additionally, a missense mutation in the A3 domain has been reported to reduce VWF binding to collagen, thereby reducing platelet adhesion and possibly causing VWD type 2M [5].

Recent large studies focusing on the molecular genetic basis of VWD type 1 have demonstrated the lack of a clear distinction between some VWD subtypes, namely type 1, type 2M and type 2A [6–8]. In other studies, some patients who were originally diagnosed with type 1 were reclassified later with VWD type 2 after more exhaustive analysis [9, 10]. This distinction is important because of the variability in response to treatment, e.g. desmopressin (DDAVP). The overlap of these subtypes is apparent-

Fig. 3. Multimeric and densitometric analyses of plasma VWF using low (a) and high (b) resolution SDS-agarose gels in a normal subject (N), VWD type 2A (2A), and in patients with VWD 2M caused by the R1374C mutation (III:10, III:16, IV:13 and V:4). In low-resolution SDS-agarose gels (a), the patients with the R1374C mutation show a quantitative distribution between N and 2A. In high-resolution agarose gels (b), a decreased but almost normal satellite banding pattern is observed in the patient with the R1374C mutation compared to the increased triplet satellite banding pattern in type 2A (IIA) of VWD (Penas et al. [10]).
ly not restricted to the phenotypic level, but also occurs at the genotypic level, thus raising important questions regarding the definition of the subtypes.

The diagnosis and classification of VWD relies on its phenotypic characterization and is supplemented by genetic analysis of causative mutations. The phenotypic characterization commonly involves the determination of FVIII in plasma and VWF:Ag, and assessment of the functional ability of VWF to bind platelets, e.g. determined by VWF:RCo. A presumptive diagnosis of type 2 VWD is investigated by analyzing the VWF:RCo/VWF:Ag ratio, investigation of plasma VWF multimers and the ristocetin-induced platelet agglutination (RIPA) assay. Type 1 is classically identified by a concordant VWF:RCo/VWF:Ag ratio $\geq 0.7$, whereas this ratio is characteristically discrepant ($<0.7$) in type 2 except for type 2N.

As previously reviewed by Michiels et al. [17] and in line with our experience, patients with VWD 2M are characterized by near normal values for FVIII coagulant activity (FVIII:C), subnormal levels for VWF:Ag and

Table 1. Phenotypic characterization of VWD type 1, 2A and 2M

<table>
<thead>
<tr>
<th>Assay</th>
<th>VWD 1</th>
<th>VWD 2A</th>
<th>VWD 2M</th>
</tr>
</thead>
<tbody>
<tr>
<td>FVIII:C</td>
<td>normal/low</td>
<td>normal/low</td>
<td>normal/low</td>
</tr>
<tr>
<td>VWF:Ag</td>
<td>low</td>
<td>low</td>
<td>normal/low</td>
</tr>
<tr>
<td>VWF:RCo</td>
<td>low</td>
<td>low</td>
<td>low</td>
</tr>
<tr>
<td>VWF:CB</td>
<td>low</td>
<td>low</td>
<td>normal/low</td>
</tr>
<tr>
<td>VWF:RCo/VWF:Ag ratio</td>
<td>$&gt;0.7$</td>
<td>$&lt;0.7$</td>
<td>$&lt;0.6$</td>
</tr>
<tr>
<td>VWF:CB/VWF:Ag ratio</td>
<td>$&gt;0.7$</td>
<td>$&lt;0.7$</td>
<td>normal</td>
</tr>
<tr>
<td>VWF multimeric pattern,</td>
<td>normal</td>
<td>loss of large</td>
<td>presence but loss of large multimers</td>
</tr>
<tr>
<td>relative multimers</td>
<td></td>
<td>multimers</td>
<td></td>
</tr>
</tbody>
</table>

Fig. 4. Despite poor or transiently restricted responses of VWF:RCo compared to restricted/normal responses of FVIII:C in 21 patients with VWD type 2M (VWD R1205H, Vicenza in 1, R1315C in 3, R1374C in 9, R1374H in 2 and undefined in 6), slightly/moderately prolonged bleeding times frequently approached normal levels 2h after DDAVP (Federici et al. [20]).
consistently very low levels for VWF:RCo with decreased VWF:RCo/Ag ratios. RIPA is uniformly decreased. Most patients show normal HMWM (table 1) [17].

Due to limitations in the sensitivity, reproducibility and variability in the interlaboratory VWF:RCo assay, the VWF:CB assay has been proposed to determine VWF activity [18]. However, the VWF:CB assay is unable to discriminate defective platelet-binding VWD variants with normal multimeric patterns such as VWD type 2M [19]. Moreover, it has been observed that the results for VWF:CB are dependent on the type of collagen used [10]. The VWF:CB/VWF:Ag ratio was indeed found to be higher (0.7) for type I collagen, while the results for type III collagen were variable [10]. This is important, given that, if only the values for the type I collagen VWF:CB results are taken into account, they would confirm a diagnosis of type I VWD. With regard to type III collagen, the variability in the results does not allow to draw conclusions about the utility of this assay in detecting 2A variants of VWD.

VWF:CB detects qualitative defects associated with a loss of HMWM in type 2A and 2B of VWD. It also detects defects in the VWF-collagen adhesive function, as it occurs in mutations at the CB site. However, in type 2M of VWD, although VWF-platelet interaction is altered, HMWM are present so that VWF:CB reflects the presence of these multimers. In spite of the recognized limitations of the VWF:RCo assay, VWF:CB should not be considered as a substitute for VWF:RCo in the diagnosis and classification of the disease, and both of them should be assessed for the diagnosis of VWD. It is therefore recommended that the VWF:CB assay be used in association with rather than as a replacement for the VWF:RCo assay [19].

The phenotype of the R1374C VWF mutation in 25 members of 4 unrelated families showed several definite characteristics: (1) a decreased VWF:RCo/VWF:Ag ratio with a VWF:CB/VWF:Ag ratio usually within the normal range, particularly after DDAVP, and (2) the presence of all-size multimers, although with a lower relative proportion of the HMWM (fig. 3). A certain degree of smearing is observed in the low-resolution VWF pattern, with medium-resolution gels showing a normal satellite pattern, suggesting normal proteolytic VWF processing (fig. 3) [17].

Except for type 2N, type 2 is classically identified by a discrepant VWF:RCo/VWF:Ag ratio <0.7. In a recently published study involving a Canadian VWD population, a ratio <0.40 (in individuals with normal multimers) was strongly associated with mutations in the A1 domain of VWF [6]. Cases with ratios >0.40 or perhaps >0.50 were found to more accurately be classified as type 1 VWD, because of the heterogeneity within this group. These authors suggest that an RCo/Ag ratio <0.50 be used to distinguish VWD type 2M from type 1.

It is also important to consider the impact of multimer analysis on the distinction between type 2A and type 2M of VWD. The multimers in type 2M are considered to be approximately normal [2] even if subtle abnormalities in triplet structure or the presence of increased HMWM are acceptable. Multimer analysis is technically challenging, and subtle distinctions may not always be appreciable. Interestingly, in a recent study, type 2M mutants and wild-type recombinant VWF exhibited a similar sensitivity to recombinant ADAMTS13-mediated proteolysis, in agreement with the normal multimeric pattern in vivo.

Using the results of a DDAVP challenge test, the typical laboratory features of VWD type 2M according to Michiels et al. [17] are (1) severe type 1, (2) decreased RIPA in the presence of a normal or near normal VWF multimeric pattern on a low-resolution agarose gel, (3) a poor response to DDAVP of VWF:RCo, and (4) a good response of both VWF:CB and VWF:Ag to DDAVP, which is consistent with the presence of all VWF multimers and can explain the normal/slightly prolonged Ivy bleeding times before and after DDAVP (fig. 4) [17, 20].

In conclusion, VWD 2M is a distinct entity and clearly differs from type 1. The genotype-phenotype correlation for cases with RCo/Ag ratios <0.60 is clear. The phenotypic definition of type 2M may need to be more stringent, and should be the subject of an international standardization initiative.

Acknowledgment

We are grateful to Prof. Ian R. Peake (University of Sheffield, Sheffield, UK) as well as to all the MCMDM-VWD European Union Project members. The study was supported by a grant (PI07/0229) from the Instituto de Salud Carlos III (Sanitarian Investigation Fund, Spain).
References


Dominant von Willebrand Disease Type 2M and 2U Are Variable Expressions of One Distinct Disease Entity Caused by Loss-of-Function Mutations in the A1 Domain of the von Willebrand Factor Gene

Alain Gadisseur a Marc van der Planken a Wilfried Schroyens a Zwi Berneman a Jan Jacques Michiels a, b

aHemostasis Unit, Department of Hematology, Antwerp University Hospital, Edegem, Belgium; bHemostasis and Thrombosis Science Center, Goodheart Institute, Rotterdam, The Netherlands

Introduction

von Willebrand disease (VWD) is a common, inherited bleeding disorder caused by a deficiency in or dysfunction of von Willebrand factor (VWF). VWF is a large, multimeric plasma glycoprotein that plays an important role in hemostasis, both as a mediator of platelet adhesion to the vascular subendothelium and as a carrier of factor VIII [FVIII coagulant activity (FVIII:C)] supporting its activity [1–5]. There are three main subtypes of VWD, classically characterized by excessive mucocutaneous bleeding, a positive family history and abnormal VWF laboratory studies. Patients with type 1 VWD present a partial deficiency in qualitatively normal VWF, type 2 VWD is caused by functionally abnormal VWF, and type 3 VWD is characterized by a virtual absence of the VWF protein. The diagnosis and classification of VWD relies on the phenotypic characterization and is complemented by genetic analysis of causative mutations [4–7].

Key Words
Collagen binding • Ristocetin cofactor activity • Ristocetin-induced platelet aggregation • von Willebrand disease • von Willebrand factor • VWF A1 domain • VWF multimers

Abstract
A complete set of laboratory investigations, including bleeding time, PFA-100 closure time, factor VIII coagulant activity (FVIII:C), von Willebrand factor (VWF), ristocetin cofactor activity (RCO), collagen binding (CB) and antigen concentration (Ag), ristocetin-induced platelet aggregation (RIPA) and multimeric analysis of VWF in low and medium SDS-agarose resolution gels, is warranted to diagnose and classify all variants of von Willebrand disease (VWD). VWD type 2M and 2U are typically characterized by decreased RIPA and a poor response of VWF:RCO to desmopressin (DDAVP), but normal VWF:CB and good responses of VWF:CB, VWF:Ag and FVIII:C to DDAVP. VWF multimeric analysis in patients with VWD 2M and 2U show relative decreases in large VWF multimers with less resolved triplet structure of each of the multimeric bands in low-, medium- or high-resolution gels. VWD type 2M or 2U are caused by a loss-of-function mutation in the A1 domain. The laboratory manifestations and molecular defects in the A1 domain causing VWD type 2M and 2U are clearly distinct from all variants of type 1 VWD and also from all other variants (VWD type 2A, 2B, 2E [IIE] and 2C [IIC]).
tified by a concordant VWF:RCo/VWF:Ag ratio ≥0.7, whereas this ratio is characteristically discrepant (<0.7) in type 2 VWD except for type 2N [1, 2]. Type 2A VWD refers to the loss of function (VWF:RCo) attributed to the absence of high-molecular-weight (HMW) multimers. All variants of type 2A VWD show a qualitative defect in VWF with absence of HMW multimers in low-resolution gels, normal or decreased RIPA, combined with decreased VWF:RCo/VWF:Ag and VWF:CB/VWF:Ag ratios. Type 2B refers to qualitatively defective VWF with increased RIPA and decreased VWF:RCo/VWF:Ag and VWF:CB/VWF:Ag ratios reflecting the absence of large VWF multimers. VWD type 2M is due to loss of function (VWF:RCo) not caused by the loss of HMW multimers in low-resolution agarose gels. VWF:CB is decreased in VWD type 2A and 2B but normal compared to VWF:Ag in VWD 2M and 2U.

A presumptive diagnosis of type 2 VWD is investigated by assessing the VWF:RCo/VWF:Ag and VWF:CB/VWF:Ag ratios, plasma VWF multimers and RIPA [6, 7]. The VWF:CB assay detects qualitative defects associated with a loss of HMW VWF multimers in VWD 2A, 2B and 2C. Although the VWF:RCo and RIPA assay is severely altered in type 2M and 2U, the HMW multimers are relatively decreased but present, thus a normal VWF:CB reflects the presence of these multimers. It is therefore recommended that the VWF:CB assay should be used in association with rather than as a replacement for the VWF:RCo assay in the differential diagnosis of VWD type 2M from VWD type 2A and 2B. In this critical appraisal, evidence accumulates that VWD 2M and 2U reflect a distinct entity of VWD with a selective VWF:RCo defect in plasma and platelets.

**Laboratory Features of Autosomal Dominant VWD 2M and 2U**

Severe type 1 ‘platelet-discrepant’ VWD, formerly labeled as 1b by Hoyer et al. [8] and described in great detail by Mannucci et al. [9] in 1985 (fig. 1), has been reclassified as VWD 2M in 2000 [1] and as VWD 2U by Sadler et al. [2] in 2006. As demonstrated by Mannucci et al. [9] in 1985 and by Castaman et al. [11] in 1993, the response to desmopressin (DDAVP) in type 1 platelet discrepant VWD was very poor for VWF:RCo but good for FVIII:C and VWF:Ag (fig. 2), consistent with the diagnosis VWD type 2M or 2U according to the SSC-ISTH classification [1, 2].

Due to the restricted use of two VWF assays (VWF:RCo and VWF:Ag), type 2M VWD is frequently misdiagnosed as severe VWD of type 1, because it can be difficult to detect discrepancies between antigenic and functional VWF, particularly when low protein levels are present in plasma or when the insensitive VWF:RCo is not correctly evaluated [12]. Using a complete set of laboratory tools for the detailed characterization of a VWF defect (table 1), three prospective studies clearly showed that the VWF:RCo/VWF:Ag ratios were discordant in VWD 2A and 2M, whereas the VWF:CB/VWF:Ag ratios...
were discordant in VWD 2A but normal in VWD 2M patients (fig. 2–4) [12–14]. All three studies uniformly demonstrated that the VWF:CB assay (preferentially collagen type I- and not collagen type III-based assays) should serve as a supplement rather than a replacement for VWF:RCo in the differential diagnosis between VWD type 2M and 2A and, even more importantly, between VWD 2M, type Vicenza and variants of VWD type 1 with a smeary pattern of VWF multimers.

The typical laboratory features of VWD type 2M recently defined by several investigators [7, 12–17] include (1) a laboratory phenotype of severe type 1 or typical type 2 VWD in plasma and platelets, (2) decreased RIPA in the presence of a normal or near normal VWF multimeric pattern in a low-resolution agarose gel, (3) a poor response to DDAVP of VWF:RCo (fig. 2–4), (4) good responses to DDAVP of both VWF:CB and VWF:Ag and FVIII:C (fig. 2–4), (5) relative decreases in the large VWF multimers with less resolved triplet structure of each of the multimeric bands in medium- or high-resolution gels. The appearance of most of the large VWF multimers in heterozygous autosomal dominant VWD type 2M can readily explain the transient correction of prolonged PFA-100 closure times and Ivy bleeding times approaching normal values after DDAVP (fig. 2). VWD type 2M and 2U share common laboratory features due to the selective loss of RCo function mutation in the A1 domain (tables 1, 2).

**VWD 2M and 2U Are Caused by Mutations in the A1 Domain**

The R1374C, R1374H and R1315C mutations add much confusion to the nomenclature because insensitive tests are used for the classification of VWD patients (table 2). Since 1995, >100 patients from more than a dozen unrelated families with a genetic defect in R1374H or R1374C have been described (table 2) and confirmed by others [14–23]. Lethagen et al. [20] found the R1374C mutation in 72 patients from 4 families diagnosed as severe type 1 VWD (mean values for VWF:Ag 0.21, vWF:RCo

---

**Table 1. Laboratory features of autosomal dominant VWD type 2M or 2U [7, 12–17]**

<p>| | |</p>
<table>
<thead>
<tr>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Low/very low levels of VWF:RCo in plasma and platelets (defective RIPA)</td>
</tr>
<tr>
<td>2</td>
<td>Decreased VWF:RCo/VWF:Ag ratio (&lt;0.6), particularly after DDAVP</td>
</tr>
<tr>
<td>3</td>
<td>Normal VWF:CB/VWF:Ag ratio (&gt;0.7–1.2), particularly after DDAVP</td>
</tr>
<tr>
<td>4</td>
<td>Prolonged PFA-100 (Coll/ADP and Coll/EPI) closure times (&gt;300 s)</td>
</tr>
<tr>
<td>5</td>
<td>Near-normal to prolonged bleeding times (variable)</td>
</tr>
<tr>
<td>6</td>
<td>Response to DDAVP: poor for VWF:RCo but good for VWF:Ag, VWF:CB and FVIII:C</td>
</tr>
<tr>
<td>7</td>
<td>Relative decrease in large VWF multimers, relative lack of triplet structure in a medium-resolution gel with or without a smeary pattern or ultralarge multimers</td>
</tr>
<tr>
<td>8</td>
<td>Loss of ristocetin cofactor functions (VWF:RCo and RIPA) due to mutations in the A1 domain</td>
</tr>
</tbody>
</table>

---

Dominant VWD 2M and 2U and Mutations in the A1 Domain
The values for VWF:Ag in 63 affected family members ranged from 0.12 to 0.72 and the values for VWF:RCo in 33 affected family members from 0.01 to 0.88. Castaman et al. [18] classified the R1374H mutation in 2 families as type 1 platelet discordant VWD (re-labeled as 2M), a subtype characterized by disproportionately low VWF:RCo in plasma and platelets compared to VWF:Ag levels (table 2). On the other hand, Hilbert et al. [19] and Nishikubo et al. [21] classified the R1374H and R1374C mutations as a variant of type 2 VWD char-
Characterized by low VWF:RCo levels, a discrepant VWF:RCo/VWF:Ag ratio, decreased or absent RIPA and a moderate decrease in large VWF multimers together with a significant increase in the lower VWF multimers due to slightly increased proteolysis of the VWF (Table 2).

DDAVP induced increases in FVIII:C, VWF:Ag and VWF:CB but did not normalize the glycoprotein (GP) Ib-dependent VWF functions expressed as RIPA and VWF:RCo (Fig. 2–4). It has been observed that the results for VWF:CB are dependent on the type of collagen used [24, 25]. In the study by Penas et al. [14], the VWF:CB/VWF:Ag ratio was indeed found to be higher (0.7) in 23 patients with VWD 2M for type I collagen, while the results for type III collagen were variable.

The pattern of proteolytic fragments of plasma VWF (the triplet structure of each band seen in high-resolution gels) is reduced in patients with the R1374H or R1374C mutation (Fig. 3), whereas it is increased in VWD 2A (IIA) [14, 16]. The relative loss of large multimers with a normal banding pattern of each multimer clearly differentiates the VWD 2M patients with the R1374H or R1374C mutation from VWD subtypes 2A and 2B with respect to the absence of large VWF multimers and pronounced triplet structure of bands from VWD subtypes 2C (IIC) and 2E (IIE), where satellite bands are absent or reduced in intensity, and from subtype 2D, where satellite bands migrate to abnormal positions [4–7]. The R1374H and R1374C mutations are difficult to classify as either 2M (in view of their abnormal VWF multimers in medium-resolution gel) or any other type 2 and therefore are best categorized as VWD type 2U (unclassifiable) [7, 19].

The laboratory phenotype of VWD 2U is characterized by decreased values for FVIII:C and VWF:Ag, very low

### Table 2. VWD ‘severe type 1’, 2M or 2A variant caused by loss-of-function mutations in the A1 domain of the VWF gene

<table>
<thead>
<tr>
<th>Patients</th>
<th>Ref. Families</th>
<th>FVIII %</th>
<th>VWF:Ag %</th>
<th>VWF:RCo %</th>
<th>VWF:RCo/Ag ratio</th>
<th>VWF:CB Ag ratio</th>
<th>RIPA Mutations VWD type MM pattern</th>
</tr>
</thead>
<tbody>
<tr>
<td>18</td>
<td>2 3</td>
<td>43–56</td>
<td>25–36</td>
<td>&lt;3–10</td>
<td>0.12–0.28</td>
<td>–</td>
<td>R1374C 1 discordant</td>
</tr>
<tr>
<td>18</td>
<td>1 5</td>
<td>13–40</td>
<td>10/40</td>
<td>&lt;10</td>
<td>0.1–0.56</td>
<td>–</td>
<td>R1374C 1, 2 variant</td>
</tr>
<tr>
<td>20</td>
<td>1 72</td>
<td>–</td>
<td>13/21</td>
<td>2/88</td>
<td>↘/N</td>
<td>–</td>
<td>R1374C severe 1</td>
</tr>
<tr>
<td>21</td>
<td>1 5</td>
<td>38–49</td>
<td>15/28</td>
<td>&lt;5</td>
<td>0.15–0.33</td>
<td>↓</td>
<td>R1374C 2 variant</td>
</tr>
<tr>
<td>22</td>
<td>1 1</td>
<td>33</td>
<td>16</td>
<td>–</td>
<td>–</td>
<td>N</td>
<td>↓ R1374C</td>
</tr>
<tr>
<td>16</td>
<td>1 2</td>
<td>30–40</td>
<td>40–40</td>
<td>13–15</td>
<td>0.33–0.38 N</td>
<td>↓</td>
<td>R1374C 2M, abN MM</td>
</tr>
<tr>
<td>14</td>
<td>2 23</td>
<td>17–51</td>
<td>16–38</td>
<td>&lt;3–19</td>
<td>0.06–0.65 N</td>
<td>↓</td>
<td>R1374C 2M, abN MM</td>
</tr>
<tr>
<td>19</td>
<td>5 14</td>
<td>12–44</td>
<td>12–23</td>
<td>&lt;10</td>
<td>&lt;0.19–0.59</td>
<td>–</td>
<td>R1374H 1, 2 variant</td>
</tr>
<tr>
<td>21</td>
<td>2 5</td>
<td>22–44</td>
<td>11–25</td>
<td>&lt;5–10</td>
<td>&lt;0.22</td>
<td>↓</td>
<td>R1374H 2M</td>
</tr>
<tr>
<td>20</td>
<td>1 11</td>
<td>4–42</td>
<td>&lt;10–18</td>
<td>↓</td>
<td>↘/N</td>
<td>–</td>
<td>R1374C severe 1</td>
</tr>
<tr>
<td>15</td>
<td>1 2</td>
<td>43–29</td>
<td>25–12</td>
<td>6–&lt;5</td>
<td>0.4</td>
<td>–</td>
<td>↓ R1315C</td>
</tr>
<tr>
<td>21</td>
<td>1 2</td>
<td>48–54</td>
<td>14–17</td>
<td>&lt;5</td>
<td>0.30</td>
<td>N</td>
<td>↓ R1315C</td>
</tr>
<tr>
<td>23</td>
<td>6 10</td>
<td>24–59</td>
<td>17–30</td>
<td>6–18</td>
<td>0.20–0.50</td>
<td>–</td>
<td>R1315C 2 variant</td>
</tr>
<tr>
<td>27</td>
<td>1 1</td>
<td>N</td>
<td>N</td>
<td>0</td>
<td>&lt;0.10</td>
<td>–</td>
<td>G1324S/null 2M</td>
</tr>
<tr>
<td>28</td>
<td>1 5</td>
<td>–</td>
<td>14–36</td>
<td>3–12</td>
<td>0.11–0.30</td>
<td>–</td>
<td>Q1191del11 2M</td>
</tr>
<tr>
<td>29</td>
<td>1 3</td>
<td>32–49</td>
<td>13–36</td>
<td>&lt;3–9</td>
<td>0.31–0.47</td>
<td>–</td>
<td>F1369I 2M</td>
</tr>
<tr>
<td>29</td>
<td>1 3</td>
<td>43–73</td>
<td>36–62</td>
<td>11–22</td>
<td>0.33–0.33</td>
<td>–</td>
<td>I1425F 2M</td>
</tr>
<tr>
<td>30</td>
<td>2 5</td>
<td>36–96</td>
<td>20–62</td>
<td>8–28</td>
<td>0.40–0.52</td>
<td>↓</td>
<td>K1408delK 2M</td>
</tr>
<tr>
<td>21</td>
<td>1 2</td>
<td>30–40</td>
<td>14–19</td>
<td>&lt;5</td>
<td>0.30–0.35</td>
<td>↓</td>
<td>Y1321D 2M</td>
</tr>
<tr>
<td>21</td>
<td>1 3</td>
<td>24–50</td>
<td>33–40</td>
<td>&lt;5–6</td>
<td>0.13–0.17</td>
<td>↓</td>
<td>I1326T 2A 2M</td>
</tr>
<tr>
<td>21</td>
<td>1 1</td>
<td>68</td>
<td>65</td>
<td>14</td>
<td>0.21</td>
<td>–</td>
<td>R1399C 2M</td>
</tr>
<tr>
<td>31</td>
<td>1 4</td>
<td>56–73</td>
<td>33–42</td>
<td>14–18</td>
<td>0.43–0.52</td>
<td>–</td>
<td>A1437F 2M</td>
</tr>
<tr>
<td>32</td>
<td>1 3</td>
<td>22–38</td>
<td>11–19</td>
<td>5–9</td>
<td>0.47–0.57</td>
<td>N</td>
<td>↓ L1446P 2M</td>
</tr>
<tr>
<td>33</td>
<td>1 1</td>
<td>33</td>
<td>19</td>
<td>3</td>
<td>0.15</td>
<td>–</td>
<td>↓ L1382P 2M</td>
</tr>
<tr>
<td>17</td>
<td>1 1</td>
<td>86</td>
<td>90</td>
<td>14</td>
<td>0.16</td>
<td>N</td>
<td>↓ L1361S 2M</td>
</tr>
<tr>
<td>33</td>
<td>1 1</td>
<td>22</td>
<td>18</td>
<td>3</td>
<td>0.17</td>
<td>N</td>
<td>– D127–E78delNsl 2M</td>
</tr>
<tr>
<td>33</td>
<td>1 1</td>
<td>43</td>
<td>20</td>
<td>3</td>
<td>0.15</td>
<td>N</td>
<td>– R1342C 2C</td>
</tr>
<tr>
<td>33</td>
<td>1 1</td>
<td>43</td>
<td>19</td>
<td>3</td>
<td>0.16</td>
<td>N</td>
<td>– I1416N 2M</td>
</tr>
</tbody>
</table>

N = Normal; abN MM = abnormal MM; ↓ = decrease, – = no data.

---

Dominant VWD 2M and 2U and Mutations in the A1 Domain


149
or undetectable levels of VWF:RCo, and uniformly decreased or absent RIPA in all cases (table 2). The response to DDAVP in case of the R1374H or R1374C mutation appears to be poor for VWF:RCo and RIPA and good for VWF:Ag and FVIII:C (fig. 4) [7, 14–17], which is associated with transient correction of slightly increased bleeding times to normal values [12, 25].

Since 1998, data on 25 patients from 9 unrelated families presenting the R1315C genetic defect have been published (table 2) [11, 15, 20, 22]. The laboratory phenotype of the R1315C mutation is characterized by subnormal values for FVIII:C (mean 41 U/dl), low levels for VWF:Ag (mean 20 U/dl), very low or undetectable levels for VWF:RCo (<0.10 U/dl) and a decreased VWF:RCo/Ag ratio. RIPA was uniformly decreased or absent in all cases with the R1315C mutation (table 2). The R1315C mutation has been labeled as type 2M in 4 cases [12, 28–30]. In two studies on VWD 2M patients, a quantitative loss of the highest VWF multimers [22, 28–30] has been reported in 19 VWD 2M patients [22, 28–30]. In two studies on VWD 2M patients, a quantitative loss of the highest VWF multimers [29, 30] due to R1374C, R1374H or R1315C mutations was noted.

VWD 2M or 2U due to Loss- and 2B due to Gain-of-Function Mutations in the A1 Domain

The A1 domain (amino acid, aa 1260–1479) is structurally delineated by a disulfide bridge between Cys1272 and Cys1458. X-ray diffraction studies of the A1 crystal revealed a globular shape comprising a central core comprised of 6 hydrophobic α-strands, surrounded by 6 amphipathic α-helices [31]. Analysis of naturally occurring loss-of-function mutations (VWD 2M) combined with mutagenesis and GPIbα peptide docking studies have identified a central front groove on the A1 domain next to strand α3 as part of the binding site for GPIbα. VWD type 2M mutations, characterized by loss of function for VWF but maintaining a normal multimerization pattern, primarily cluster around the VWF interaction site with its platelet receptor GPIbα. Two main clusters of VWD 2M with loss-of-function mutations in the A1 domain are located in aa 1272–1302 and 1359–1437 (table 3). Three VWD 2M loss-of-function mutations in aa 1315, 1324 and 1462–1467 can be identified as isolated among VWD 2B gain-of-function mutations (table 3). The crystal structure of a gain-of-function A1 domain mutant R1306Q combined with the amino-terminal domain of GPIbα (also containing a gain-of-function mutation) confirmed that the frontal part of A1 constitutes the contact area for GPIbα [31]. Two distinct areas of tight interaction were revealed: the first and most extensive contact site being located near the top of A1 and the second involving residues near the bottom of A1. A main cluster of VWD 2B gain-of-function mutations (increased binding of VWF-A1 and platelet-GPIbα) is located between aa 1304 and 1341 with two exceptions (aa 1315 and 1324; table 3) [32]. Two minor clusters of VWD 2B mutations are located at aa 1268–1272 and 1460–1462 (table 3) [32]. Several mutations in exon 28 of the A1 domain in patients with VWD type 2M have been described recently, e.g. L1382P, D1277-E78delinsG, R1342C, G1415D and I1416N [17, 33–36]. The mutations S1285P and L1307P in the study of Goodeve et al. [35] and P1413L in the study of James et al. [36] very likely belong to the VWD 2M category. VWD cases compound heterozygous for R854Q-R1351C in the study of James et al. [17] and compound heterozygous for R924Q-R1315C, P2145S-R1315C, P1266L-R1315C and I1481fs-Y1584C in the European VWD type 1 study of Goodeve et al. [35] all show predominant features of VWD type 2M.

The 2006 ISTH registry of VWD has included the R1347C, R1374H, R1374S, R1379C, K1405del and P1462A mutations as unclassified (U) VWD group. L1382 was labeled as 2U or 2A, and F1369I and R1315C as 2M or 2U (table 3) [32]. The mutations L1282R, S1285F, L1296P, D1302G, G1324S, G1324A, R1392-Q1402del, E1352K, K1362T, P1475S and P2781S were labeled as VWD 2M (table 3) [32]. We have to consider that the C1101R, C1101W, Y1107C, W1120S, C1130F, C1149R, T1136M, C1159F, E1171Q, R1205L and C1196R muta-
Table 3: VWF missense mutations in the A1 domain are related to VWD 2M, 2U and 2B [32]

<table>
<thead>
<tr>
<th>VWD type</th>
<th>Exon</th>
<th>Mutation</th>
<th>VWD type</th>
<th>Exon</th>
<th>Mutation</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>28</td>
<td>3686T→G</td>
<td>V1229G</td>
<td>2B</td>
<td>28</td>
</tr>
<tr>
<td>1</td>
<td>28</td>
<td>3692A→C</td>
<td>N1231T</td>
<td>2M</td>
<td>28</td>
</tr>
<tr>
<td>2U</td>
<td>28</td>
<td>3702T→G</td>
<td>C1234W</td>
<td>2M</td>
<td>28</td>
</tr>
<tr>
<td>2B</td>
<td>28</td>
<td>3797C→A</td>
<td>P1266Q</td>
<td>2B</td>
<td>28</td>
</tr>
<tr>
<td>1/2B</td>
<td>28</td>
<td>3797C→T</td>
<td>P1266L</td>
<td>2B</td>
<td>28</td>
</tr>
<tr>
<td>2B</td>
<td>28</td>
<td>3802C→G</td>
<td>H1268D</td>
<td>2B</td>
<td>28</td>
</tr>
<tr>
<td>2B</td>
<td>28</td>
<td>3802C→A</td>
<td>H1268N</td>
<td>2B</td>
<td>28</td>
</tr>
<tr>
<td>2B</td>
<td>28</td>
<td>3814T→G</td>
<td>C1272G</td>
<td>2B</td>
<td>28</td>
</tr>
<tr>
<td>2B</td>
<td>28</td>
<td>3814T→C</td>
<td>C1272R</td>
<td>2M</td>
<td>28</td>
</tr>
<tr>
<td>2A</td>
<td>28</td>
<td>3815G→C</td>
<td>C1272S</td>
<td>2M</td>
<td>28</td>
</tr>
<tr>
<td>2A</td>
<td>28</td>
<td>3827T→C</td>
<td>L1276P</td>
<td>2M</td>
<td>28</td>
</tr>
<tr>
<td>2B?</td>
<td>28</td>
<td>3835G→A</td>
<td>V1279I</td>
<td>2U</td>
<td>28</td>
</tr>
<tr>
<td>2M</td>
<td>28</td>
<td>3835G→A</td>
<td>V1279I</td>
<td>2U</td>
<td>28</td>
</tr>
<tr>
<td>2M</td>
<td>28</td>
<td>3835G→A</td>
<td>V1279I</td>
<td>2U</td>
<td>28</td>
</tr>
<tr>
<td>2M</td>
<td>28</td>
<td>3845T→G</td>
<td>L1282R</td>
<td>2U</td>
<td>28</td>
</tr>
<tr>
<td>2M</td>
<td>28</td>
<td>3854C→T</td>
<td>S1285F</td>
<td>2U</td>
<td>28</td>
</tr>
<tr>
<td>2M</td>
<td>28</td>
<td>3887T→C</td>
<td>L1296P</td>
<td>2U</td>
<td>28</td>
</tr>
<tr>
<td>2M</td>
<td>28</td>
<td>3905A→G</td>
<td>D1302G</td>
<td>2M</td>
<td>28</td>
</tr>
<tr>
<td>2B</td>
<td>28</td>
<td>3912Ins</td>
<td>1304Ins</td>
<td>2A</td>
<td>28</td>
</tr>
<tr>
<td>2B</td>
<td>28</td>
<td>3916C→T</td>
<td>R1306W</td>
<td>2M</td>
<td>28</td>
</tr>
<tr>
<td>2B</td>
<td>28</td>
<td>3917G→A</td>
<td>R1306Q</td>
<td>2U</td>
<td>28</td>
</tr>
<tr>
<td>2B</td>
<td>28</td>
<td>3917T→G</td>
<td>R1306L</td>
<td>2U</td>
<td>28</td>
</tr>
<tr>
<td>2B</td>
<td>28</td>
<td>3922C→T</td>
<td>R1308C</td>
<td>2U</td>
<td>28</td>
</tr>
<tr>
<td>2A?</td>
<td>28</td>
<td>3923G→A</td>
<td>R1308H</td>
<td>2M</td>
<td>28</td>
</tr>
<tr>
<td>2B</td>
<td>28</td>
<td>3925A→G</td>
<td>I1309V</td>
<td>2M</td>
<td>28</td>
</tr>
<tr>
<td>2B</td>
<td>28</td>
<td>3929C→T</td>
<td>S1310F</td>
<td>1</td>
<td>28</td>
</tr>
<tr>
<td>2B</td>
<td>28</td>
<td>3939G→C</td>
<td>W1313C</td>
<td>2A</td>
<td>28</td>
</tr>
<tr>
<td>2B</td>
<td>28</td>
<td>3940G→T</td>
<td>V1314F</td>
<td>2B</td>
<td>28</td>
</tr>
<tr>
<td>2B</td>
<td>28</td>
<td>3940G→C</td>
<td>V1314L</td>
<td>2B</td>
<td>28</td>
</tr>
<tr>
<td>2B</td>
<td>28</td>
<td>3941T→A</td>
<td>V1314D</td>
<td>2U</td>
<td>28</td>
</tr>
<tr>
<td>2U</td>
<td>28</td>
<td>3943C→T</td>
<td>R1315C</td>
<td>2M</td>
<td>28</td>
</tr>
<tr>
<td>2M</td>
<td>28</td>
<td>3943C→T</td>
<td>R1315C</td>
<td>2U</td>
<td>28</td>
</tr>
</tbody>
</table>

Discussion

Patients with VWD type 2M and 2U are characterized by near normal values for FVIII:C, subnormal levels for VWF:Ag and consistently very low levels for VWF:RCo with decreased VWF:RCo/Ag ratios [1, 2]. RIPA is uniformly decreased [6, 7], and most patients show normal VWF:CB/VWF:Ag ratios and a relative loss of HMW multimers in low-resolution gels (agarose 1%) [1, 2, 6, 7]. With the advent of molecular and more sophisticated laboratory tools, e.g. medium-/high-resolution VWF multimeric analysis, and the VWF:RCo and VWF:CB assays, the updated 2008 classification of VWD patients proposed during the European VWF and VWD Workshop 2007 not only allows to subdivide VWD 2A into IIA, IIC, IID and IIE [2–5], but also to clearly differentiate between VWD type 2M and 2U versus 2A subtypes and between VWD 2M and 2U versus the various but not yet clearly defined variants of type 1 [6, 7]. All type 2 VWD (2A, 2B and 2C) show qualitatively defective VWF with absence of HMW multimers in low-, medium- and high-resolution gels, normal or decreased RIPA, together with decreased VWF:RCo/Ag and decreased VWF:CB/Ag ratios.

Dominant VWD 2M and 2U and Mutations in the A1 Domain


151
Type 2B refers to qualitatively defective VWF with increased RIPA and decreased VWF:RCo/Ag and VWF:CB/Ag ratios reflecting the absence of large VWF multimers. The present critical analysis of the literature demonstrates that VWD type 2M and 2U belong to the same group of qualitatively defective VWF with selective loss of VWF functions VWF:RCo and RIPA, but with normal VWF:CB and the relative loss of large VWF multimers and less pronounced triplet structure in low- and medium-resolution SDS gels. Although VWF-platelet interaction measured by the VWF:RCo and RIPA assays is severely altered in VWD type 2M and 2U, the majority of the HMW multimers are present. It is therefore recommended that the VWF:CB assay should be used as a supplement rather than as a replacement for the VWF:RCo assay in the differential diagnosis of VWD type 2M and 2U from VWD type 2A (IIA), 2B (IIIB), 2C (IIC) and 2E (IIE) [12, 14]. With regard to the VWF:CB assay, it is important to realize that only the values for the type I collagen VWF:CB results are to be taken into account (and VWF:RCo) to confirm a diagnosis of type I VWD [6, 7, 12, 13]. With regard to type III collagen, the variability in the results does not allow to draw conclusions about the utility of this assay in the detection of type 2 variants of VWD and the differential diagnosis between VWD type 2M or 2U versus type 2A and 2B [23, 24]. VWD type 2M and 2U are caused by mutations in the A1 domain, which are typically associated with decreased or absent RIPA, a low VWF:RCo/VWF:Ag (<0.60) combined with a normal VWF:CB/VWF:Ag ratio before and after DDAVP. The multimers in VWD type 2M and 2U typically show a relative decrease in the large VWF multimers in a medium- or high-resolution gel (SDS-agarose gel >1.6%) as well as subtle abnormalities in less pronounced triplet structure or even may show increased HMW multimers after DDAVP. Recent expression studies demonstrated that in comparison with wild-type recombinant VWF, type 2M mutants show loss of VWF binding to GPIb and exhibit a similar sensitivity to recombinant ADAMTS13-mediated proteolysis, in agreement with the near normal multimeric pattern in vivo. In table 3, we propose more stringent criteria for the phenotypic definition of VWD type 2M and 2U clearly showing a specific VWF RCo defect before and after DDAVP (fig. 3, 4). The VWD 2M and 2U appear to be clearly distinct from all variants of VWD type I and all other variants of type 2. The new definition and delineation of VWD type 2M and 2U as a specific VWF:RCo defect (table 1) should be the subject of an international standardization initiative during future SSC meetings of the ISTH.

References


32 University of Sheffield: ISTH SSC VWF Database. http://www.vwf.group.shef.ac.uk/index.html


Dominant von Willebrand Disease Type 2A Groups I and II due to Missense Mutations in the A2 Domain of the von Willebrand Factor Gene: Diagnosis and Management

Jan Jacques Michiels a, c, Huub H.D.M. van Vliet b

 a Hemostasis and Thrombosis Research Center, Antwerp University Hospital, Edegem, Belgium; b Hemostasis and Thrombosis Research, Erasmus University Medical Center Rotterdam, and c Hemostasis and Thrombosis Science Center, Goodheart Institute, Rotterdam, The Netherlands

Abstract
Pertinent findings in patients with von Willebrand disease (VWD) type 2A include prolonged bleeding time (BT), consistently low von Willebrand factor (VWF):ristocetin cofactor activity (RCo)/antigen concentration (Ag) and VWF:collagen binding (CB)/Ag ratios, absence of high, and (depending on severity) intermediate and large VWF multimers, the presence of pronounced triplet structure of individual bands and increased VWF degradation products due to increased proteolysis caused by mutations in the A2 domain of VWF. Two categories of VWD type 2A can be distinguished: group I with severe and group II with mild VWD. A minority of VWD type 2A patients have pronounced BT, normal factor VIII coagulant activity and VWF:Ag, low VWF:RCo and VWF:CB, a normal ristocetin-induced platelet aggregation and complete but transient correction of BT and functional VWF parameters to normal levels for only a few hours due to short half-lives for VWF:RCo and CWF:CB. Such transient complete responses to desmopressin (DDAVP) lasting only a few hours may facilitate treatment and prophylaxis of minor bleedings, but may not be able to prevent bleeding during minor and major surgery. Most VWD type 2A patients have pronounced VWD with very low VWF:RCo, prolonged BT, PFA-100 closure times $>$250 s, and response to DDAVP is only transient, minor, poor or absent, with no correction of the BT despite some increase in VWF:RCo, thus being candidates for factor VIII-VWF concentrate substitution for the acute and prophylactic treatment of bleeding symptoms.

Introduction
In 1980, Ruggeri et al. [1] described heightened interaction between platelets and functionally abnormal factor VIII (FVIII)/von Willebrand factor (VWF) protein in a new subtype of type II von Willebrand disease (VWD) and labeled it type IIB, and VWD type II in which the interaction of platelets and abnormal FVIII/VWF on crossed immunoelectrophoresis is decreased or absent in the presence of ristocetin were classified as type II A. They demonstrated that the mean ristocetin con-
centration for a normal ristocetin-induced platelet agglutination (RIPA) curve in platelet-rich plasma was 0.60 mg/ml in patients with VWD type IIB (2B), whereas RIPA was absent at a ristocetin concentration of >2 mg/ml in patients with severe type IIA (2A) [1]. Using high-resolution SDS-agarose multimeric analysis of VWF in plasma in combination with immunoblots of VWF proteolytic degradation products, Zimmerman et al. [2] nicely demonstrated that proteolysis of VWF is a normal event in normal individuals, increased in VWD type IIA (2A) and IIB (2B) with increased triplet structure of each visible band as the result of proteolysis, and that proteolysis of VWF is minimal in type IIC (2C), IID (2D) and IIE (2E) variants with aberrant multimeric structure of individual oligomers [2]. VWD type IIA, IIB, IIC, IID and IIE according to Ruggeri et al. [1] and Zimmerman et al. [2] are reclassified by Schneppenheim et al. [3] as 2A, 2B, 2C, 2D and 2E. In this article, we report on the clinical manifestations, laboratory phenotype and molecular defects of severe VWD 2A group I and mild VWD 2A group II.

**VWD Type 2A Group I and Type 2A Group II**


The absence of high-molecular-weight VWF multimers and increased triplet structure is the consequence of increased proteolysis of large VWF multimers (fig. 1, 2). Structural changes within the A2 domain can produce two different characteristic phenotypes of VWD type 2A [11, 12]. Expression studies in Coss cells demonstrated that the single missense mutations V1607D, S1506L, L1540P and G1505R resulted in no secretion of high-molecular-weight multimers due to impaired transport of VWF multimers between the endoplasmatic reticulum and the Golgi complex (so-called VWD 2A group I defect) with very likely intracellular proteolysis of large VWF multimers [11–13]. Expression studies demonstrated that at least five missense mutations in the A2 domain (R1597W, G1505E, E1638K, I1628T and L1503Q) result in normal secretion of high-molecular-weight multimers.
similar to wild-type multimers indicating that subsequent loss of large VWF multimers is caused by hypersensitivity to ADMATS13-induced increased proteolysis in plasma (so-called VWD 2A group II defect) [11, 12, 14]. Interestingly, platelet lysates demonstrated a decrease in large VWF multimers for G1505L and S1506L mutants of VWD 2A group I, but a normal pattern for the G1505E and R1597W mutants of VWD 2A group II. Federici et al. [15] showed that in patients with VWD type 2A group I due to the mutations S1506L and V1665E, desmopressin (DDAVP) induced poor responses of functional VWF parameters with no reappearance of the large multimers and persistence of a strongly prolonged BT, whereas patients with VWD 2A group II due to the mutations R1597W and G1629R responded better to DDAVP, with transient increases in large multimers associated with transient (1–2 h) correction of BT and VWF:RCo to low normal values. VWD type 2A subgroups I and II show a typical proteolytic pattern with lack of large VWF multimers and the presence of pronounced triplet structure indicating that proteolysis of large multimers occurs already intracellularly in group I of VWD type 2A and does occur in plasma after secretion by endothelial cells in group II of VWD type 2A (fig. 1, 2).

**DDAVP Response Curves in VWD 2A**

Batlle et al. [16, 17] and Michiels et al. [4, 5] observed heterogeneity of mild/moderate to moderate/severe type 2A VWD with regard to bleeding symptoms, laboratory phenotypes and typical responses to DDAVP (table 1). Patients with pronounced VWD 2A group I have low values of VWF:Ag, very low or undetectable levels of VWF:RCo and VWF:CB and no RIPA at high concentration of ristocetin 1.75 or 2.0 mg/ml, and present minor or poor responses of functional VWF:RCo and VWF:CB with no correction of VWF multimers and BT after DDAVP (fig. 1).
Patients with mild VWD 2A group II are characterized by normal or subnormal values of FVIII:C and VWF:Ag, VWF:RCo values >0.20, normal RIPA at ristocetin concentrations of 1.2 or 1.75 mg/ml, complete but transient correction of BT, FVIII:C and VWF parameters, and large multimers for a few hours after DDAVP (fig. 2). 

We investigated in the 1990s three affected members, cases II-1 (uncle in table 1), III-1 (proband in table 1) and III-7 (nephew in table 1) [4], who belong to a large Dutch family recently diagnosed as VWD type 2A due to a novel mutation V1499E in the A2 domain [18]. As RIPA was normal but not increased (table 1), laboratory findings are consistent with mild VWD type 2A group II. Case III-7 [18] (proband in table 1 [4]), born in 1961, presented with a history of bruises, gum bleeding, bleeding after tooth extraction for a few to several hours since early childhood and moderate menorrhagia since her first 

---

**Table 1.** Results of VWF:Ag and propeptide (pp) in 10 affected members of a large Dutch family with dominant VWD type 2A group II caused by the V1499E mutation in the A2 domain [19].

<table>
<thead>
<tr>
<th>Patient</th>
<th>VWD 2A</th>
<th>VWF:Ag</th>
<th>VWFpp</th>
<th>VWFpp/VWF ratio</th>
</tr>
</thead>
<tbody>
<tr>
<td>II-1</td>
<td>59</td>
<td>76</td>
<td>1.3</td>
<td></td>
</tr>
<tr>
<td>III-1</td>
<td>–</td>
<td>45</td>
<td>–</td>
<td></td>
</tr>
<tr>
<td>III-4</td>
<td>33</td>
<td>40</td>
<td>1.3</td>
<td></td>
</tr>
<tr>
<td>III-6</td>
<td>31</td>
<td>51</td>
<td>1.6</td>
<td></td>
</tr>
<tr>
<td>III-7</td>
<td>22</td>
<td>44</td>
<td>2.0</td>
<td></td>
</tr>
<tr>
<td>III-8</td>
<td>28</td>
<td>54</td>
<td>1.9</td>
<td></td>
</tr>
<tr>
<td>III-9</td>
<td>24</td>
<td>38</td>
<td>1.6</td>
<td></td>
</tr>
<tr>
<td>IV-8</td>
<td>29</td>
<td>43</td>
<td>1.5</td>
<td></td>
</tr>
<tr>
<td>IV-11</td>
<td>27</td>
<td>56</td>
<td>2.1</td>
<td></td>
</tr>
<tr>
<td>IV-12</td>
<td>20</td>
<td>35</td>
<td>1.6</td>
<td></td>
</tr>
<tr>
<td>Mean (range)</td>
<td>30 (20–59)</td>
<td>48 (35–76)</td>
<td>1.8 (1.3–2.1)</td>
<td></td>
</tr>
<tr>
<td>Normal</td>
<td>50–150</td>
<td>60–140</td>
<td>1.0</td>
<td></td>
</tr>
</tbody>
</table>

---

Diagnosis and Management of VWD 2A

**Fig. 3.** Two related cases, II-1 (left curve) and III-1 (right curve) [16, 18], of a large family with autosomal dominant VWD type 2A group II caused by a novel mutation (V1499E) in the A2 domain [18]. DDAVP induced normal FVIII:C and VWF parameters followed by normal half-lives, a transient correction of VWF:RCo and VWF:CB followed by shortened half-lives after DDAVP [4]. Medium-resolution gel in the laboratory of Budde (2005) showed the absence of large VWF multimers and increased triplet structure of each of the band consistent with VWD type 2A group II [12–15]. P = Patient; NP = normal plasma.
menstruation (menarche). She showed a good response of the VWF parameters 1 and 2 h after DDAVP with correction of strongly prolonged Ivy BT to normal at two occasions, giving the impression of a very good response to DDAVP (fig. 2, left). During pregnancy in 1994, VWF:Ag rose to normal, whereas the values for VWF:RCo and VWF:CB remained below the lower level of normal (fig. 2, left) with the persistence of prolonged BT and the absence of high-molecular-weight VWF multimers consistent with VWD type 2A. Her first pregnancy and postpartum period were uneventful with Haemate-P treatment.

Case II-1 [18] (uncle in table 1 [4]; male born in 1940) had a history of easy bruising, epistaxis and gum bleeding since early childhood. Several tooth extractions were followed by prolonged bleeding for a few hours or even longer for which surgical suturing was needed. Minor surgeries while on Haemate-P treatment were not followed by bleeding complications. The VWF multimeric pattern in low-resolution gels in case II-1 shows the absence of large VWF multimers and increased VWF degradation indicating increased proteolysis (fig. 2, right). The medium-resolution gels according to Schnepenheim and Budde [9] show the absence of some of the large multimers and a pronounced triplet structure of each visible band due to increased proteolysis consistent with VWD 2 (fig. 3, right).

Case III-1 [18] (nephew in table 1 [4]) presented in 1994 with a severe knife cut with persistent bleeding for several hours from a wound a few centimeters in length in the top of his thumb. DDAVP was followed by a good response of VWF parameters and transient correction of Ivy BT (fig. 3 right). Ivy BT became abnormal when VWF:Ag was still above 1.3 U/ml and VWF:CB fell to around 0.70 U/ml, consistent with defective VWF function due to the absence large VWF multimers. Despite careful surgical suturing, the wound started bleeding again within 6 h while VWF parameters were still normal but Ivy BT was again prolonged. The bleeding stopped immediately after Haemate-P treatment (2,000 units; 30 U/kg).

The responses to DDAVP in cases II-1 and III-1 in figure 3 were identical. The response to DDAVP was completely normal with maximal values of 2.0 U/ml for FVIII:C and VWF:Ag followed by normal or near normal half-lives. DDAVP induced transient good responses of the functional parameters VWF:RCo and VWF:CB, with peak levels around 1.0 IU/ml, followed by shortened half-lives <4 h due to increased proteolysis (fig. 3). The VWF:RCo/VWF:Ag ratio remained decreased (0.5) directly after DDAVP (fig. 3). After DDAVP, prolonged Ivy BT transiently reached normal values for a few to several hours (fig. 3), associated with a transient reappearance of most
of the large VWF multimers a few hours after DDAVP followed by increased proteolysis of large VWF multimers (fig. 2, right).

The absolute values of VWF:Ag in 10 affected members of the large Dutch family with VWD 2A are decreased in 9 of 10 cases of the VWD type 2A group II, suggesting a secretion and/or clearance defect of VWF:Ag caused by the V1499E mutation. The ratio of VWF propeptide/VWF:Ag was between 1 and 2.0 (<2), which is consistent with the near normal half-life of VWF:Ag after DDAVP indicating a mild clearance defect of VWF:Ag. DDAVP response curves (fig. 2, 3) clearly demonstrate that mild VWD type 2A group II due to the mutation V1499E in the A2 domain is indeed mainly caused by increased proteolysis of secreted mutant VWF.

Table 2. Protocol for a pharmacokinetic study of FVIII/VWF concentrate substitution in patients with VWD type 2 undergoing elective surgery or for the treatment of bleeding [20–22]

<table>
<thead>
<tr>
<th>Blood sample assay</th>
<th>Before VWF-FVIII</th>
<th>After substitution of VWF-FVIII concentrate</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ivy BT</td>
<td>+ + + + +</td>
<td>+ + + + + + +</td>
</tr>
<tr>
<td>PFC</td>
<td>+ + + +</td>
<td>+ + + + + + +</td>
</tr>
<tr>
<td>RIPA</td>
<td>+ + – + +</td>
<td>+ + + + + + +</td>
</tr>
<tr>
<td>FVIII:C</td>
<td>+ + + + +</td>
<td>+ + + + + + +</td>
</tr>
<tr>
<td>VWF:Ag</td>
<td>+ + + + +</td>
<td>+ + + + + + +</td>
</tr>
<tr>
<td>VWF:RCo</td>
<td>+ + + + +</td>
<td>+ + + + + + +</td>
</tr>
<tr>
<td>VWF:CB</td>
<td>+ + + + +</td>
<td>+ + + + + + +</td>
</tr>
<tr>
<td>VWF:multimer</td>
<td>+ + + + +</td>
<td>+ + + + + + +</td>
</tr>
</tbody>
</table>

Fig. 5. Dose-response relation between PFC and FVIII-VWF parameters in 14 patients with VWD type 2 before (type 2: △; type 2M: ○) and 1 h after DDAVP (type 2: ▲; type 2M: □) [19].
Federici et al. [15] demonstrated a poor response of VWF:RCo compared to restricted or good responses of FVIII:C in 15 patients with VWD type 2A, with correction of strongly prolonged BT only in 2 of the 15 patients (fig. 4). These observations strongly indicate the need of purified VWF concentrate or VWF-FVIII concentrate with a high ratio of VWF:RCo/VWF:Ag with the presence of large VWF multimers and a high ratio of VWF:RCo/FVIII:coagulant activity (C) >2 for the management and prevention of bleeding during surgery or trauma in VWD type 2A patients and in type 2B, too.

We evaluated dose-response curves of PFA, VWF and FVIII before and after DDAVP in 14 patients with VWD type 2 in routine clinical practice [19]. Pretreatment values of functional VWF:RCo and VWF:CB are significantly lower compared to VWF:Ag and FVIII:C in the 14 patients with VWD type 2. PFA-100 closure times (PFCs) were prolonged (<300 s) in all VWD type 2 patients except for 2 VWD 2M patients (open symbols, fig. 4). In 4 patients with type 2M and 2 with mild type 2, PFCs was corrected after DDAVP (closed symbols, fig. 5). None of the VWD type 2A patients showed a shortening of the prolonged PFC, which is consistent with the diagnosis of VWD type 2 (2A and 2B). In contrast, in all the VWD type 2M or mild type 2 patients (n = 6), PFC reached near normal or normal values (<150 s; fig. 5). The results in figure 4 are in agreement with published data and substantiate the conclusion that VWD type 2A needs to be treated with VWF-FVIII concentrates for minor and major bleedings [4, 15]. VWD 2M patients may profit from DDAVP treatment for minor bleeding, but confirmation in a prospective study is required.

Fig. 6. Response of FVIII:C and VWF parameters to DDAVP and VWF-FVIII concentrate in case 1 with pronounced VWD type 2A group II.
Prospective Studies on VWF-FVIII Concentrate Substitution in VWD Type 2

Candidates for VWF:FVIII concentrate substitution to treat spontaneous bleeding and prevent bleeding in surgical settings and trauma should include all type 2 and 3 and severe type 1 VWD patients, with transient, poor or no responses to DDAVP. The in vivo responses of VWF parameters to VWF-FVIII concentrates have to be evaluated according to standardized recommendations proposed by the Scientific Standardization Committee of the International Society on Thrombosis and Hemostasis for studies on ex vivo biological effects of virus-inactivated concentrates in VWD patients [20–22]. Infusion of FVIII/VWF concentrate using both FVIII:C and VWF:RCo dosing (in IU/kg body weight, BW) for the treatment of spontaneous bleeding or bleeding prophylaxis during surgery has to be evaluated prospectively in every VWD patient for recovery after the first loading dose during daytime, evening, night and at the weekend by the responsible hematologist in the hospital (table 2). Each dose of FVIII/VWF concentrate has to be administered by intravenous infusion over a period of 15–30 min. After the first loading dose, blood samples have to be taken for FVIII:C and VWF parameters before infusion and 1 h (not earlier), 3, 6, 12 and 24 h after infusion together with assessment of BT or PFC at the discretion of the responsible physician. In case two or more FVIII/VWF infusions are needed during one treatment period, subsequent blood samples should be taken before and 1 h after infusion (table 2).

Each lot of FVIII/VWF concentrate should be labeled and/or FVIII:C, VWF parameters and VWF multimers should be evaluated. Blood sampling at 24 h is optional but strongly recommended for pharmacokinetic evaluation. All VWD patients who are to be treated for bleeding or prophylaxis of bleeding during elective surgery or after trauma are candidates for monitoring and pharmacokinetic evaluation of FVIII/VWF concentrate substitution before and after the first loading dose using VWF:RCo dosing per kg BW.

A minority of VWD type 2A have mild VWD group II characterized by near normal to prolonged values for BT, normal FVIII:C and VWF:Ag, low VWF:RCo and VWF:CB, a normal RIPA and complete correction of BT and functional VWF parameters to normal for only a few
hours followed by short half-lives for VWF:RCo and VWF:CB [4, 5, 9, 10]. Such patients with mild VWD type 2A group II as well as patients with VWD type 1/2E or 2M have transiently complete responses of FVIII:C, VWF parameters and BT to DDAVP that may be efficacious in the acute and prophylactic treatment of spontaneous minor bleedings but fail as prophylactic treatment in surgical settings and trauma (table 3). In contrast, patients with pronounced VWD type 2A group I have pronounced or very low VWF:RCo, prolonged to strongly BT, PFC >250 s and show only a transient minor or a poor response to DDAVP with no correction of the BT despite some increase in VWF:RCo, and therefore are candidates for VWF-FVIII concentrate substitution for the acute and prophylactic treatment of bleeding symptoms (table 3).

In the 1980s and 1990s, the recommended dose for Haemate-P in surgical settings or trauma was 40–50 IU FVIII:C per kg BW for Haemate-P to reach peak levels of FVIII:C around 1.2–1.5 IU/ml. As the ratio of VWF:RCo/FVIII:C is ~2.2, this strategy predicts overtreatment of patients with VWD type 2A with regard to VWF levels. To confirm this hypothesis, we performed prospective evaluation studies of VWF-FVIII substitution during major surgery in patients with severe VWD 2A and 2B (fig. 6, 7).

Case 1. A 70-year-old male with pronounced VWD type 2A poorly responsive to DDAVP (fig. 6, left) has to undergo major surgery for correction of abdominal aortic aneurysm. Haemate-P treatment using FVIII:C dosing, 50 IU FVIII:C/kg BW two times daily on day 1, 40 IU FVIII:C/kg BW two times daily on day 2 and 20 IU FVIII:C/kg BW two times daily for several days to reach
peak levels of FVIII:C of ~1.2–1.8 IU/ml resulted in very high levels of VWF:RCo and complete correction of strongly prolonged BT to normal (fig. 6, right).

Case 2. A 46-year-old female with VWD type 2B unresponsive to DDAVP (fig. 7, left) has to undergo major surgery. Haemate-P treatment using FVIII:C dosing, 40 IU FVIII:C/kg BW preoperatively followed by 20 IU FVIII:C/kg BW two times daily, resulted in FVIII:C levels of ~1.5 IU/ml and in much higher values for both VWF:RCo and VWF:CB indicating significant overtreatment with regard to VWF parameters (fig. 7, right). This prompted us to adapt the recommendations for VWD patients using VWF:RCo dosing with the compelling need for prospective evaluation studies according to a standardized protocol (table 3) [4, 21, 22].

Case 3. A 33-year-old female had pronounced VWD 2A group I due to the S1506L mutation (fig. 1). DDAVP induced top levels of VWF:Ag of 1.25 IU/ml, VWF:RCo of 0.55 and no response of VWF:CB, 0.10 IU/ml, and no correction of the prolonged BT (fig. 1). At the time of investigation, she was pregnant and gave her consent to evaluate the hemostatic effect of one loading dose Haemate-P on BT, FVIII:C and VWF parameters. After one loading dose, Haemate-P 40 IU FVIII:C/kg BW = 88 IU VWF:RCo/kg, the strongly prolonged BT was corrected to normal 1 and 3 h after infusion. BT was slightly prolonged 6 h after transfusion at normal values of VWF:CB, 1.12 IU/ml, and VWF:RCo, 1.2 IU/ml (fig. 8, left) and was significantly prolonged 18 h after infusion with borderline values for VWF:RCo of 0.50 IU/ml. Consequently, during delivery she was treated with a loading dose of Haemate-P 40 IU/ml FVIII:C/kg BW = 88 IU VWF:RCo/kg followed by with 14 IU FVIII:C/kg BW = 30 IU VWF:RCo two times daily for 5 days (fig. 8, right). Both delivery and the postpartum period were uneventful with no excessive blood loss. After the loading dose, the strongly

Diagnosis and Management of VWD 2A

Acta Haematol 2009;121:154–166
prolonged BT corrected to normal, but was again significantly prolonged 10 h after infusion (fig. 8, right). Interestingly, BT could again be corrected to normal by repeated maintenance treatment with Haemate-P 30 IU VWF/kg every 12 h (fig. 8, right).

Case 4. A 63-year-old female with VWD type 2A was referred for preoperative evaluation (elective minor surgery). Intravenous DDAVP induced normal secretion of FVIII:C and VWF:Ag amounting to 2.40 IU/ml followed by a normal biological half-life of about 12 h, whereas peak levels of VWF:RCo and VWF:CB were 0.9 and 0.8 IU/ml, respectively, followed by shortened biological half-lives of 4 h for VWF:RCo and <2 h for VWF:CB. This type of DDAVP response curve indicates rapid proteolysis of the high- and intermediate-molecular-weight VWF multimers after release of normal VWF by endothelial cells consistent with the diagnosis of VWD 2A group II (fig. 9, left). This was associated with a complete correction of the very prolonged Ivy BT for only about 1 h (fig. 9, left). After a loading dose of Haemate-P 50 IU FVIII:C/kg BW (110 IU VWF:RCo/kg), the strongly prolonged BTs were within the normal range 1, 3, 6 and 12 h after infusion (fig. 9, right). At 24 h there was a prolongation of the BT grade at values of VWF:RCo just above the lower limit of normal and a decreased value of VWF:CB of 0.35 U/ml (fig. 9 right). Therefore, she received prophylactic treatment to prevent bleeding during surgery (cataract extirpation and lens replacement) with Haemate-P 40 IU FVIII:C/kg BW followed by Haemate-P 30 IU VWF:RCo/kg every 12 and 24 h postoperatively (fig. 10). At FVIII:C and VWF:RCo levels of ~2.0 IU/ml ~30 h postoperatively, BT and VWF:CB were within the normal range (0.60–1.25 IU/ml; fig. 10). BT was again prolonged 1 day (24 h) after infusion at time 44 h, when the VWF:RCo level was still normal (0.92 IU/ml) but the VWF:CB was decreased (0.34 IU/ml; fig. 10).

Fig. 9. Response of FVIII:C and VWF parameters to DDAVP and one loading dose of VWF-FVIII concentrate in case 4 with VWD type 2A group II.
These and other observations prompted us to propose adjusted guidelines using VWF:RCo dosing based on a previously published prospective study [20–22] (table 3). Our studies showed that a loading dose of Haemate-P completely corrected both VWF:RCo and VWF:CB levels and strongly prolonged Ivy BT to normal values for 6–12 h in mild and pronounced VWD type 2A, but for 6–8 h in severe VWD 2A with absent RIPA. One explanation may be that in patients with severe VWD type 2A group I lacking intermediate/large VWF multimers and RIPA, platelets show nonfunctional VWF and therefore only transiently corrected BT, in agreement with a previous study in patients with VWD type 3 [20], whereas mild VWD type 2A group II patients do have normal platelet VWF content. Another explanation may be that following Haemate-P transfusion the mixture of endogenous mutant and infused normal VWF still will show decreased ratios for VWF:RCo and VWF:CB a few to several hours after infusion. We have introduced adjusted recommendations to dose FVIII/VWF concentrates in international units of VWF:RCo per kilogram BW for the acute and prophylactic treatment of bleeding in VWD type 2 and extended it to recessive VWD type 1 and type 3 (table 3) [18]. The adjusted recommendations are to be tailored to the patient regarding the severity of bleeding complications, the severity of VWD, the type of surgery (minor or major) and the type of VWD (type 2 or 3; table 2) [4, 20–22]. Such recommendations have to be confirmed regarding safety and cost-effectiveness in prospective studies and are to be amended if necessary to improve the management of VWD patients [24, 25].

**Fig. 10.** Response of FVIII:C and VWF parameters to VWF-FVIII concentrate substitution to prevent bleeding during and after elective surgery in case 4.

**References**


Managing Patients with von Willebrand Disease Type 1, 2 and 3 with Desmopressin and von Willebrand Factor-Factor VIII Concentrate in Surgical Settings

Jan Jacques Michiels\textsuperscript{a, c}, Huub H.D.M. van Vliet\textsuperscript{b}, Zwi Berneman\textsuperscript{a}

Wilfried Schroyens\textsuperscript{a}, Alain Gadisseur\textsuperscript{a}

\textsuperscript{a}Hemostasis and Thrombosis Research Center, Antwerp University Hospital, Edegem, Belgium;
\textsuperscript{b}Hemostasis and Thrombosis Research, Erasmus University Medical Center Rotterdam, and
\textsuperscript{c}Hemostasis and Thrombosis Science Center, Goodheart Institute, Rotterdam, The Netherlands

Introduction

Patients with von Willebrand disease (VWD) have an increased bleeding tendency following surgery or other invasive procedures. It is generally agreed that the low von Willebrand factor (VWF) level is most important for mucous membrane bleeds, whereas decreased factor VIII (FVIII) is more important for soft tissue and joint bleeds [1–3]. VWF levels should be normalized in the peri-operative and early postoperative period, especially when there is a risk for mucous membrane bleeds. FVIII should be normalized in connection with major surgery, both during the surgical procedure and ~7–10 days postoperatively [4].

Key Words
Bleeding severity · Desmopressin · Pharmacokinetic studies · Trauma · von Willebrand factor/factor VIII concentrates

Abstract
Guidelines and recommendations for the acute and prophylactic treatment of bleeding in von Willebrand disease (VWD) patients with von Willebrand factor (VWF)/factor VIII (FVIII) concentrates should be based on the analysis of the content of VWF/FVIII concentrates and on pharmacokinetic studies in patients with different severity of VWD (type 1, type 2 or type 3). The VWF/FVIII concentrates should be assessed using the parameters FVIII:coagulant activity (C), VWF:ristocetin cofactor activity (RCo), VWF:collagen binding and VWF multimeric patterns for the presence of large multimers to determine their predicted efficacy and safety in prospective management studies. As the bleeding tendency is moderate in VWD type 2 and severe in type 3 and because the FVIII:C levels are subnormal in type 2 but very low in type 3 VWD patients, new guidelines using VWF:RCO unit dosing for the acute and prophylactic treatment of bleeding episodes are proposed. Such guidelines should be stratified for the severity of bleeding, the type of surgery (minor or major) and also for the bleeding score in either VWD type 1, 2 or 3.
Factor levels can be raised either by stimulating the endogenous release of FVIII and VWF with desmopressin (DDAVP, 1-desamino-8-D-arginine vasopressin), or by substituting the deficient factors with a coagulation factor concentrate containing VWF and FVIII.

**Desmopressin: DDAVP**

DDAVP is a widely used hemostatic drug [5]. It is a synthetic analogue of the natural hormone vasopressin, but it has no pressor activity, being in contrast to vasopressin. The effect is virtually immediate, usually with 2- to 4-fold increases in the plasma concentrations of FVIII, VWF and tissue plasminogen activator [6]. Most patients with VWD type 1 respond adequately to DDAVP (fig. 1; table 1). Some patients with type 2 may respond sufficiently, but most type 2 patients will have an insufficient response due to functional abnormalities in the VWF. In the classical form of type 2B, DDAVP causes platelet aggregation and thrombocytopenia, and should be avoided [7]. Type 3 patients do not respond to DDAVP. The response rate in type 1 and 2 is dependent on the severity of the disorder. Thus, even if the majority of type 1 patients may respond well, only a minority of those with severe type 1 will be responders [8]. The European multicenter study Molecular and Clinical Markers for the Diagnosis and Management of Type 1 von Willebrand disease (MCMDM-1VWD; www.shef.ac.uk/euvwd/) recently demonstrated that there is a clear correlation between mutations in the VWF gene and the response to DDAVP in type 1 VWD.

All patients should be given a test dose to ensure that the response is sufficient for clinical use. DDAVP can be used to treat major bleeds or to prevent bleeding in connection with surgery or other invasive procedures, if VWF:ristocetin cofactor activity (RCo) and FVIII:coagulant activity (C) reach normal levels after DDAVP. If response is lower or the duration is short, a VWF/FVIII concentrate should be considered [8–16].

In case of surgery, DDAVP (0.3 µg/kg) may be given intravenously about 30–60 min before surgery, or subcu-

---

**Fig. 1.** Response to DDAVP in 26 patients diagnosed with VWD type 1 according to SSC ISTH criteria [8]. The responses of FVIII:C to DDAVP are good in 9 cases, but restrictive or even poor or non-responsive in more than half of these patients with VWD type 1, indicating that various degrees of severity of either autosomal recessive or dominant type 1 VWD patients are lumped when the SSC ISTH classification is applied [11, 12]. Among VWD type 1 patients, there is a group of mild VWD type 1 with a good response of VWF:RCo and complete correction of VWF parameters and BT after DDAVP [11, 12]. There is another well-recognized group of severe type 1 VWD patients with no or very poor responses of VWF:RCo and no correction of BT after DDAVP indicating the need of VWF/FVIII concentrate to manage and prevent bleeding during minor and major surgery or trauma [12].
taneously about 1–2 h preoperatively. The intranasal spray may be used in connection with minor procedures, and should be taken 1–2 h in advance in a dose of 300 μg (150 μg in patients weighing <30 kg) [8–16].

DDAVP dosing can be repeated at 12- or 24-hour intervals if necessary, but repeated dosing for several days should be avoided. If repeated, once-daily dosing of DDAVP over several days is required, tachyphylaxis and antidiuretic effects must be considered. It may be necessary to switch to a VWF/FVIII concentrate in some cases.

A single dose of DDAVP is often sufficient for minor surgery. In connection with tooth extractions and other procedures involving mucous membranes, concomitant treatment with an antifibrinolytic agent, e.g. tranexamic acid, may be of benefit.

**General Recommendations for the Evaluation of Prospective Studies [8–16]**

Recessive VWD type 3 and severe type 1 patients with no response of VWF parameters and FVIII to DDAVP are candidates for prophylactic treatment of hemophilic bleeding in joints or soft tissues with low-dose VWF/FVIII substitution 2–3 times a week.

VWD type 2A, 2C and 2D patients with poor response of functional VWF parameters to DDAVP are candidates for VWF/FVIII concentrate substitution to prevent bleeding during minor and major surgery and/or trauma, and to treat spontaneous serious bleeding manifestations.

VWD type 2E, 2M and 2A group II patients with short or restricted good responses of functional VWF parameters to normal with correction of PFA-100 and Ivy bleeding time (BT) for a few to several hours are candidates for DDAVP treatment of minor bleeds or prevention of bleeding during minor surgery, but should receive additional VWF/FVIII substitution therapy for maintained correction of functional VWF parameters during and after major surgery or trauma.

VWD patients with good responses to DDAVP of FVIII:C and all VWF parameters to normal values, including correction of PFA-100 and Ivy BT for >6–12 h after DDAVP treatment, are candidates for DDAVP for the prevention of excessive bleeding associated with minor and major surgery or trauma.

<table>
<thead>
<tr>
<th>VWD classification</th>
<th>Mutation location</th>
<th>Response to DDAVP of FVIII:C, VWF and BT</th>
<th>VWF:Ag</th>
<th>VWF:RCo</th>
<th>VWF:CB</th>
<th>BT correction</th>
</tr>
</thead>
<tbody>
<tr>
<td>VWD type 1, normal MM</td>
<td>variable</td>
<td>good</td>
<td>good</td>
<td>good</td>
<td>good</td>
<td>yes</td>
</tr>
<tr>
<td>VWD type 1, abnormal MM</td>
<td>D4, B1–B3, C1–C2</td>
<td>good</td>
<td>good/restricted</td>
<td>good/restricted</td>
<td>good/restricted</td>
<td>yes</td>
</tr>
<tr>
<td>Dominant VWD, abnormal MM</td>
<td>D3</td>
<td>good</td>
<td>transient</td>
<td>transient</td>
<td>transient</td>
<td>transient</td>
</tr>
<tr>
<td>1/2E</td>
<td>D3</td>
<td>short</td>
<td>short/transient</td>
<td>short/transient</td>
<td>short/transient</td>
<td>transient</td>
</tr>
<tr>
<td>1/Vicenza</td>
<td>D3</td>
<td>short</td>
<td>short/transient</td>
<td>short/transient</td>
<td>short/transient</td>
<td>short/transient</td>
</tr>
<tr>
<td>2M</td>
<td>A2</td>
<td>good</td>
<td>restricted</td>
<td>restricted</td>
<td>restricted</td>
<td>restricted</td>
</tr>
<tr>
<td>2A group I</td>
<td>A2</td>
<td>good</td>
<td>restricted</td>
<td>restricted</td>
<td>restricted</td>
<td>restricted</td>
</tr>
<tr>
<td>2B</td>
<td>A1</td>
<td>good</td>
<td>partial</td>
<td>partial</td>
<td>partial</td>
<td>partial</td>
</tr>
<tr>
<td>2D</td>
<td>CK</td>
<td>partial</td>
<td>poor</td>
<td>poor</td>
<td>poor</td>
<td>no</td>
</tr>
<tr>
<td>Recessive VWD 3</td>
<td>D1, D2, D3</td>
<td>partial</td>
<td>poor</td>
<td>poor</td>
<td>poor</td>
<td>no</td>
</tr>
<tr>
<td>Severe 1</td>
<td>D1, D2, CK</td>
<td>partial</td>
<td>partial</td>
<td>partial</td>
<td>partial</td>
<td>partial</td>
</tr>
<tr>
<td>Secretion defect</td>
<td>D1, D2, CK</td>
<td>partial</td>
<td>partial</td>
<td>partial</td>
<td>partial</td>
<td>partial</td>
</tr>
<tr>
<td>Severe 2C, 2D</td>
<td>D1, D2, CK</td>
<td>partial</td>
<td>partial</td>
<td>partial</td>
<td>partial</td>
<td>partial</td>
</tr>
<tr>
<td>2N</td>
<td>D1, D2, D3</td>
<td>poor/short</td>
<td>good</td>
<td>good</td>
<td>good</td>
<td>normal BT</td>
</tr>
</tbody>
</table>
VWF/FVIII Concentrate

Patients who are unresponsive to DDAVP, i.e. severe type 1 patients, the majority of those with type 2, and all type 3 patients, require concentrates containing large amounts of VWF in case of bleeds or surgery. Some type 1 patients may also require VWF concentrates if they need prolonged treatment, or if they have contraindications to DDAVP, such as cardiovascular disease. Physicians treating VWD patients must be aware of the large differences between different VWF/FVIII concentrates.

Proper recommendations for prospective outcome studies to evaluate the pharmacokinetics (PK) and dynamics combined with clinical efficacy of VWF/FVIII concentrates in VWD patients are required (table 2) [15].

The in vitro characteristics of a VWF/FVIII concentrate should be labeled by its content of FVIII:C, VWF: antigen (Ag), VWF:RCo; VWF:collagen binding (CB; collagen type 1, 95%, and type 3, 5%) and the presence of large VWF multimers compared to normal control plasma [17–19]. The in vivo responses of VWF parameters to VWF/FVIII concentrates have to be evaluated according to the protocol proposed by the Scientific Standardization Committee (SSC) of the International Society on Thrombosis and Hemostasis (ISTH) for studies on ex vivo biological effects of virus-inactivated concentrates in VWD patients [13–17]. Ideally, in each VWD patient who needs the recommended infusion of VWF/FVIII concentrate using both FVIII:C and VWF:RCo dosing (in IU/kg body weight) to treat or prevent spontaneous or surgery-induced bleeding, PK should be evaluated prospectively after the first loading dose during daytime, evening, night and at the weekend by the responsible hematologist in the hospital for future reference when the same concentrate is used (table 2). Each dose of VWF/FVIII concentrate has to be administered by intravenous infusion over a period of 15–30 min. Blood samples before and after the first loading dose have to be taken for FVIII:C and VWF parameters before infusion and 1 (not earlier), 3, 6, 12 and 24 h after infusion combined with assessment of BT or PFA-100 closure times at the discretion of the responsible physician (table 2) [15]. In case two or more VWF/FVIII infusions are needed during one treatment interval, subsequent blood samples should be taken before and 1 h after infusion.

For each lot of a VWF/FVIII concentrate, FVIII:C, VWF:Ag, VWF:RCo, VWF:CB and the presence of large VWF multimers in low- and medium-resolution SDS-agarose gels should be determined [16–19]. Blood sampling before and 1, 3, 6 and 12 h after infusion and before and after each following infusion is required for clinical outcome and efficacy studies. For the prophylactic treatment of bleeding during elective surgery, real field PK study of VWF/FVIII concentrates for 12 h starting from the administration of the first loading dose using VWF:RCo dosing per kg body weight (60 or 80 VWF:RCo U kg⁻¹) is mandatory [15].

Lethagen et al. [18] compared the in vitro characteristics of six von Willebrand concentrates (fig. 2 left). The content of high-molecular-weight (HMW) multimers (presence of large multimers) was normal, with a ratio of VWF:RCo/VWF:Ag >0.7 in Haemate-P (CS Behring, Marburg, Germany), Innobrand (CAF, Brussels, Germany) and Facteur Willebrand (LFB, Lille, France), being moderately reduced in Koate and 8Y and significantly reduced in Immunate. Budde et al. [19] compared 12 VWF/FVIII concentrates to investigate content and activities of FVIII:C and VWF parameters as well as the content of HMW VWF multimers. Compared to controls, HMW multimers were present in 93.6% for Haemate-P, 79.2% for Innobrand and <36% for the remaining VWF/FVIII concentrates. VWF:RCo per unit FVIII:C was 2.88 for Haemate-P, 2.28 for Green Eight, 2.20 for Innobrand and 0.82 for Alphanate. Conversely, the FVIII/VWF:RCo ratio was 0.35 for Haemate-P, 0.44 for Green Eight, 0.45 for Innobrand and 1.2 for Alphanate. The ratio of VWF:RCo/VWF:Ag was 0.94 for Haemate-P, 0.81 for Innobrand and 0.43 for Alphanate, which simply reflects the presence or absence of large VWF multimers in the con-
centrate. The ratio of VWF:CB/VWF:Ag was 0.89 for Haemate-P, 0.64 for Innobrand and 0.49 for Alphanate [19]. Since the ratio of VWF:RCo/VWF:Ag for Immunate is <0.50, it is no longer used for the prevention and treatment of bleeding in VWD patients. In the treatment of VWD patients, one should be aware that endogenous FVIII:C will bind to the amount of VWF:Ag infused, a main determinant for a much higher recovery and longer half-life of FVIII:C, indicating rather complex PK of the VWF-FVIII concentrates infused in VWD patients. Consequently, substitution of a VWF/FVIII concentrate with a low ratio of VWF:RCo/VWF:Ag (<0.70) and/or a high ratio of FVIII:C/VWF:RCo (>1) for several days after surgery or trauma are to be preferred in order to prevent unusually high FVIII:C levels, a major risk factor for postoperative deep vein thrombosis (fig. 3) [20].

The French PK study evaluated Wilfactin 100 VWF:RCo/kg⁻¹ in 8 VWD type 3 patients. The calculated incremental recoveries of VWF:RCo (2.1%) and VWF:Ag (1.8%), the half-lives of VWF:RCo (12.4 h) and VWF:Ag (15.9 h), and the VWF:RCo and VWF:Ag curves were equal in terms of bioequivalence and PK. The FVIII:C levels increased slowly, with peak levels at 12–24 h of about 50%, which is 3 times lower compared to VWF peak levels after infusion of Wilfactin (fig. 4) [17]. As a consequence of this discrepancy, Innobrand, a mixture of FVIII/purified VWF with a ratio of FVIII/VWF:RCo of 0.40 which resembles Haemate-P, was introduced. The European PK study compared Wilfactin and human factor VIII concentrate (Haemate-P or Innobrand) in 17 VWD patients (2 VWD type 1, 9 type 2A/B and 6 type 3

Fig. 2. Multimeric composition of VWF in the available VWF/FVIII concentrates (left) [18] and in Wilate (right) [21] compared to normal plasma. SHP = Standard human plasma; lots 1/2 = Wilate.

Fig. 3. Representative example of the effect of VWF/FVIII concentrate with a high FVIII-VWF:RCo ratio on plasma FVIII:C and VWF:RCo levels during long-term daily treatment for up to 8 days after major surgery. At levels of normal VWF:RCo maintained at ~1 IU/ml (100%), FVIII:C rose to levels >3 IU/ml (300%) [20].
patients) [17]. The administration of Wilfactin or VWF/FVIII concentrate resulted in a transient shortening of Ivy or Simplate BT in 71 and 82% of the patients, respectively. The shortening of BT was maximal 1–3 h after infusion and was lost after 24 h. The measured VWF content of Wilfactin versus Haemate-P/Innobrand as well as the calculated incremental recoveries of VWF:RCo (1.9 vs. 1.9) and VWF:Ag (2.2 vs. 2.2%), and the half-lives of VWF:RCo (11.7 vs. 12.8 h) and VWF:Ag (14.8 vs. 17.8 h, respectively) were bioequivalent in terms of efficacy. This confirms that the PK profile of VWF does not depend on the amount of FVIII in the concentrate or in the VWD type 2 patient. This is true for the comparison of Wilfactin and Haemate-P containing the large VWF multimers.

**Fig. 4.** Direct comparison of the in vivo responses of one loading dose of Wilfactin and VWF-FVIII concentrate (Haemate-P or Innobrand) in 17 patients with VWD: Wilfactin (upper left) and VWF/FVIII concentrate (Haemate-P or Innobrand, lower left) [17]. Direct comparison of FVIII:C response to Wilfactin versus VWF/FVIII (Haemate-P or Innobrand, upper right) and responses of FVIII:C subdivided in VWD type 1, type 2A, type 2B and type 3 [17].
as reflected by the normal VWF:RCo/VWF:Ag ratios. As shown in the French and European VWD studies, the pattern of FVIII:C kinetics was typically very different after infusion of a VWF/FVIII concentrate (Haemate-P or Innobrand) compared to a purified VWF concentrate (Wilfactin; fig. 4). The human VWF/FVIII concentrates Haemate-P (FVIII:C/VWF:RCo ratio 0.34) and Innobrand (FVIII:C/VWF:RCo ratio 0.40) was followed by the predicted incremental recoveries resulting in a two times higher concentration of VWF parameters compared to FVIII:C immediately after the loading dose (fig. 4). In contrast, after infusion of Wilfactin containing a very small amount of FVIII:C (FVIII:C/VWF:RCo ratio <0.04), the maximal FVIII:C levels were progressively attained between 12 and 24 h after infusion, reaching subnormal to low normal levels (fig. 4) [17]. This delayed increase is due to the progressive stabilization of endogenous FVIII by its binding to the purified VWF:Ag in the
concentrate infused. Depending on the FVIII:C levels before treatment, type 2A and 2B had significantly higher FVIII:C levels during the first 12 h after infusion of Wilfactin compared to VWD type 3 (fig. 4, lower left). This will predict equal efficacy and safety in the acute and prophylactic treatment of bleeding in VWD type 2 with subnormal or low normal FVIII:C levels. Comparing the FVIII:C levels after infusion of Wilfactin (endogenous FVIII:C) and Haemate-P or Innobrand (exogenous and endogenous FVIII:C), the curves of FVIII:C reached equal levels 24–48 h after infusion, and subsequent decay did not differ until 72 h after infusion (fig. 4) [17]. This has important implications for the treatment of acute bleeding episodes and may also have an impact on the efficacy of long-term prophylaxis of joint bleeds in VWD type 3. The much higher levels of FVIII:C following each infusion lasting 2–3 days with Haemate-P or Innobrand compared to Wilfactin surely will have consequences with regard to dosing of each of the products for the acute and prophylactic treatment of bleeding in severe VWD type 3 and 1 patients.

Using the VWF/FVIII concentrate Wilate, large VWF multimers were absent (fig. 2, right) [19]. The VWF:RCo/FVIII:C ratio in 15 Wilate batches was 0.84 [21] (FVIII/ VWF:RCo ratio 1.1) compared to a VWF:RCo/FVIII:C ratio of 2.2 (FVIII/VWF:Ag ratio 0.45) in Haemate-P [22].

Two prospective studies on one of the most used VWF/FVIII concentrates (Haemate-P) showed that preoperative PK assessment in each individual VWD patient was useful to determine the loading dose for minor or major surgery (fig. 5) [22, 23]. In the first small prospective study of Haemate-P used in VWD type 2 patients undergoing elective major surgery, the mean loading dose was 90 IU/kg VWF:RCo. From that PK study, it was concluded that a lower loading dose could be effective and recommendations for both loading dose (60 IU/kg VWF:RCo for major and 40 IU/kg VWF:RCo for minor surgery) and for maintenance dosing were proposed depending on the severity of VWD and the type of surgery. The second prospective study demonstrated for the first time that the initial in vivo recovery of VWF parameters and FVIII:C is constant.

### Table 3. Efficacy of VWF/FVIII concentrate replacement regarding surgery and major bleeding events

<table>
<thead>
<tr>
<th>Source</th>
<th>VWF/FVIII concentrate</th>
<th>Patients/surgical procedures</th>
<th>Type of study</th>
<th>Loading dose VWF:RCo</th>
<th>Outcome</th>
</tr>
</thead>
<tbody>
<tr>
<td>Michiels et al. 2004 [22]</td>
<td>Haemate-P</td>
<td>5/surgery</td>
<td>prospective</td>
<td>60–80 U kg⁻¹</td>
<td>excellent/good 100%</td>
</tr>
<tr>
<td>Dobrkovska et al. 1998 [24]</td>
<td>Humate-P</td>
<td>97/surgery</td>
<td>retrospective</td>
<td>80 U kg⁻¹</td>
<td>excellent/good 99%</td>
</tr>
<tr>
<td>Lillicrap et al. 2002 [25]</td>
<td>Humate-P</td>
<td>73/bleeding, 344 events</td>
<td>prospective</td>
<td>55/69 U kg⁻¹</td>
<td>excellent/good 99%</td>
</tr>
<tr>
<td>Goudemand et al. 2005 [17]</td>
<td>Wilfactin</td>
<td>direct comparison</td>
<td>prospective</td>
<td>60/100 U kg⁻¹</td>
<td>PK study figure 4</td>
</tr>
<tr>
<td>Mannucci et al. 2002 [26]</td>
<td>Alphanate</td>
<td>39/71 surgical or invasive</td>
<td>prospective</td>
<td>60 U kg⁻¹</td>
<td>good clinical response</td>
</tr>
<tr>
<td>Franchini et al. 2003 [27]</td>
<td>Haemate-P</td>
<td>26/43 procedures</td>
<td>retrospective</td>
<td>35/61 U kg⁻¹</td>
<td>excellent/good 98%</td>
</tr>
<tr>
<td>Thompson et al. 2004 [28]</td>
<td>Humate-P</td>
<td>39/42 surgical procedures</td>
<td>prospective</td>
<td>82 U kg⁻¹</td>
<td>excellent/good 100%</td>
</tr>
<tr>
<td>Borel-Derlon et al. (2007)</td>
<td>Wilfactin</td>
<td>50/139 non-surgical bleeds</td>
<td>prospective</td>
<td>50/60 U kg⁻¹</td>
<td>excellent/good 88%</td>
</tr>
<tr>
<td>Federici et al. (2007)</td>
<td>Haemate-P</td>
<td>56/73 surgical procedures</td>
<td>retrospective</td>
<td></td>
<td>excellent/good 97%</td>
</tr>
<tr>
<td>Lethagen et al. (2007)</td>
<td>Haemate-P</td>
<td>29/27 surgical procedures</td>
<td>prospective</td>
<td>62 U kg⁻¹</td>
<td>excellent/good 96.3% PK</td>
</tr>
</tbody>
</table>

over a wide range of doses and that PK determinations can provide a reliable basis for serial dosing decisions. In this prospective study, 29 patients with severe VWD received a loading dose of 79 IU/kg 2 weeks prior to major surgery (fig. 5). From the in vivo recovery and PK in figure 5, a loading dose of 60–65 IU/kg was calculated as appropriate for successful hemostasis in these 29 VWD patients during subsequent elective surgery. On the day of surgery, hemostasis was indeed rated as excellent or good in 96%, being 100% on the first postoperative day [23].

There may be considerable differences in the relative concentration of VWF and FVIII and of the functional activity of VWF in the available VWF/FVIII concentrates (table 3) [18, 19, 21]. The dosing of a concentrate is dependent on the patients’ own basal level, and the nature and severity of the bleeding or the procedure. Successful hemostasis will be attained with a median VWF:RCo loading dose of 40–60 IU kg\(^{-1}\) in subjects with various types of VWD undergoing minor and major surgery, respectively [14–16, 21–30]. The median in vivo recovery of VWF:RCo was 1.9 IU·dl\(^{-1}\)·(IU·kg\(^{-1}\)) after the loading dose [15, 20]. Median half-lives of VWF:RCo and VWF:Ag may range from 10 to 25 h. Postoperative mean trough VWF:RCo levels of 60–80 IU dl\(^{-1}\) were sufficient to prevent bleeding in several studies.

VWF concentrate administration is usually repeated every 12–24 h postoperatively. A VWF concentrate can also be administered as a continuous infusion. Levels of VWF:RCo and FVIII:C should be monitored when treatment is protracted. During extended substitution with a purified VWF or VWF/FVIII concentrate, monitoring of FVIII:C and VWF parameters is mandatory aiming to keep peak FVIII:C levels in the upper range of normal and VWF:RCo levels just above the lower range of normal (>0.60 IU/ml). Long-lasting FVIII:C levels >1.5–2.0 IU/ml should be avoided due to the risk of thrombosis (fig. 3).

**Antifibrinolytic Agents**

Tranexamic acid is probably the most widely used antifibrinolytic agent in VWD. More adverse effects hamper the alternative antifibrinolytic, ε-aminocaproic acid. Tranexamic acid can be used either alone or minor procedures or in combination with DDAVP or a VWF concentrate, when mucous membranes are involved.

**Conclusion**

Surgical procedures can be safely performed in VWD patients receiving sufficient hemostatic treatment. DDAVP can be used for selected responders. When choosing a VWF concentrate, the relative content of FVIII and VWF, and the functional capacity of VWF must be considered. A PK test may help to tailor the preoperative loading dose of a VWF concentrate.

---

**References**


Causes, Etiology and Diagnosis of Acquired von Willebrand Disease: A Prospective Diagnostic Workup to Establish the Most Effective Therapeutic Strategies

Christoph Sucker a  Jan Jacques Michiels c  Rainer B. Zotz b

a LaboMed Coagulation Center, Berlin, and b Hemostasis Institute Düsseldorf, Düsseldorf, Germany; c Hemostasis and Thrombosis Research Center, Antwerp University Hospital, Edegem, Belgium, and Hemostasis Thrombosis Science Center, Goodheart Institute, Rotterdam, The Netherlands

Key Words
Acquired von Willebrand disease • Benign monoclonal gammopathy • Immunoglobulin • Systemic lupus erythematosus

Abstract
Acquired von Willebrand disease (aVWD) occurs in association with a variety of underlying disorders, most frequently in lymphoproliferative and myeloproliferative disorders, other malignancies, and cardiovascular disease. aVWD is a complex and heterogeneous defect with a multifactorial etiology and the pathophysiologic mechanisms remain unclear in many cases. Assays for anti-factor VIII (FVIII)/von Willebrand factor (VWF) activities often yield negative results although antibodies may be present in autoimmune disease and some lymphoproliferative disorders. Functional assays of VWF in patients’ plasma and particularly in heart valve disease, VWF multimer analysis are important for aVWD diagnosis. In patients with normal partial thromboplastin times and normal VWF activity, the diagnosis of aVWD is based on clinical suspicion and a careful bleeding history, which should prompt the clinician to initiate further laboratory investigations. Management of bleeding in aVWD relies mainly on desmopressin, FVIII/VWF concentrates and high-dose intravenous immunoglobulin. The half-life of VWF may be very short, and in bleeding episodes high doses of FVIII/VWF concentrates at short intervals may be necessary even when high-dose intravenous immunoglobulin was applied before. Since the optimal treatment strategy has not yet been defined for aVWD of different etiology, controlled multicenter trials aiming at the development of standardized treatment protocols are urgently needed.

Introduction

In 1968, Simone et al. [1] reported the first case of acquired von Willebrand disease (aVWD) in a 7-year-old child suffering from systemic lupus erythematosus (SLE). Since then, numerous case reports and large case series have been published regarding this entity and associations with a number of diseases such as hematologic disorders, solid tumors, autoimmune disorders, cardiac defects and the use of some therapeutic drugs have been reported [2–5]. To gain more knowledge on this rare entity, an international aVWD registry was founded and the first data obtained from 186 patients were published in 2000 [5]. According to the registry data, aVWD is most commonly associated with lymphoproliferative (48%) and myeloproliferative disorders (15%), neoplasia (5%), and immunological (2%), cardiovascular (21%) and miscellaneous disorders (9%). The registry data confirm the heterogeneous nature of aVWD. Due to the lack of epidemiologic studies, the prevalence of aVWD is currently unknown. It has...
been speculated that 5% of all patients with VWD suffer from the acquired variant of the disease. Some authors, however, reported an even higher percentage of up to 20% of acquired forms among all patients with VWD. Based on this assumption, a prevalence of aVWD of 0.04–0.2% in the general population can be assumed [6].

aVWD is clinically characterized by an acquired bleeding tendency that manifests in association with predisposing diseases and conditions. Similar to the congenital form of VWD, the bleeding pattern is dominated by bleeding of mucosal origin, with epistaxis, bleeding from oral mucosa, menorrhagia, hemotoma, and traumatic or perioperative bleeding complications being the most common symptoms [3]. In contrast to hemophilia, joint and muscle bleeding is not a characteristic of aVWD and argues against aVWD [2, 3].

In cases of acquired hemorrhage with predominating mucosal bleeding, aVWD should be considered and further laboratory procedures should be initiated. The laboratory diagnostic workup of aVWD has not yet been standardized. Reduced von Willebrand factor (VWF) activity, reduced VWF antigen (Ag) levels, a pathologic ratio of VWF activity to VWF:Ag levels and a reduction or absence of high-molecular-weight VWF multimers are often found in patients with aVWD [3, 7–9]. However, these assays are not useful to differentiate aVWD from congenital forms. Assays detecting VWF autoantibodies could play a future role, but have not yet been introduced in clinical practice [2, 10]. Increased clearance of VWF can be detected in kinetic studies, assessing the half-life of desmopressin (DDAVP)-liberated endogenous or exogenously administered VWF [8, 10]. In this approach, a significant reduction in the VWF half-life is suspicious for inhibitors directed against this plasma protein.

**Classification of aVWD**

Classification, diagnosis, mechanism of the VWF deficiency and treatment outcome in patients with aVWD largely depend on the underlying disorder or causative agent [2, 4, 8–10]. In cases of hypothyroidism, aVWD is typically a type I VWF deficiency due to decreased synthesis of the VWF protein. Treatment of hypothyroidism with thyroxine was associated with the disappearance of the aVWD and resolution of the bleeding diathesis [2, 11]. In children with Wilms tumor, aVWD is characterized by undetectable or very low levels of VWF:Ag and VWF: ristocetin cofactor activity (RCO) and a moderate deficiency in factor VIII (FVIII):coagulant activity (C) [2, 11].

Multimeric analysis of VWF shows a normal pattern consistent with VWD type I or type III. The absence of VWD in the family and the return of FVIII/VWF parameters to normal after chemotherapy and/or surgical removal of the Wilms tumor support that aVWD is causally related to the Wilms tumor. The etiology of aVWD in Wilms tumor remains elusive, but hyaluronic acid, secreted by nephroblastoma cells, may be responsible for the atypical variant of aVWD in Wilms tumor [11].

The laboratory features of aVWD associated with SLE and aVWD associated with IgG benign monoclonal gammopathy (BMG) are quite similar due to a common etiology of immune-mediated deficiency of the factor VIII/VWF complex, but they differ with regard to the response to corticosteroids [2, 10]. The similarities and differences of aVWD in SLE and IgG BMG are described in the following:

1. The pertinent laboratory findings of aVWD in SLE and IgG BMG are similar and characterized by a prolonged bleeding time, normal platelet count and a decreased or absent ristocetin-induced platelet aggregation together with a normal prothrombin time and prolonged activated partial thromboplastin time due to a combined FVIII:C and a type II VWF deficiency in most or type I VWF deficiency in a few.

2. Absence of FVIII:C inhibitor in the Bethesda assay is a prerequisite to exclude acquired hemophilia A due to an autoantibody against FVIII:C.

3. Inhibition of VWF:RCo or ristocetin-induced platelet aggregation in a mixture of patient plasma and normal platelet-poor or platelet-rich plasma, respectively, is sometimes detectable in aVWD associated with SLE but not in aVWD associated with IgG BMG.

4. Using a simple ELISA, an IgG antibody against VWF is detectable in aVWD associated with SLE or IgG BMG. In both of the associated diseases, IgG acts as an autoantibody that binds tightly to circulating FVIII/VWF (fig. 1). We could demonstrate that the IgG autoantibody was directed against the high VWF multimers (fig. 1). Accordingly, it was postulated that the IgG-autoantibody-FVIII/VWF complex thereby rapidly clears the large and intermediate VWF multimers from the circulation by the reticuloendothelial system. This concept can readily explain the combined FVIII:C and VWF deficiency and the poor responses of FVIII:C and VWF parameters to DDAVP and FVIII/VWF concentrate in aVWD associated with SLE or IgG BMG (fig. 2) [2, 8, 10].

5. A complete but temporary correction of FVIII:C and VWF parameters for one to a few weeks after high-
dose intravenous immunoglobulin is seen in immune-mediated aVWD associated with SLE or IgG BMG (fig. 1, 2) [10, 12]. A uniform good response to prednisone (corticosteroid) treatment with complete correction of the FVIII:C and VWF parameters together with the relief of SLE signs and symptoms was observed in aVWD combined with SLE, but not in aVWD associated with IgG BMG. No response to chemotherapy was observed in aVWD associated with IgG BMG (fig. 3) [10].

aVWD associated with IgM BMG is characterized by normal or slightly decreased FVIII:C and VWF:RCo levels, but with a pronounced deficiency in VWF:RCo consistent with type II aVWD on multimeric analysis. aVWD type II in IgM BMG does not respond to any conventional treatment including DDAVP, FVIII/VWF concentrate, high-dose intravenous immunoglobulin and very likely also not to chemotherapy [2, 8, 10].

The laboratory features of aVWD with spontaneous bleeding symptoms in cases of hemorrhagic thrombocytopenia [2, 13] are characterized by: a very high platelet count, prolonged bleeding time, normal FVIII:C and VWF:Ag concentration, low VWF:RCo and collagen binding (CB) activity, and absence of large and intermediate VWF multimers simulating a platelet type 2B VWD.

Correction of the platelet counts to normal (<400 × 10^9/l) is associated with complete correction of the VWF multimeric pattern and correction of all VWF parameters to normal values [2, 12]. A less common cause of aVWD has been observed in patients with cardiovascular disease. Aortic stenosis combined with bleeding from gastrointestinal angiodysplasia (Heyde syndrome) has recently been associated with a type 2-like aVWD characterized by normal FVIII:C and VWF:Ag, subnormal VWF:RCo and absence of large VWF multimers (type 2-like aVWD) [14–19]. Increased proteolysis of large VWF multimers secondary to high shear stress rates may be responsible for the type 2-like aVWD in patients with aortic stenosis. Strikingly, aortic valve replacement cures both the gastrointestinal bleeding and the aVWD defect. Surgical repair of the ventricular defect was followed by normalization of the VWF multimeric pattern. In a systematic investigation, Rauch et al. [20] observed a type 2-like aVWD in 4 of 12 children with persistent patent ductus arteriosis (PDA) [20]. After interventional PDA occlusion, the VWF multimers had normalized in all 4 with pathologic VWF, confirming the acquired nature of aVWD in PDA.

Drug-induced aVWD has been described in association with the use of valproic acid, ciprofloxacin, griseofulvin, tetracycline, pesticide, thrombolytic agents and
hydroxyethyl starch, and discontinuation of the causative agent resolves the VWF deficiency [2, 3].

In many cases of aVWD, standard laboratory assays like VWF:RCo, VWF:Ag or the VWF:RCo/Ag ratio have a low sensitivity and are not useful to differentiate aVWD from congenital forms. In a study evaluating 35 patients with recent onset of bleeding and abnormal plasma VWF multimers, PFA-100 was inconclusive, due to anemia or thrombocytopenia, in 29%. However, the sensitivity of PFA-100 was 80% in the remaining patients. In contrast, sensitivity of VWF:Ag (23%), VWF:RCo/Ag ratio <0.7 (26%), VWF:CB/Ag ratio <0.7 (46%), anti-VWF antibodies (15%) and VWF propeptide/Ag ratio (22%) was too low to rule out the disease. A combination of VWF:Ag <50 IU/dl, VWF:RCo/Ag ratio <0.7 and VWF:CB/Ag ratio <0.8 yielded a sensitivity of 86% [21]. Patients diagnosed only because of abnormal VWF multimers showed similar clinical characteristics as other patients. Early diagnosis of aVWD is difficult due to the low sensitivity of the tests used. A substantial number of patients present with normal or increased test results, emphasizing the importance of multimer analysis in all patients with suspected aVWD.

Pathophysiology of aVWD

As reflected by the heterogeneous etiology of aVWD, the pathogenesis of this disorder is complex and nonuniform. Related to the underlying disorder or condition, a number of pathogenetic mechanisms are involved.

1) Autoantibodies directed against VWF are a frequent cause of aVWD in patients with IgG BMG or autoimmune disorders (fig. 1) [2, 8, 10]. They can be directed either against functional or non-functional domains of the VWF. Consequently, VWF autoantibodies may induce a functional impairment in plasma VWF but usually lead to reduced FVIII and VWF levels due to the formation of Ag-antibody complexes that are rapidly cleared from the circulation (fig. 2). Whereas in-

---

Fig. 2. Poor response to Haemate-P and DDAVP and good response to high-dose intravenous IgG (IVIG) in a case of aVWD with IgG BMG [10].
hibitory autoantibodies can be detected by mixing studies with normal human plasma, increased VWF clearance can only be detected by kinetic studies determining the half-life of administered VWF/VIII:C concentrates or the half-life of endogenous VWF upon stimulated release through DDAVP application (fig. 2). ELISA assays directly detecting VWF autoantibodies have been occasionally introduced in clinical practice, improving the diagnostic workup and demonstrating the autoimmune etiology of aVWD (fig. 1) [2]. ELISA techniques, however, might be compromised by false positive results due to natural antibodies against blood group antigens bound to VWF.

Autoantibodies directed against VWF are preferentially found in aVWD due to hematologic disorders, particularly monoclonal gammopathies of different causes (including multiple myeloma and BMG of undetermined significance). In these cases, multimeric analysis often shows a significant reduction or even loss of high-molecular-weight VWF multimers, resembling the pattern of inherited VWD type 2A. In addition, inhibitors of VWF may also be the cause of aVWD found in association with SLE and other autoimmune disorders [2, 8, 10].

Adsorption of VWF to malign cells can also cause aVWD. This pathomechanism may be relevant in some cases associated with hematologic disorders and solid tumors. As demonstrated, the tumor cells may show an aberrant expression of adhesion molecules that bind VWF and reduce its concentration in the blood.

Proteolysis of VWF can cause aVWD. In the presence of thrombocytosis, particularly in patients with essential thrombocythemia, increased activity of platelet proteases (calpains) can lead to abnormal cleavage of the VWF and induce a bleeding tendency. In the presence of cardiac defects, high shear stress can lead to a conformational change in VWF, making this adhesive protein more susceptible to proteolytic cleavage. Consequently, enhanced VWF proteolysis by ADAMTS13 can result in a significant reduction in hemostatically most competent highest-molecular-weight VWF multimers, inducing a bleeding tendency [14, 16]. Among others, this pathomechanism has been suggested as a link between aortic valve stenosis and gastrointestinal bleeding, termed Heyde syndrome [18].

In rare cases, reduced VWF synthesis has been proposed as a pathogenic factor in aVWD, particularly in

Fig. 3. No response to FVIII concentrate, prednisone, Cytoxan and rituximab and good response to intravenous high-dose IgG (IVIG) in a case of aVWD with IgG BMG (source: Moll [23]).
cases associated with hypothyreosis and antiepileptic treatment with valproic acid.

Due to the scarcity of clinical studies, the therapy of aVWD has not yet been standardized. Since this bleeding disorder is a secondary complication due to predisposing conditions, successful treatment of the underlying disease or cessation of causative medication can lead to remission of aVWD and, thus, absence of bleeding. However, given the association with hematologic and solid malignancies, the causal treatment options are often limited. As symptomatic measures, DDAVP and VWF-containing concentrates have been used for the treatment of bleeding episodes in patients with aVWD. However, these agents are mostly not efficient, due to the shortened half-life of transfused or DDAVP-liberated endogenous VWF. In these cases, immunoglobulins and immunosuppressive agents (such as azathioprine, cyclophosphamide and steroids) and, in rare cases, immunoadsorption can be used for titer reduction or elimination of autoantibodies against the VWF. Finally, off-label use of recombinant activated factor VII (rFVIIa, NovoSeven®) has been successfully used in patients with aVWD and could be a promising option if other therapeutic measures fail [22].

Conclusion

Due to the low prevalence and heterogeneity of the disease, no single center will be able to characterize and evaluate symptoms, laboratory features and different treatment options in a large number of patients. Plasma samples from clinically well-characterized patients should be stored centrally (with informed consent of the patient) to be provided to scientists worldwide to develop and evaluate new laboratory assays. To improve the management of aVWD, it is recommended to register all cases in the international registry on aVWD (http://www.intreavws.com). A prospective study is needed to establish the most effective therapy.

References

ADAMTS13 in Health and Disease

Hendrik B. Feys  Hans Deckmyn  Karen Vanhoorelbeke
Laboratory for Thrombosis Research, Interdisciplinary Research Center, Catholic University of Leuven, Campus Kortrijk, Kortrijk, Belgium

Key Words
ADAMTS13  ·  ADAMTS13 activity test  ·  Collagen binding assay  ·  Microthrombus formation  ·  Thrombocytopenia

Abstract
We raised a set of monoclonal antibodies (mAb) against recombinant human ADAMTS13, constructed the first antigen test fully based on mAbs and compared ADAMTS13 antigen and enzymatic activity levels in a large set of plasma samples collected from different patients and healthy controls. Assessing both ADAMTS13 antigen and activity helps to understand whether or not the protease is fully active in pathological conditions, e.g. liver cirrhosis, inflammatory bowel disease, cardiac surgery, pregnancy and oral contraceptive intake, in the neonatal state and in normal individuals. Our ADAMTS13 antigen assay showed less variability than the collagen binding-based activity assay. Antigen values correlated well with activity in normal individuals, but differed to various degrees in neonates, pregnancies at later maternal age and cardiac surgery. No discrepancies were noted in liver cirrhosis and inflammatory bowel disease, which were both associated with low plasma levels of ADAMTS13. In conclusion, parallel measurement of ADAMTS13 activity and antigen provides a new tool for understanding the behavior of the VWF-cleaving protease in health and disease.

Thrombotic thrombocytopenic purpura (TTP) is a rare disease with an annual incidence of approximately 3.7 cases per million in the USA (registered from 1968 to 1991) [1]. Symptoms include severe thrombocytopenia with platelet counts <20,000/μl and hemolytic anemia with fragmented red blood cells (schistocytes) in peripheral blood smear. In some cases, other symptoms like global or focal ischemic neurological signs, fever and/or renal dysfunction are prominent. Hereditary TTP is very rare and usually sets in during infancy or childhood, although patients with later onset have been reported [2]. Patients with hereditary TTP exhibit a chronic relapsing course and respond well to plasma infusion treatment.

Acquired TTP (aTTP) is the most common form of the disease and mostly occurs in older children and adults. In aTTP patients, the relapse rate is high and can occur several years after having suffered a first bout. Most patients with aTTP respond well to plasma exchange treatment, although some patients are refractory to treatment [3–5]. Since aTTP is almost always autoimmune mediated, patients refractory to plasma exchange are often treated with immunosuppressive agents and if necessary splenectomy.

The pathologic hallmark of TTP is the occurrence of platelet- and von Willebrand factor (VWF)-rich microthrombi in the small vessels of multiple organs [3–5]. VWF is a large multimeric plasma glycoprotein that is
synthesized in megakaryocytes and endothelial cells. It plays a major role in primary hemostasis serving as the first adhesive link between platelets and damaged vessel wall. Moreover, it indirectly contributes to coagulation by stabilizing coagulation factor VIII. In endothelial cells, VWF is either constitutively secreted or stored in Weibel-Palade bodies. In response to stimuli, VWF is released from its storage granules in the form of ultralarge (UL) multimers, sizing up to 20,000 kDa and exhibiting outspoken adhesiveness to glycoprotein Ib-IX [6]. These UL multimers are normally not found in the circulation since they are rapidly proteolyzed into smaller, less reactive multimers upon release from endothelial cells. This process is catalyzed by the plasma metalloprotease ADAMTS13 (a disintegrin-like metalloprotease with thrombospondin type 1 motif 13) [7] which targets a specific peptide bond located between Tyr1605-Met1606 [8] in the VWF A2 domain.

A first link between VWF proteolysis and TTP pathogenesis was proposed in 1982 by Moake et al. [9], who observed 'unusually' large VWF multimers in plasma

Table 1. ADAMTS13 activity, antigen and VWF antigen (VWF:Ag) in physiological and pathological conditions

<table>
<thead>
<tr>
<th>Condition</th>
<th>n</th>
<th>VWF:Ag</th>
<th>ADAMTS13 activity, %</th>
<th>ADAMTS13 antigen, %</th>
<th>Activity/antigen ratio</th>
</tr>
</thead>
<tbody>
<tr>
<td>Healthy individuals</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>&lt;35 years</td>
<td>33</td>
<td>118 ± 35***</td>
<td>117 ± 24</td>
<td>107 ± 17</td>
<td>1.10 ± 0.20</td>
</tr>
<tr>
<td>36–50 years</td>
<td>35</td>
<td>140 ± 54</td>
<td>110 ± 30</td>
<td>106 ± 19</td>
<td>1.05 ± 0.25</td>
</tr>
<tr>
<td>51–65 years</td>
<td>32</td>
<td>142 ± 46</td>
<td>113 ± 32</td>
<td>104 ± 19</td>
<td>1.10 ± 0.30</td>
</tr>
<tr>
<td>&gt;65 years</td>
<td>32</td>
<td>149 ± 41</td>
<td>86 ± 20**</td>
<td>90 ± 15*</td>
<td>0.98 ± 0.24</td>
</tr>
<tr>
<td>Neonates</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Full term</td>
<td>41</td>
<td>112 ± 27</td>
<td>43 ± 20*</td>
<td>73 ± 9*</td>
<td>0.59 ± 0.25*</td>
</tr>
<tr>
<td>Pregnancy</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>3rd trimester</td>
<td>42</td>
<td>290 ± 102*</td>
<td>85 ± 36**</td>
<td>92 ± 14**</td>
<td>0.90 ± 0.32***</td>
</tr>
<tr>
<td>Oral contraceptive intake</td>
<td>33</td>
<td>141 ± 42</td>
<td>103 ± 26</td>
<td>97 ± 23</td>
<td>1.09 ± 0.26</td>
</tr>
<tr>
<td>Liver cirrhosis</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Child A</td>
<td>33</td>
<td>295 ± 80*</td>
<td>93 ± 41***</td>
<td>94 ± 35</td>
<td>0.99 ± 0.29</td>
</tr>
<tr>
<td>Child B</td>
<td>32</td>
<td>319 ± 108*</td>
<td>89 ± 34**</td>
<td>90 ± 32**</td>
<td>1.05 ± 0.33</td>
</tr>
<tr>
<td>Child C</td>
<td>25</td>
<td>442 ± 262*</td>
<td>67 ± 38*</td>
<td>63 ± 35*</td>
<td>1.13 ± 0.39</td>
</tr>
<tr>
<td>Inflammatory bowel disease</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>C-reactive protein &lt; 1</td>
<td>32</td>
<td>139 ± 49</td>
<td>99 ± 29</td>
<td>97 ± 15</td>
<td>1.04 ± 0.33</td>
</tr>
<tr>
<td>C-reactive protein &gt; 1</td>
<td>12</td>
<td>167 ± 66***</td>
<td>82 ± 28**</td>
<td>82 ± 20**</td>
<td>1.02 ± 0.33</td>
</tr>
<tr>
<td>Cardiac surgery</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Baseline</td>
<td>30</td>
<td>146 ± 64</td>
<td>80 ± 24*</td>
<td>95 ± 15</td>
<td>0.87 ± 0.21*</td>
</tr>
<tr>
<td>During surgery</td>
<td>30</td>
<td>174 ± 65***</td>
<td>53 ± 21*</td>
<td>67 ± 14*</td>
<td>0.81 ± 0.34*</td>
</tr>
<tr>
<td>After 4 days</td>
<td>30</td>
<td>350 ± 95*</td>
<td>48 ± 15*</td>
<td>72 ± 14*</td>
<td>0.71 ± 0.31*</td>
</tr>
</tbody>
</table>

All values are expressed as a percentage of normal pooled human plasma. Results are given as means ± SD. For each condition and measurement, statistically significant differences are shown: * p < 0.001, ** p < 0.01, *** p < 0.05, vs. normal healthy controls. Printed with permission (British Journal of Haematology, Wiley-Blackwell [15]).

Fig. 1. ADAMTS13 antigen determination using an mAb-based immunoassay. mAb 2G3 was coated on a microtiter plate. Different plasma dilutions of normal human plasma (NHP), plasma of a congenital TTP patient (cTTP) and plasma of an aTTP patient were added. Bound ADAMTS13 was detected using two biotinylated anti-ADAMTS13 mAbs followed by addition of streptavidin-HRP. Colorimetric development was with o-phenylenediamine dihydrochloride and H$_2$O$_2$. synthesized in megakaryocytes and endothelial cells. It plays a major role in primary hemostasis serving as the first adhesive link between platelets and damaged vessel wall. Moreover, it indirectly contributes to coagulation by stabilizing coagulation factor VIII. In endothelial cells, VWF is either constitutively secreted or stored in Weibel-Palade bodies. In response to stimuli, VWF is released from its storage granules in the form of ultralarge (UL) multimers, sizing up to 20,000 kDa and exhibiting outspoken adhesiveness to glycoprotein Ib-IX [6]. These UL multimers are normally not found in the circulation since they are rapidly proteolyzed into smaller, less reactive multimers upon release from endothelial cells. This process is catalyzed by the plasma metalloprotease ADAMTS13 (a disintegrin-like metalloprotease with thrombospondin type 1 motif 13) [7] which targets a specific peptide bond located between Tyr1605-Met1606 [8] in the VWF A2 domain.

A first link between VWF proteolysis and TTP pathogenesis was proposed in 1982 by Moake et al. [9], who observed 'unusually' large VWF multimers in plasma.
samples taken repeatedly from 4 patients with chronic relapsing TTP. It was hypothesized that these UL-VWF multimers spontaneously form platelet- and VWF-rich thrombi characteristic of TTP. The patients were believed to have a defective VWF ‘depolymerase’. In 1996, it was found eventually that VWF was proteolytically cleaved. However, in laboratory conditions, proteolysis could only be achieved under slightly denaturing conditions or in the presence of high shear stress [10, 11]. Finally, in 2001, several independent groups could identify this VWF-cleaving protease as ADAMTS13 [7, 12, 13].

ADAMTS13 deficiency is found in most patients with TTP and is thought to be indirectly causing platelet aggregation and microthrombus formation in the circulation leading to thrombotic microangiopathy. It is also possible that a secondary deficiency in ADAMTS13 may account for the development of microthrombi in non-TTP diseases. Many studies have therefore aimed to determine links between ADAMTS13 deficiency and primary disease states or physiological conditions.

We have raised a set of monoclonal antibodies (mAbs) against recombinant human ADAMTS13 and subsequently constructed the first antigen test fully based on mAbs (fig. 1) [14]. Next we compared ADAMTS13 antigen and enzymatic activity levels in a large set of plasma samples collected from healthy controls and different patients, most of them being associated with an increased tendency to thrombosis [15]. Measuring both ADAMTS13 antigen [14] and activity helps to understand whether or not the protease is fully active. Pathological conditions included liver cirrhosis (n = 90), inflammatory bowel disease (n = 44) and cardiac surgery (n = 30). Non-pathological conditions were pregnancy (n = 42), oral contraceptive intake (n = 33) and neonatal state (n = 41). Normal individuals of different ages were used as controls (n = 132). Our antigen assay showed less variability than the collagen binding-based activity assay [15]. Antigen values correlated well with activity in normal individuals, but differed to various degrees in neonates, pregnancy at later maternal age and cardiac surgery. No discrepancies were noted in liver cirrhosis and inflammatory bowel disease, which were both associated with low plasma levels of ADAMTS13 (table 1). The parallel measurement of ADAMTS13 activity and antigen provides a new tool for understanding the behavior of the VWF-cleaving protease in health and disease [15].

References

Author Index Vol. 121, No. 2–3, 2009

Batlle, J. 139
Berneman, Z. 71, 85, 111, 119, 128, 145, 167
Bowen, D.J. 98
Castaman, G. 106
Collins, P.W. 98
Davies, J.A. 98
Deckmyn, H. 71, 183
Feys, H.B. 183
Gadisseur, A. 71, 85, 111, 119, 128, 145, 167
Giacomelli, S. 106
Hathaway, L.S. 98
Hermans, C. 71, 139
Jacquemin, M. 102
Michiels, J.J. 71, 85, 111, 119, 128, 145, 154, 167, 177
Rodeghiero, F. 106
Schroyens, W. 71, 85, 111, 119, 128, 145, 167
Sucker, C. 177
van der Planken, M. 85, 111, 145
van Vliet, H.H.D.M. 85, 154, 167
Vangenegten, I. 119
Vanhoorelbeke, K. 183
Zotz, R.B. 177

Subject Index Vol. 121, No. 2–3, 2009

Acquired von Willebrand disease 177
ADAMTS13 98, 183
– activity test 183
Autosomal recessive disease 106
Benign monoclonal gammopathy 177
Bleeding severity 167
Blood group O 85, 98
C1584 98
– mutation 85
C2362F 106
Classification of von Willebrand disease 71
Collagen binding 145
– assay 183
D1/D2 domain 111
Desmopressin 102, 167
– response 106, 139
Dominant disease 71
Double heterozygous mutations 111
Factor VIII binding 102
– clearance 119
Genotypes 128
Hemophilia A 102
Immunoglobulin 177
International Society on Thrombosis and Haemostasis 71
Laboratory parameters 71
Management of bleeding 154
Microthrombus formation 183
Mild hemophilia 119
Molecular classification 71
Multimeric patterns 106, 139
Normandy 102, 119
Pharmacokinetic studies 167
Phenotype(s) 128, 139
Platelet adhesion 139
Prolonged bleeding time 154
Propeptides 128
Prophylactic treatment 154
Proteolysis 98
Recessive disease 71
Ristocetin cofactor activity 145
Ristocetin-induced platelet aggregation 145
Subtype IIC 111
Systemic lupus erythematosus 177
Thrombocytopenia 183
Trauma 167
Treatment of bleeding 154
Type Vicenza 85
Type I Vicenza 128
– 2A 111
– 2M 139
– 2N 119
von Willebrand disease 128, 139, 145
– type I 85
– 2A 154
– 2C 111
– 2N 102, 119
– 3 106
– factor 71, 145
– multimer 85
– replacement 154
– /factor VIII concentrates 167
VWF A1 domain 145
– multimers 145
Y/C1584 98