

# Characterization of Recessive Severe Type 1 and 3 von Willebrand Disease (VWD), Asymptomatic Heterozygous Carriers Versus Bloodgroup O-Related von Willebrand Factor Deficiency, and Dominant Type 1 VWD

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**Summary:** Recessive type 3 von Willebrand disease (VWD) is caused by homozygosity or double heterozygosity for two non-sense mutations (null alleles). Type 3 VWD is easy to diagnose by the combination of a strongly prolonged bleeding time (BT), absence of ristocetine-induced platelet aggregation (RIPA), absence of von Willebrand factor (VWF) protein, and prolonged activated partial thromboplastin time (aPTT) due to factor VIII:coagulant (FVIII:C) deficiency. VWD type 3 is associated with a pronounced tendency to mucocutaneous and musculoskeletal bleedings since early childhood. Carriers of one null allele are usually asymptomatic at VWF levels of 50% of normal. Recessive severe type 1 VWD is caused by homozygosity or double heterozygosity for a missense mutation. Recessive type 1 VWD differs from type 3 VWD by the presence of detectable von Willebrand factor: antigen VWF:Ag and FVIII:C levels between 0.09 and 0.40 U/mL. Patients with recessive type 1 VWD show an abnormal VWF multimeric pattern in plasma and/or platelets consistent with severe type 2 VWD. Carriers of a missense mutation may have mild bleeding and mild VWF deficiency and can be diagnosed by a double VWF peak on cross immunoelectrophoresis (CIE). There will be cases of mild and moderate recessive type 1 VWD due to dou-

ble heterozygosity of two missense mutations, or with the combination of one missense mutation with a non-sense or bloodgroup O. Mild deficiency of VWF in the range of 0.20 to 0.60 U/mL, with normal ratios of von Willebrand factor: ristocetine cofactor/antigen VWF:RCo/Ag and VWF:collagen binding/antigen (VWF:CB/Ag), normal VWF multimers, and a completely normal response to desmopressin acetate (DDAVP) with VWF level rising from below to above 1.00 U/mL are very likely cases of so-called pseudo-VWF deficiency in individuals with normal VWF protein and gene. Autosomal dominant type 1 VWD variants are in fact type 2 variants caused by a heterozygous missense mutation in the VWF gene that produces a mutant VWF protein that has a dominant effect on normal VWF protein produced by the normal VWF allele with regard to the synthesis, processing, storage, secretion, and/or proteolysis of VWF in endothelial cells. A DDAVP challenge test clearly differentiates between dominant type 1 VWD phenotype and dominant type 2 M VWD.

**Key Words:** von Willebrand factor—von Willebrand disease—Ristocetin cofactor activity—von Willebrand collagen-binding activity—Factor VIII:C—Bleeding time—DDAVP.

The von Willebrand factor (VWF) is a multi-meric plasma glycoprotein that plays a central

role in hemostasis. VWF acts both as carrier for coagulation factor VIII in the plasma and as a mediator of platelet adhesion to subendothelium after vascular injury. A number of distinct functional domains have been identified within the VWF, including regions involved in binding to factor VIII, to platelet receptor GPIb, to platelet GP IIb-IIIa, to components of extracellular matrix such as collagen and heparin, regions involved in multimerization and dimerization of VWF, and the A1 and A2 domains involved in the

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majority of type 2 von VWF defects (1). The classification of congenital von Willebrand disease (VWD) as recommended by the VWF Scientific Standardization Committee (VWF-SSC) of the International Society on Thrombosis and Haemostasis (ISTH) is based on laboratory phenotyping using the combination of factor VIII:C and VWF: antigen (VWF:Ag) levels, ristocetine-induced platelet aggregation (RIPA), and rather insensitive tests for VWF: ristocetine cofactor (VWF:RCo) and VWF multimeric pattern in a low-resolution agarose gel (2). In the last decade, accumulating data on the structure and function relationship between laboratory phenotype and expression VWF gene mutations, improved tools, including VWF: collagen binding (VWF:CB) assay and VWF multimeric analysis in high-resolution agarose gels. The contribution of a desmopressin acetate (DDAVP) challenge test has become available for clinicians to better distinguish and treat the various type 1 and type 2 VWDs. In this study, we have evaluated the clinical features, laboratory phenotypes, and genotypes of severe autosomal recessive type 3 and 1 von Willebrand disease (VWD), asymptomatic carriers of a nonsense or missense mutation in the VWF gene, bloodgroup O-related VWF deficiency, and mild or moderate dominant type 1 VWD.

### Diagnosis and Classification of Type 1 and 3 VWD Patients

For the categorization of type 1 and 3 VWD according to the SSC/ISTH 1993 the following considerations have to be taken into account

1. Inherited VWD is caused by genetic mutations at the VWF locus.
2. Type 1 VWD refers to partial quantitative deficiency of VWF, type 2 VWD refers to qualitative deficiency of VWF, and type 3 refers to virtually complete deficiency of VWF.
3. Using better laboratory tools like high-resolution VWF multimeric analysis and more sensitive functional assays like VWF collagen-binding assay (VWF:CB), in view of new data on structure, function, and VWF gene defects, the "splitting" approach is clearly superior in elucidating specific variants of VWD and to differentiate type 1 from type 2 VWD.
4. Using molecular tools, the "splitting" approach will surely elucidate the differences between autosomal recessive type 3 and severe type 1 VWD, and will clarify much better the clinical relevance and therapeutic implications of the different type 1 and type 2 VWD patients.

### A Simple Scoring System for Severity of Bleeding Symptoms

Experienced clinical coagulation-oriented hematologists can easily assess the degree of an acquired or congenital bleeding tendency by using a simple scoring system for bleeding severity in patients with congenital VWD according to Zhang et al (3) and Eikenboom et al (4):

*Very mild:* The patient has only one or two unclear minor bleeding symptoms without bleedings after tooth extraction or surgery.

*Mild:* The patient has one or two obvious mucocutaneous symptoms, i.e, frequent episodes of epistaxis, and/or prolonged or profuse menstruation or frequent hematomas, which usually do not require medical treatment or FVIII/VWF concentrate treatment.

*Moderate:* A congenital bleeding tendency is usually recognized since early childhood. The patient has recurrent mucocutaneous more than two bleedings after tooth extraction, trauma, or surgery and bleedings that needed medical treatment and/or factor VIII/VWF (FVIII/VWF) concentrate transfusion because of abnormal bleeding after an operation and/or trauma, or has bleed for more than 24 hours after a tooth extraction. In patients with congenital VWD,

*Severe:* The patient has mucocutaneous bleeding since early childhood plus, hemarthrosis, muscle bleeding, and a need for prophylactic treatment with FVIII/VWF concentrate that refers to a pseudo-hemophilia bleeding type, which is usually seen in type 3 VWD.

### Laboratory Methods to Diagnose and Phenotype VWD

Bleeding times (BT) are usually measured according to Ivy (5) or a standardized template device, mainly Simplate (6). BT measurements have to be performed preferentially by experienced technicians in reference coagulation laboratories. Ivy and Simplate BT are recorded as normal; prolonged between normal and 15, 20, or 30 minutes; and strongly prolonged in excess of 15, 20, or 30 minutes. Platelets are counted in ethylene-diamine-tetra acid (EDTA) blood samples using a Platelet Analyzer. Platelet aggregation induced by collagen, adenosine diphosphate (ADP), epinephrine, and ristocetine-induced platelet aggregation (RIPA) is performed by using various aggregometers according to standard procedures to exclude platelet function abnormalities.

The PFA-100® (platelet function analyzer) is a sensitive tool for the investigation of primary hemostasis. The PFA-100 uses whole blood flow through a capillary device to mimic high shear-stress conditions that occur *in vivo* in the end-arterial microvasculature. The PFA-100 measures the closure time when high-shear blood flows through membrane cartridges coated with platelet agonists and ceases as a result of platelet adhesion and aggregation. There are two cartridge types available; both utilize a membrane coated with collagen (C: fibrillar type 1 equine tendon) in addition, one cartridge type is coated with ADP (C/ADP) and the other with epinephrine (C/Epi). The two cartridges have slightly differing sensitivities to test variables and accordingly yield slightly different normal ranges. Any value of closure times above 250–300 seconds (s) can be considered as maximally prolonged, and values between the upper limit of normal and 250 s as prolonged.

Both BT and PFA-100 are global test systems used for screening of platelet and VWF disorders, but they are not specific for, or predictive of, any congenital or acquired platelet or VWF dysfunction. Once the diagnosis of VWD is made, BT and PFA-100 can be used to estimate the severity of bleeding tendency and for therapeutic monitoring of bleedings.

Laboratory methods to diagnose von Willebrand disease include factor VIII: coagulant (FVIII:C), VWF:Ag, VWF:RCo, VWF:CB, low- and high-resolution VWF multimeric analysis, and the result of a DDACP challenge test. Factor VIII coagulant (FVIII:C) activity is assayed by means of automatic coagulation machines using FVIII-deficient plasma. VWF:Ag can be measured by a variety of immunologic methods. The Laurel rocked electrophoresis has been replaced widely by an enzyme-linked immunosorbent assay (ELISA) (7,8). The VWF:RCo is determined either by agglutination of fixed platelets measured in an aggregometer or by a macroscopic slide technique (8). The various VWF:RCo tests are quantitative and allow identification of qualitative VWF abnormalities because the ability of ristocetine to influence the VWF interaction with platelet membrane GPIIb/IIIa depends on its multimerization. The VWF:RCo is rather insensitive to measure levels lower than 0.10 U/mL owing to an unacceptable degree of interassay and interlaboratory variability. The VWF:RCo and VWF:CB assays are complementary to assess the functional intactness or defects of the VWF in type 1 and type 2 VWD, respectively. There is

also very good evidence that the VWF:CB assay using equine collagen type 1<sup>3</sup> or a type I/III (95%/5%) mixture (9–14) is much more sensitive than the VWF:RCo for the measurement of the hemostatic more potent high VWF multimers present in plasma and FVIII/VWF concentrates.

The multimeric composition of VWF in most studies is usually analyzed by sodium dodecyl sulfate (SDS) agarose gel electrophoresis. A low-resolution gel (0.6 to 1.2% agarose) has been used as the first-line method because it clearly differentiates between loss of the largest multimers, the presence of supranormal multimers, and normal samples but has limited power for the relative loss of large multimers and to detect the different banding patterns in various type 2 VWD patients. The medium-resolution gels (1.4 to 2.0% agarose), followed by quantitative evaluation by densitometry, which show the presence or absence of multimers (including supranormal), together with the triplet structure of individual multimers, are currently the basis of proper laboratory phenotyping and classification of type 1 and its differentiation from various specific type 2 variants of VWD patients (8,15,16).

Laboratory phenotyping of VWD is based on measurements of FVIII:C, VWF:Ag, VWF:RCo, VWF:CB, RIPA, and analysis of VWF multimeric pattern to classify VWD according to established criteria (2,8,16,17). VWD type 1 is a quantitative VWF deficiency with equally decreased values of all VWF parameters (<0.60 U/mL) and a normal ratio of about 1 for VWF:RCo/Ag and VWF:CB/Ag. 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 a decreased ratio for both VWF:RCo/Ag and VWF:CB/Ag (<0.60). Type 2 can easily be subclassified as 2A by a normal or decreased RIPA and absence of large VWF multimers, as type 2B by increased RIPA and absence of large VWF multimers, and as 2M by decreased or zero RIPA and presence of most of the large VWF multimers.

By definition, the concentration in plasma of FVIII:C and VWF:Ag is 1 U/mL (18). Consequently the ratio FVIII:C/VWF:Ag in normal plasma is by this definition 1 in all normal individuals with blood group O and non-O (18). In homozygous or double heterozygous type 2N VWD, the ratio FVIII:C/VWF:Ag is decreased owing to a FVIII:C binding defect of the VWF protein. The ratio of FVIII:C to VWF:Ag on a molecular basis is 1:50, indicating that many potential FVIII:C

binding sites on VWF:Ag are free. As the VWF:Ag is 50% of normal owing to decreased biosynthesis in quantitative VWD type 1 heterozygous for VWF null alleles, the ratio of FVIII:C/VWF:Ag will increase to about 2 (18). As demonstrated by Eikenboom et al (18), VWD type 1 heterozygous for the VWF null allele indeed has a ratio of 2.06 for FVIII:C/VWF:Ag, and this ratio appears 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 of <30, between 30–60, and above 60 U/mL (18).

The protocol for DDAVP challenge testing can be performed in the outpatient setting after informed consent and counseling of the VWD patient by the responsible nurse and physician. DDAVP 4 ug/mL is dissolved in sterile saline to a total volume of 50 mL and infused over a 30-minute period at a dose of 0.3 ug/kg. Measuring the BT, FVIII:C, and all VWF parameters before DDAVP (t=0), and at time points 0.25, 1, 2, 4, 6, and 12 hours postinfusion of DDAVP, allows a

much better characterization of the various variants of type 1 and type 2 VWD based on the type of response and calculation of the biological half-life times of FVIII:C and the VWF parameters (17).

### Autosomal Recessive Type 3 VWD and Asymptomatic Heterozygous Type 1 “VWD”

The inheritance of VWD type 3 is autosomal recessive (3,19,20). Patients with type 3 VWD typically have strongly prolonged BT and aPTT; FVIII:C levels between 1% and 9%; undetectable VWF:Ag, VWF:Ag, and VWF:CB levels; and absence of RIPA (3,19–22). In 31 cases with type 3 VWD, (ages 2 to 80, median 15 years) described by Schneppenheim et al (19), bleeding manifestations were recorded as easy bruising and prolonged epistaxis in 31 (100%), spontaneous joint bleedings in 23 (74%), muscle bleedings in seven (22%), and gastrointestinal bleedings in three (10%). As shown in Table 1, the bleeding manifestations and complications of childbirth have

**TABLE 1.** Bleeding Manifestations and Complications of Childbirth in 385 Iranian Patients with Type 3 VWD22

	Type 3 VWD	Hemophilia A
FVIII:C	1% to 9%	1%
VWF:Ag	Undetectable	Normal
VWF:RCo	Undetectable	Normal
RIPA	Absent	Normal
Bleeding time	Strongly prolonged	Normal
Number of patients (pts)	385	100
	Age 2–72	Age matched
Type of bleedings:		
Spontaneous hemarthroses	37%	86%
Spontaneous muscle bleeding	52%	93%
Epistaxis >10 minutes	77%	20%
Oral cavity bleeding after tooth extraction or trauma or laceration of tongue and lip	70%	64%
Menorrhagia usually controlled by oral contraceptives in women	69%	—
Gastrointestinal bleeding	20%	10%
Postoperative bleeding in 205, who underwent surgery	42%	36%

FVIII:C = Factor VIII coagulant deficiency, VWF:Ag = VWF: antigen, VWF:RCo = VWF: ristocetine cofactor, RIPA = ristocetine-induced platelet aggregation.

been nicely evaluated by Lak et al (21) in 385 Iranian patients with autosomal recessive type 3 VWD as compared to age-matched severe hemophilia A. Among patients with type 3 VWD the incidences of spontaneous hemarthroses (37%) and muscle bleedings (52%) were lower, very likely because FVIII:C levels were higher (1% to 9%) as compared to severe hemophilia A (1%). Patients with type 3 VWD are homozygous or compound heterozygous for two null alleles (gene deletions, stop codons, frame shift mutations, splice site mutations, and absence of messenger RNA (mRNA) in the vast majority of reported cases (3,19,20). Compound heterozygosity for a null allele and a missense mutation or homozygosity for a missense mutation are rare in type 3 VWD (19).

The only objective and correct way to characterize true type 1 VWF deficiency heterozygous for the VWF null allele is to analyse the bleeding manifestations and FVIII:C/VWF parameters in obligate heterozygous parents of patients with type 3 VWF. In the study of 27 patients with congenital type 1 VWF deficiency associated with one null allele analyzed by Schneppenheim et al

(19), 22 were asymptomatic and only seven presented very mild bleeding, mainly bruising and epistaxis. All, except one had normal BT. The mean values for FVIII:C, VWF:Ag, and VWF:RCo were 0.76, 0.39, and 0.39 U/mL, respectively, with an increased FVIII:C/VWF:Ag ratio of 1.9 and a normal VWF:RCo/Ag ratio of 1 consistent with true type 1 VWF deficiency (Table 2). In the study of Zhang et al (3) including 25 patients heterozygous for the VWF null allele and blood group non-O, 12 had no history of bleeding and 13 presented with very mild bleedings (one or two bleeding symptoms, mainly epistaxis, bruises, and/or prolonged menstruations with no abnormal bleeding after tooth extraction). The mean values for FVIII:C and VWF:Ag were 0.81 and 0.45, respectively, with an increased ratio for FVIII:C/VWF:Ag of 1.8 (Table 2). In the same study of Zhang et al (3) including 17 patients heterozygous for the VWF null allele and blood group A, eight had no bleeding history and 11 presented minor bleedings. The mean values for FVIII:C and VWF:Ag were 0.74 and 0.32, respectively, with an increased ratio for FVIII:C/VWF:Ag of 2.3 (Table 2). In each of these two

**TABLE 2.** Laboratory Phenotype and Clinical Symptoms in 69 Patients with True VWF Deficiency Type 1 Heterozygous for the VWF Null Allele (Parents of Type III VWD)

Author	Schneppenheim (19)	Zhang (3)	Zhang (2)	Eikenboom (22)	6
Number of Patients	27	25	17	14	6
Blood group	Not specified	A	O	A	O
FVIII:C, % (range)	76 (35–118)	81 (37–121)	74 (11–128)	93 (69–138)	81 (58–93)
VWF:Ag, % (range)	39 (27–68)	45 (13–94)	32 (12–70)	61 (37–98)	52 (40–66)
VWF:RCo, % (range)	39 (16–57)	— —	— —	56 (30–92)	53 (39–68)
Ratio, FVIII:C/VWF:Ag,	1.9	1.8	2.3	1.52	1.56
Ratio, VWF:RCo/Ag (range)	1 (0.55–1.92)	—	—	1.66	1.52
Mild bleedings,* n (%)	7 (26%)	13 (52%)	11 (65%)	1 (7%)	1 (17%)

\*Mild bleeding is defined by one or two bleeding symptoms, mainly epistaxis, bruises, and/or prolonged menstruations without abnormal bleeding after both extraction or surgery, hemarthros, or muscle bleeding. Abbreviations: See Table 1.

studies (3,20), there was a wide range of values from 0.11 to 1.28 U/mL for FVIII:C, from 12 to 0.94 for VWF:Ag, with ratios of FVIII:C/VWF:Ag ranging from normal to increased above 2, indicating the difficulty in distinguishing true congenital type 1 VWF deficiency from VWF deficiency related to blood group O. In the study of Schneppenheim (19), the VWF parameters in the seven mildly symptomatic cases heterozygous for the VWF allele ranged from 0.22 to 0.45 U/mL for VWF:Ag and from 0.24 to 0.48 for VWF:RCo. In the study of Zhang (3), 24 individuals heterozygous for the VWF allele (true type 1 VWF deficiency) had VWF:Ag levels above 0.50 U/mL in six and ranged from 0.13 to 0.45 U/mL in 18. From these data it can be concluded that half of the individuals with true VWF deficiency type 1 heterozygous for the VWF null allele are at risk for very mild bleedings at VWF values between 0.20 to 0.50 U/mL, do have normal BT, and are predicted to have a completely normal response of FVIII:C and VWF parameters from below 0.50 to above 1.00 U/mL before and after DDAVP.

We studied a consanguine family with type 3 VWD. The propositus was a young girl and presented with mucocutaneous bleeding and hemarthroses of the ankle joints, FVIII:C 1%, and absence of VWF due to homozygosity for the non-sense splice site mutation IV7+1 G>A in intron 7. Both parents were heterozygous for the non-sense mutation and completely asymptomatic with near-normal to normal values for FVIII:C, VWF parameters, and normal ratios for FVIII:C/VWF:Ag (Table 3). After DDAVP, the FVIII:C levels rose to much higher levels as compared to VWF:Ag, VWF:RCo, and VWF:CB levels,

resulting in high ratios above 3 for FVIII:C as compared to each of the VWF parameters, but VWF:Rco/Ag and VWF:CB/Ag were normal before and after DDAVP, consistent with true type 1 VWF deficiency (Table 3). This demonstrates that an increased FVIII:C/VWF:Ag ratio after DDAVP is typically diagnostic for true VWF deficiency type 1 heterozygous for a null allele.

#### Autosomal Recessive Severe Type 1 VWD

In 1975 Gralnick et al (23) described a large family with three cases (two brothers, one sister) of autosomal of recessive severe VWD featured by strongly prolonged BT (>30 minutes), FVIII:C levels of 8% to 16%, and undetectable VWF activity (Table 4). The mother and another two brothers and one sister had slightly prolonged BT normal values for FVIII:C and FVIII:RA, subnormal levels for VWF activity, and an abnormal pattern in the cross immunoelectrophoresis (CIE) using VWF antibodies, which is very suggestive for a functional type 2 VWF defect of one allele (Table 4). Such patients are carriers of a missense mutation and have mild von Willebrand disease type 2. A sixth brother and a third sister of this large family showed completely normal values for FVIII:C, FVIII:RA, and VWF activity, and a normal pattern on CIE. These data indicate that in this family the three cases of severe recessive VWD are very likely double heterozygous for a missense mutation and a null allele, or double heterozygous for two missense mutations.

In 1979, Armitage and Rizza (24) described a case with severe type VWD (BT 12 minutes, FVIII:C 26%, and VWF:RCo <0.05) showing the absence of a normal peak (= absence of normal

**TABLE 3.** DDAVP Challenge Test VWD Type 1 Heterozygous for Null Allele

Antwerp Hemostasis Laboratory Marc van der Planken DDAVP:						
	Before	After 1	2	3	4	6 Hours
FVIII:C	84	496	540	592	528	496
VWF:Ag	64	128	174	168	146	140
VWF:RCo	67	176	200	184	136	120
Budde Coagulation Laboratory Hamburg						
VWF:CB	55	116	164	150	128	117
VWF:Ag	52	102	150	144	117	105

Abbreviations: See Table 1. DDAVP: desmopressin acetate.

**TABLE 4.** Autosomal Recessive Severe Type 1 VWD in Two Large Families with Autosomal Dominant Trait of Asymptomatic or Mild Type 2 VWD

Gralnick et al, 1975 (23)	BT	FVIII:C	FVIII:RA	VWF Activity	CIE VWF Protein
Propositus (male)	>30	0.09	0	0	No VWF protein
Brother 1	>30	0.10	0	0	No VWF protein
Sister 1	>30	0.16	0	0	No VWF protein
Mother*	16-20	0.90	—	0.10	Abn VWF protein
Brother 2*	9-15	1.30	—	0.42	Abn VWF protein
Brother 3*	11	1.10	—	0.42	Abn VWF protein
Sister 2*	8	1.15	—	0.56	Abn VWF protein
Normal	<8	>0.60	>0.60	>0.60	Normal VWF peak

\* = Asymptomatic, but slightly prolonged BT and abnormal (abn) VWF protein on CIE.

Armitage and Rizza, 1979 (24)	BT	FVIII:C	VWF:RCo	Bleeding	CIE VWF Protein
Propositus (male)	12	0.26	<0.05	Moderate	Normal peak absent Abn peak present
Sister	5	1.04	Normal	None	Double VWF peak
Mother	6	0.86	Normal	Mild	Double VWF peak
Maternal uncle	5	1.01	Normal	Mild/moderate	Double VWF peak
Maternal aunt	3	0.62	Normal	Mild/moderate	Double VWF peak
Four nonaffected maternal family members	<3	Normal	Normal	None	Normal VWF peak
Father and paternal family members	<3	—	—	All asymptomatic	Not tested

Abn = abnormal, BT = bleeding time, CIE = cross immunoelectrophoresis, FVIII:C = factor VIII coagulant deficiency, FVIII:RA = factor VIII related antigen, VWF:RCo = VWF ristocetine cofactor.

VWF protein) and the presence of an abnormal peak (= presence of abnormal VWF protein on VWF CEI (Table 4). The bleeding history of his father's family was negative, but his sister, mother, and another four members of his mother's family had an autosomal dominant mild bleeding history with partial penetrance of easy bruising epistaxis, slightly prolonged BT, and a double peak on VWF CEI indicating the presence of normal and abnormal VWF protein, but the values for FVIII:C, factor VIII: related antigen (FVIII:RA), and VWF:RCo were normal. These data of abnormal VWF protein in the propositus with severe VWD and the presence of a double VWF peak with autosomal dominant inheritance in family members is consistent with homozygosity for a missense mutation or double heterozygosity for a missense/null allele of the propositus for a functional VWF defect. The dou-

ble VWF peak on CIE refers to heterozygosity for a missense mutation in the mildly symptomatic maternal family members (Table 4).

Compound heterozygosity for a null allele and a missense mutation or homozygosity for a missense mutation are rare in type 3 VWD (3,19,20), but are common and frequently traced in patients with severe autosomal recessive type 1 VWD (22). Patients with autosomal recessive severe type 1 VWD who are compound heterozygous for a null allele and a missense mutation, or homozygous or double heterozygous for a missense mutation, have detectable but very low VWF levels (Table 5).

Homozygotes for the missense mutations W377C (exon Schneppenheim et al (19) and for R273W (exon 7, Allen et al (25)) in propeptide D1 domain have been described to be associated with autosomal recessive severe type 1 or even

**TABLE 5.** Reports of Autosomal Recessive Severe Type 1 VWD Caused by Homozygous Missense Mutations or Compound Missense/Null Mutations Showed a Type 2 VWD on Multimeric Analysis of VWF Protein

Author/Mutation	F/M, Age	BT	VIII:C	VWF:Ag	VWF:RCo	VWF:CB	RIPA	VWF:MM
Schneppenheim, 1994 (19)								
W377C/W377C	2 yr	>20	0.02	0.03	0.03	—	—	nt
Eikenboom, 1998 (22), Castaman, 2000 (27)								
C2362F/C2362F	F	>30	0.19/0.44	0.02	<0.03	—	—	2A
C2362F/splice site	M	>30	0.01/0.28	0.01	<0.03	—	—	2A
C2362F/R2535*null	F	>30	0.11/0.30	0.01	<0.03	—	—	2A
C2362F/737insC	F	>30	0.08/0.21	0.02	>0.03	—	—	2A
C2671Y/del null	F	>30	0.10/0.19	0.03	<0.03	—	—	2A
4699/?	M	15 >20	0.25/0.37	0.11	<0.03	—	—	
C2671Y/W2193R (Gene conversion)	M	>30	0.18/0.33	0.6	<0.03	—	—	
Castaman, 2002 (28)								
C2362F/splice site	M 3 yr	>15	0.18 0.21	0.05 0.07	<0.03 0.07	—	—	N low resolution
Schneppenheim, 2001 (29)								
C2754W/C2754W		>20	0.12	<0.05	<0.05	—	—	2D
Allen, 2000 (25)								
R273W/R273W	Boy	15	0.20	0.06	0.06	—	—	2A or 2E
R273W/R273W	Boy	15	0.33	0.09	0.04	—	—	2A or 2E
R273W/R273W	Boy	>20	0.09	<0.01	<0.01	—	—	—

Abbreviations: See Table 1. VWF:CB = VWF: collagen binding, VWF:MM = VWF multimers.

type 3 VWD. Reported cases of severe recessive type 1 VWD are in fact autosomal recessive severe type 2 VWD as demonstrated by VWF multimer analysis (25–29). The multimeric pattern of homozygous R273W clearly showed the absence of high-molecular-weight multimers and a pronounced monomeric band consistent with type 2A or 2E VWD (25). Homozygosity for the missense mutation C2364F in a family and double heterozygosity for C2364F/null in three families have been reported to be associated with severe type 1 VWD featured by FVIII:C levels of 12 to 32 U.L, very low but detectable VWF:Ag, and undetectable VWF:RCo (Table 5) (22). In some of these patients with severe type 1 VWD, FVIII:C, VWF:Ag, and VWF:RCo reached values of >0.50, 0.11, and 0.09 U/L, respectively, after DDAVP (26). C2364F het-

erozygous carriers were asymptomatic, had normal or slightly prolonged BT, subnormal values for VWF:Ag and VWF:RCo with a normal VWF:RCo/Ag ratio, and a normal VWF multimeric pattern in a low 0.8% or 0.9% agarose resolution gel (27). However, analysis of VWF in plasma from cases with severe autosomal recessive VWD homozygous for a missense mutation C2362F or compound C2362F/null (exon 42 of the B1-3 domain), as well as heterozygous carrier of C2364F, all showed a heightened proteolytic pattern with marked increase of 176 and 140 kDa degradation products consistent with type 2A VWD (Table 5) (27). Castaman et al (28) described another case of severe autosomal recessive type 1 VWD double heterozygous for two missense mutations C2364F/splice site (Table 5).



Homozygosity for a dimerization defect in the C terminal domain of the VWF gene C2754W caused type 3 VWD with undetectable VWF:Ag, RCo, and CB (Schneppenheim et al) (29). Repeated VWF multimeric analysis of the patient's platelet could detect only low-molecular-weight multimers, and further analysis revealed intervening bands between individual bands consistent with type 2D VWD. Eikenboom (22) described a case of autosomal recessive type 1 VWD due to double heterozygosity for the mutation C2671Y in exon 49 of the dimerization region of the VWF gene for which analysis of the VWF multimeric pattern was not performed.

Finally, clinicians have to be aware that type 2N VWD may present with normal BT and equally decreased VWF:Ag and VWF:RCo simulating recessive type 1 VWD, but FVIII:C is much lower as compared to VWF:Ag owing to an FVIII binding defect in the D' domain of the VWF protein (4).

#### **Bloodgroup O-Related VWF Deficiency and Asymptomatic Carriers of a VWF Missense Mutation**

ABO blood group is a well-known significant determinant of plasma VWF concentration. In the study of Gill et al (30), 456 normal individuals with blood group O had the lowest VWF:Ag levels (mean 0.75 U/mL with an  $\pm$ SD of 0.35–1.57), followed by blood group A (mean  $1.06 \pm 2SD$  0.48–2.33), and then blood groups B and AB with completely normal VWF level. About one third of individuals with blood group O, only a few with blood group A, and hardly any with blood groups B and AB had VWF levels below 0.60 U/mL. Of 114 patients diagnosed as having type 1 VWD in the study of Gill et al (31), blood group O was found in 77%, blood group A in 18%, B in 4%, and AB in 0%, whereas the frequency of these blood groups in the normal population was significantly different: 45%, 45%, 7%, and 3%, respectively. These data strongly indicate that a large proportion of patients diagnosed as having very mild or mild type 1 VWD have decreased concentration of structurally normal VWF protein in the range of 0.25 to 0.60 U/mL with normal FVIII:C/VWF:Ag and VWF:RCo/Ag ratios just on the basis of congenitally determined blood group O and a normal wild-type sequence of the vWF gene (pseudo-VWF deficiency) (30,31). Sadler (31) calculated that most diagnoses of VWD type 1 are false positive: 25% of the individuals in the population have one or two bleeding symptoms (mild bleeding), and 2.5% of the general population have

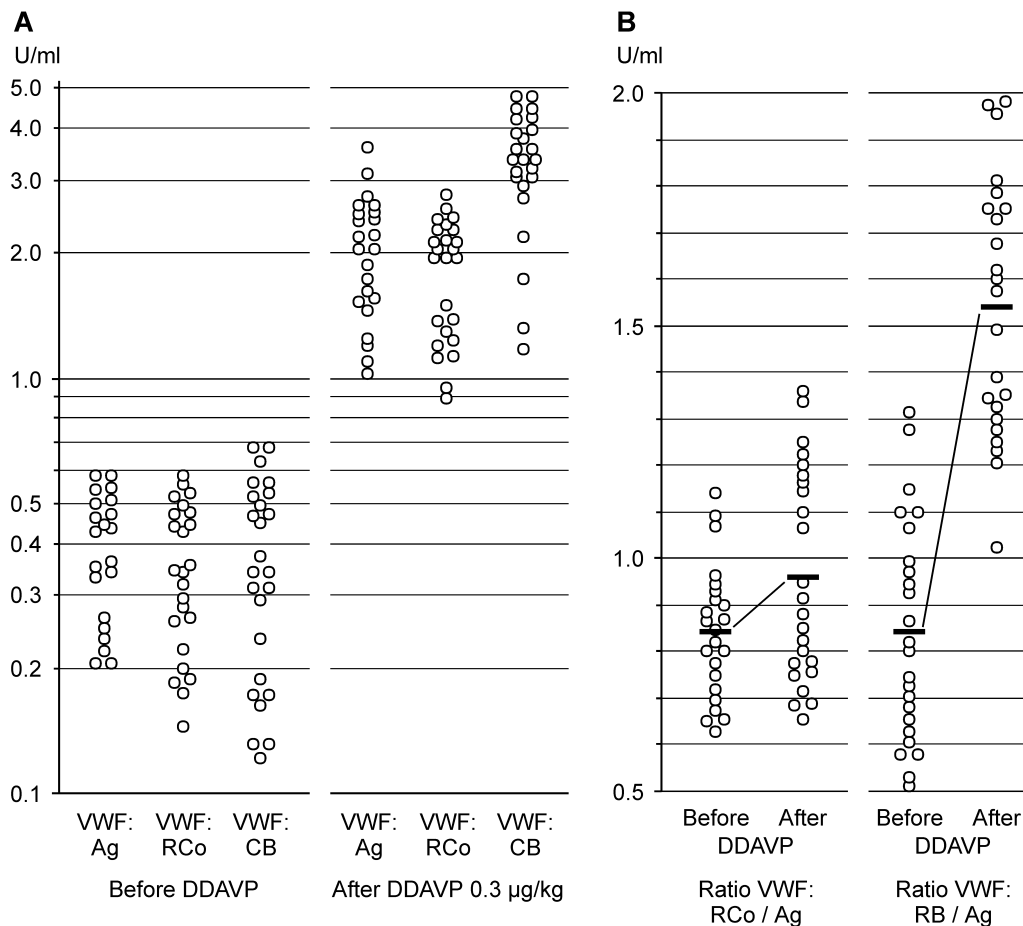
low plasma VWF concentrations. Thus  $0.25 \times 0.025 = 0.6\%$  individuals of the general population have by chance low VWF levels and mild bleeding symptoms just by chance. If a positive family history of mild bleedings is taken into account, then still 0.04% of individuals have by chance the combination of mild bleeding symptoms and low VWF levels. Heterozygous type 1 deficiency (carriers of VWF null allele) and pseudo-VWF deficiency can cause very mild or mild bleedings, but such very mild and mild bleedings may have many causes, are very common, and are not specific (31). A moderate and persistent bleeding tendency (of mucocutaneous type in the absence of petechiae) since early childhood is very suspicious and rather specific for a clinically relevant congenital VWF defect (17).

Coughlan et al (32) evaluated the presence of VWF null alleles in a cohort of 36 unrelated patients with type 1 VWD according to SSC recommendations of the ISTH and in a group of 82 control persons. VWF null alleles were not found in their type 1 VWD (and therefore labeled as pseudo-VWF deficiency) and also not in the control population. The frequency of bloodgroup O in their type 1 patients was 72%, which is higher than the 43% in their control population. Of 62 parents of type 3 patients with a documented null allele, 23 (37%) had bloodgroup O (Table 2). These observations reflect the complexity underlying the expression of a laboratory phenotype type 1 VWD and imply the involvement of asymptomatic heterozygous missense mutations in the VWF gene, genetically determined factors like blood group O, and other congenital or acquired, still unknown, factors as well. In this context two recent studies confirmed this view by the observation that the missense mutation (polymorphism) Y1584C in exon 26 of the VWF gene was enriched in symptomatic VWD type 1 (14%), and the majority of these Y1584C heterozygotes (about 90%) were of bloodgroup O (33,34). Bleeding manifestation in propositi in nine type 1 families with the Y1584 mutation and bloodgroup O were mild in two and mild to moderate seven (33). The laboratory phenotype of probands with the combination of C1584C and bloodgroup O was typically type 1 VWD with mean values of FVIII:C 0.54 (0.11 to 1.01), VWF:Ag 0.40 (0.24 to 0.50), and VWF:RCo 0.36 (0.29 to 0.46) (33). Heterozygosity for the missense mutation Y1584C was found in 2 of 200 random controls by Bowen (34), and in one asymptomatic case (VWF:Ag 0.50 U/mL) among 100 healthy controls by O'Brien (33).

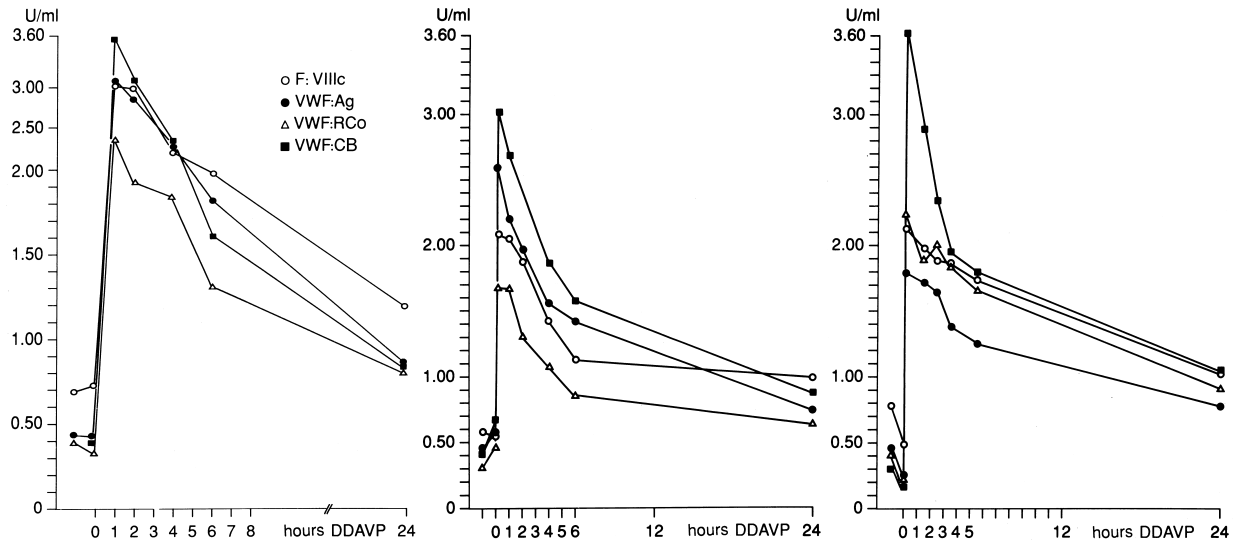
### Pseudo-VWD

We prospectively studied the probands of 24 unrelated families diagnosed as having so-called mild VWD type 1. Bleeding manifestations were mild and the BT usually normal on repeated occasions. The levels for VWF:Ag, VWF:RCo, and VWF:CB were between 0.20 to 0.60 U/mL with normal ratios for VWF:RCo/Ag (Fig. 1) (17). FVIII:C levels were somewhat higher as compared to VWF:Ag with a FVIII:C/VWF:Ag ratio of 1.48 (range 0.8–2.2). VWF:Ag levels of 0.20 and 0.60 before DDAVP reached high peak values all above 1.0 U/mL with a mean of 2.16 U/mL (range 1.15 to 3.18 U/mL) after DDAVP (Fig. 2) (17). After DDAVP the ratios of VWF:RCo/Ag remained normal (0.97, range 0.65–1.34), but the ratio of VWF:CB/Ag signif-

icantly increased to 1.60 (range 1.03–2.28), indicating the release of unusually high VWF multimers immediately after DDAVP (Fig. 1) (17). Interestingly, the ratio FVIII:C/VWF:Ag remained normal or corrected to normal after DDAVP in nearly all probands (Fig. 1), which (based on the analysis above) is very suggestive for a pseudo-VWF deficiency rather than a genetically determined true type 1 VWF deficiency (17,31). The values for FVIII:C and VWF parameters remained in the low normal range for 24 hours after DDAVP infusion (Fig. 2). There were only a few probands showing typical features suspicious for true type 1 VWD before and after DDAVP with increased FVIII:C/VWF:Ag ratios of about 2 and normal VWF:RCo/Ag ratios (Fig. 3).



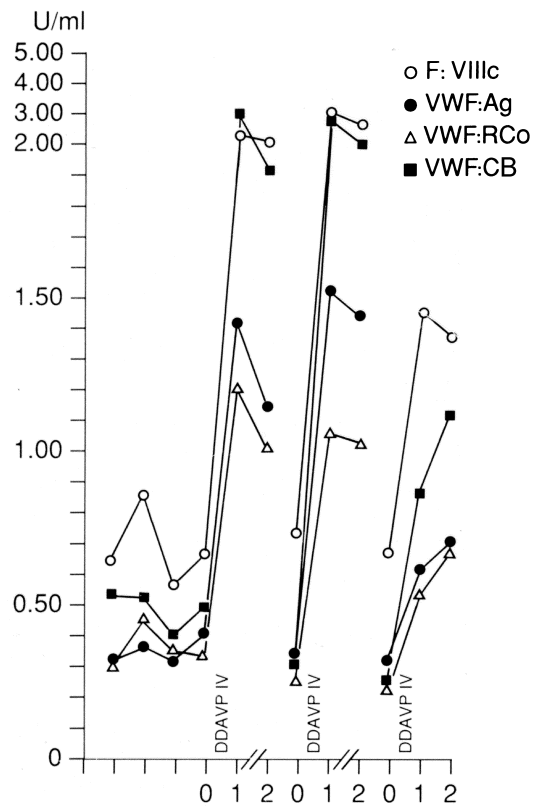
**FIG. 1.** VWF parameters before and within 15 minutes after intravenous DDAVP in 26 patients with mild VWF deficiency showing normal responses of VWF:Ag, and VWF:RCo and supernormal response of VWF:CB (mainly pseudo-VWD, see Fig. 2).



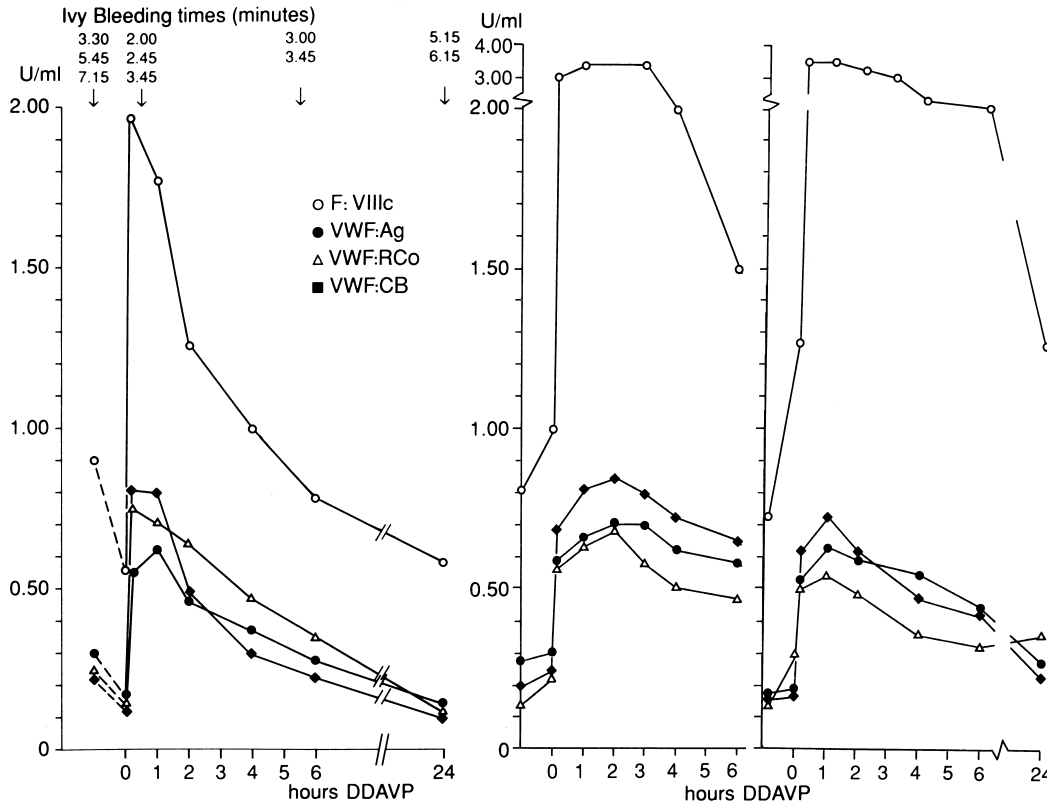
**FIG. 2.** Normal responses to DDAVP of FVIII:C and VWF parameters in 3 patients with mild VWF deficiency consistent with pseudo-VWD.

**Autosomal Dominant Type 1 VWD**

In our cohort of five VWD patients with severe type 1 VWD (17), we found three probands from different families with severe type I VWD (vWF parameters <0.20 U/mL), who had 1) a mild to moderate bleeding history since childhood, 2) a normal RIPA, 3) a normal or slightly prolonged Ivy BT, 4) a normal VWF multimeric pattern but a decreased response of VWF parameters to DDAVP suggesting a secretion defect of VWF from endothelial cells (Fig. 4). In these three probands, the ratio of FVIII:C/VWF:Ag was above 3 before and after DDAVP (Table 6), which is very suggestive for a decreased synthesis or secretion defect due to a not yet defined VWF missense mutation. We propose to label this category as autosomal dominant moderate VWD type 1 due to a secretion defect (Table 6). Eikenboom et al (35) demonstrated that the classical autosomal dominant type 1 VWD with high penetration of moderate bleeding symptoms was caused by a heterozygous missense mutation Cys<sup>367</sup> Ø Phe (C1130F) in one and Cys<sup>386</sup> Ø Arg (C1149R) in another family. These two missense mutations are located in the D3' domain and interfere with the normal VWF subunits coded by the normal VWF allele, causing a defective intracellular multimerization and degradation of VWF, and leading to a secretion defect of the VWF by endothelial cells (35,36). The autosomal dominant severe type 1 VWD (C1130F) is fea-



**FIG. 3.** Good response to DDAVP in a case of mild type 1 VWD showing an increased ratio of FVIII:C/VWF:Ag and a normal ratio of VWF:RCO/Ag before and after DDAVP.



**FIG. 4.** Normal response of FVIII:C and decreased response of VWF parameters to DDAVP in three cases (one woman, left, and one brother and sister, middle and right, respectively) showing an increased ratio of FVIII:C/VWF:Ag and a normal VWF:RCO/Ag ratio before and after DDAVP consistent with dominant type 1 VWD.

tured by low WF levels of  $<0.20$  U/mL and the presence of all sizes of VWF multimers, and an increased FVIII:C/VWF:Ag ratio of 2.0 to 2.3 consistent with a “synthesis  $\emptyset$  secretion” defect of VWF deficiency type 1 (Table 6). A similar dominant-negative mechanism of intracellular retention and degradation of VWF caused by heterodimerization of mutant and normal VWF subunits in the endoplasmic reticulum followed by proteosomal degradation in the cytoplasm has been described for the C1149R mutation, but the laboratory features of this autosomal dominant type 1 mutant are not reported (36). Castaman et al (37) screened 24 unrelated Italian patients with autosomal dominant type 1 VWD for the C1149R and C1130F mutations. None of the patients had the C1149R mutations and three apparently unrelated patients showed the presence of the C1130F mutations (formerly reported as C367F) and typical features of severe dominant

type 1 VWD with VWF values below 15 U/dL and increased FVIII:C/VWF:Ag ratios (Table 6). Casana et al (38) reported the association of dominant mild type 1 VWD with increased FVIII:C/VWF:Ag ratios in seven members of one family with heterozygosity of T1156M in the D3 domain of the VWF gene. Federici et al (39) described a family (grandfather, mother, and newborn daughter) with severe type 1 VWD, subtype ‘plasma low-platelet low,’ that could be explained by decreased synthesis of VWF in endothelial cells of cultured endothelial cells isolated from the umbilical vein of the newborn daughter. This family can now readily be reclassified as having autosomal dominant severe type 1 VWD (Table 6).

#### Diagnostic Differentiation Between Dominant Type 1 Versus 2M or Variant Type 2A VWD

In 1985 Mannucci et al (40) described 17 patients from 13 kindreds with pronounced VWD

**TABLE 6.** Laboratory Data on 6 Families with Autosomal Dominant Type 1 VWD

Author	BT	F VIIIc u/mL	vWF:Ag u/mL	Rcof u/mL	CBA u/mL	Ratio vWF-CBA/Ag	Ratio VIII:C/VWF:Ag	RIPA	Response to DDAVP	vWF MM
Michiels et al, 2002 (17)										
Family 1	N	19	9	<10	12	1.3	2.1	N	Decreased 'type 1'	N
Family 2	↑	56	17	16	13	0.8	3.3	N	Decreased 'type 1'	N
Family 3										
Mother	↑	67	19	14	21	1.1	3.5	N	Decreased 'type 1'	N
Daughter	↑	65	19	23	16	0.9	3.4	N	Decreased 'type 1'	N
Brother	↑	82	21	17	27	1.3	3.9	N	Decreased 'type 1'	N
Eikenboom et al, 1996 (35)										
						RCo/Ag				
Family 4										
Father	↑	21	10	<20	—	—	2.1	—	—	N
Son	↑	22	11	<20	—	—	2.0	—	—	N
Daughter	↑	35	15	<20	—	—	2.3	—	—	N
Federici et al, 1993 (39)										
Grandfather	↑	35	10	7	—	0.7	3.5	—	—	N
Mother	↑	28	8	6	—	0.75	3.5	—	Decreased	N
Daughter	↑	24	10	6	—	0.60	2.4	—	—	N
Dominant type 1 VWD in 7 members of one family associated with T1156M in the D3 domain of the VWF gene										
Casana et al, 2001 (38)										
II:1	nr	0.80	0.39	0.27	nt	0.7	2.1	—	Good	N
II:2	nr	0.69	0.37	0.29	nt	0.78	1.9	—		N
III:4	nr	0.62	0.28	0.17	nt	0.60	2.2	—		N
III:8	nr	0.59	0.27	0.17	nt	0.62	2.2	—		N
IV:1	nr	0.68	0.36	0.31	nt	0.86	2.2	—		N
IV:2	nr	0.70	0.35	0.20	nt	0.57	2.0	—		N

↑ = prolonged, N = normal, decr = decreased, — = not tested.

N = normal VWF multimers in low-resolution agarose gel.

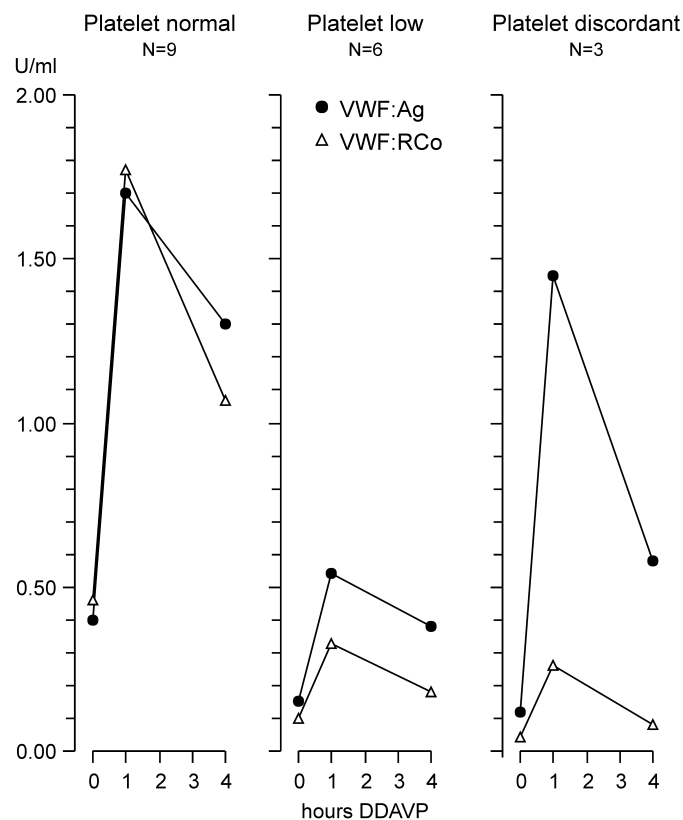
with VWF values below 0.20 in all them and identified three subtypes in this cohort according to the content of VWF in patient platelets, indicated as platelet normal, platelet discordant, and platelet low. In this study, type 1 platelet normal VWD was characterized by normal content of platelet VWF, a normal response to DDAVP, and normal BT before and after DDAVP, followed by normal half-life times of VWF parameters. Type 1 platelet discordant VWD was characterized by the absence of most of the large

VWF multimers, abnormal VWF:Ag content and deficient VWF:RCo in platelets, a discrepant response to DDAVP with a good response of FVIII:C and VWF:Ag, a poor response of VWF:RCo, and only a transient and partial correction of the strongly prolonged BT. This category has been reclassified by Sadler (31) as a variant of type 2A. The six patients with very severe type 1 VWD platelet low were featured by a strongly prolonged BT, a normal pattern of VWF multimers in the densimetric scans of a 1.4% agarose gel,

and very low plasma VWF levels in whom platelet VWF:Ag and VWF:RCo were equally low (“type 1 platelet low”). After DDAVP the BTs only partially corrected for a few hours and plasma VWF:Ag remained low (less than 0.50 U/mL), VWF:RCo remained very low (less than 0.25 U/mL) owing to defective production of VWF in cellular compartments, whereas FVIII:C levels increased to values between 0.70 to 1.30 U/mL (Fig. 5, Mannucci et al (40)) consistent with a severe secretion defect type 1 (1 SD) or autosomal recessive type 1.

Cattaneo et al (41) evaluated the response to DDAVP in nine patients with type 1 platelet normal, six patients with platelet low, and three patients with platelet discordant. The nine VWD patients with VWD type 1 platelet normal was fea-

tured by mild disease, mean values of 41 (19–64) for VWF:Ag and 43 (21–54) for VWF:RCo, and a completely normal response of VWF parameters to DDAVP followed by normal half-life times consistent with mild type 1 VWD or pseudo-VWD (Fig. 5). The six vWD patients with type 1 platelet low had low values of 16 (1–38) for VWF:Ag and 5 (5–32) for VWF:RCo and a poor response to DDAVP for both VWF:Ag and VWF:RCo (Fig. 5) consistent with a type of secretion defect. The three VWD patients with type 1 platelet discordant had mean values of 44 (11–81) for VWF:Ag and 5 (5–33) for VWF:RCo, but the response to DDAVP was good for VWF:Ag and poor for VWF:RCo (Fig. 5) and therefore consistent with 2M or 2A variant (17,31). In this cohort of VWD patients, Simplate BT was pro-



**FIG. 5.** Responses to DDAVP in type 1 DDAVP according to the classification of Mannucci et al (40) and Cattaneo et al (46). Left: Normal response to DDAVP of both VWF:Ag and VWF:RCo in mild type 1 VWD “platelet normal” consistent with either pseudo-VWD or mild type 1 VWD. Middle: Poor response to DDAVP of both VWF:Ag and VWF:RCo in type 1 VWD “platelet low” consistent with recessive type 1 or 2A-like VWD. Right: good response of VWF:Ag and poor response of VWF:RCo to DDAVP in type 1 VWD “platelet discordant” consistent with type 2M VWD.

longed in VWD type 1 platelet low, but normal in most VWD patients with platelet normal, and discordant. The PFA-100 closure times (PCT) was normal in five of the nine patients with VWD type platelet normal and prolonged in all VWD patients with type 1 platelet low and discordant. Treatment with DDAVP corrected the PCT to normal in patients with VWD type 1 platelet normal, but not in platelet low and discordant.

Fressinaud et al (42) evaluated BT (Simplat) and PCT (PFA-100) in 36 patients with type 1 VWD. VWD type 1 was defined by a quantitative deficiency of both VWF:RCo and VWF:Ag levels below 0.40 U/mL without taking into account the results of RIPA and disregarding the VWF:RCo/Ag ratios as the first steps to differentiate type 1 and type 2 VWD. In this study, 14 patients (cases 1 to 14) had very mild type 1 VWD featured by VWF:RCo levels between 0.28 and 0.39, normal VWF:RCo/Ag ratios, normal BT in 10, and prolonged PCT not exceeding 250 s in most of them. Thirteen VWD patients (cases 23 to 36) had low VWF:RCo levels between 5 and 0.16 U/mL, prolonged PCT (>250 s), and prolonged BT in seven of them. Eight of these 13 VWD patients had decreased ratios for VWF:

RCo/Ag consistent with a variant of type 2 VWD. Six patients (cases 16 to 22) had values of VWF:RCo between 0.22 and 0.27 with normal VWF:RCo/Ag ratios, prolonged BT in five, and PCT above 250 s in most of them. Eight patients (cases 29 to 36) had severe VWD with values for VWF:RCo of 0.10 U/mL or less, and at this low level it is rather difficult to differentiate between true type 1, type 2M, or a variant of type 2A.

In our cohort of five probands with severe type 1 VWD (17), we found two probands with severe type 1 VWD and decreased RIPA, that could be classified as VWD type 2M because of a very characteristic response to DDAVP (Fig. 6) (43). The typical laboratory features in our VWD type 2 M patients are 1) "severe type 1," 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, 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 slightly normal to slightly prolonged Ivy BTs before and after DDAVP (Fig. 6) (17). In a study performed in 317 patients previously registered as type 1 VWD, 30 patients

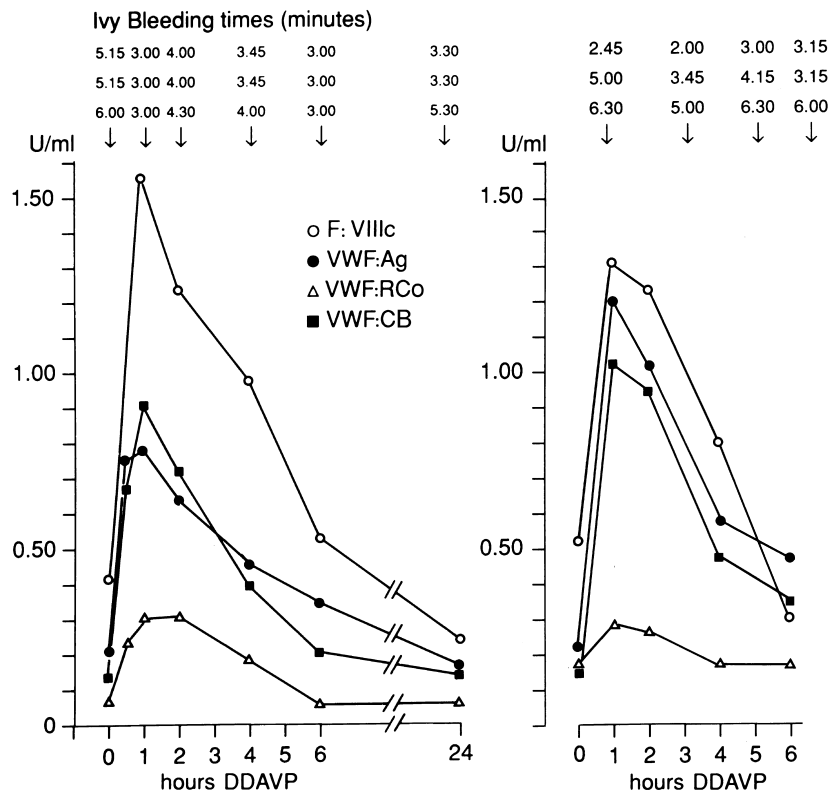


FIG. 6. Poor response of VWF:RCo and good responses of FVIII:C, VWF:AG and VWF:CB to intravenous DDAVP in two cases with type 2M VWD.

from 17 unrelated families with discrepant VWF:RCo/Ag ratios of plasma and normal VWF multimers could be reclassified as type 2M (44). RIPA assay was previously performed in 26 of 30 2M patients and an absent or decreased responsiveness to ristocetin with minor aggregation at 1.5 mg/mL was found in all cases (44). This simply may mean that all cases of severe type 1 CvWD with a normal or near-normal VWF multimeric pattern, a decreased or absent RIPA, and a decreased VWF:RCO/Ag ratio can currently be reclassified as 2M VWD. However, the diagnostic differentiation of dominant severe type 1 VWD versus type 2 M and 2 U remains a challenge that will be addressed in our manuscript on classification of type 2 VWD (43).

### **Autosomal Dominant VWD Type 1 Vicenza**

Congenital VWD Vicenza clearly differs from 2M and 2U. VWD Vicenza is featured by equally low levels of FVIII:C, VWF:Ag, and VWF:RCo; usually normal RIPA; a normal or slightly prolonged BT; and the presence of unusually large vWF multimers in plasma (44,45). Cattaneo et al (46) evaluated the Simplate BT, PFA-100 closure times (PCT), VWF parameters, and the response to DDAVP in five patients with VWD Vicenza. The values for VWF:Ag and VWF:RCo were very low (2–9) and 5 (5–6), respectively, in VWD Vicenza. Simplate BT was normal and PFA-100 closure times (PCT) were prolonged before and normalized after DDAVP in VWD Vicenza. The response to DDAVP in several cases of VWD Vicenza in the study of Casonato et al (45) and Cattaneo et al (46) was good for FVIII:C, VWF:Ag, and VWF:RCo, which was followed by unexplained very short half-life times of less than a few hours for FVIII:C and all VWF parameters consistent with a laboratory type 1 phenotype. The ratios for FVIII:C/VWF:Ag and VWF:RCo/Ag and VWF:CB/Ag remained normal before and after DDAVP (17,45,46). Schneppenheim et al (47) analyzed the complete coding region (exons 2 to 52) and adjacent intron sequences of the VWF gene in 23 patients with VWD Vicenza: three patients from the two Italian families originally described in Vicenza (45), 13 patients from five additional Italian families, and seven patients from three German families with VWD Vicenza (48). In six patients from two German families, who had significantly higher VWF:Ag and VWF:RCo levels (between 10–221 U/dL) and much higher FVIII:C levels, no genetic defect could be detected. In the other 17 patients with significantly lower VWF values

(below 10 U/dl except in two) including all Italian families, a single mutation R1205H in the D3 (multimerization) domain was found as the probable cause of VWD type Vicenza (47). The causative relationship between the mutation R1205H and the VWD type Vicenza is still unclear. Decreased proteolysis due to relative resistance to the VWF-specific protease is unlikely since the triplet structure of the individual VWF Vicenza multimers as the result of proteolysis is normal. Multimeric analysis of plasma and platelet VWF of patients with the mutant VWF Vicenza clearly show dense bands of the ultralarge and large multimers and a very dense band of degraded VWF with relative faint bands of intermediated and low multimers as compared to normal VWF multimeric pattern (44). This difference can readily be explained by the rapid and complete degradation of the FVIII/VWF complex of the mutant VWF Vicenza within a few hours, but the absence of increased triplet structure due to rapid proteolysis, as seen in types 2A and 2B, clearly indicates that the mechanism of rapid degradation of VWF Vicenza multimers is different and not caused by proteolysis. After DDAVP, FVIII:C and VWF parameters reach normal values indicating normal synthesis, but the FVIII/VWF complex of the mutant VWF Vicenza is rapidly cleared, whereas in types 2A and 2B VWD there is a normal release of FVIII:C and VWF:Ag after DDAVP with normal half-life times for FVIII:C and VWF:Ag and very short half-life times for VWF:RCo owing to proteolysis of large VWF multimers (17).

### **CONCLUSIONS**

Classification of type 1 and type 3 VWD based on clinical, laboratory, and molecular features is shown in Table 7. Recessive type 3 VWD is caused by homozygous or double heterozygous non-sense mutations in the VWF gene (null alleles). The complete absence of VWF is compatible with life. The loss of VWF results in FVIII:C deficiency ranging from 1% to 9%. Type 3 VWD is easy to diagnose by the combination of a strongly prolonged BT, absence of RIPA, absence of VWF, and FVIII:C deficiency and is associated since early childhood with a pronounced tendency to mucocutaneous and musculoskeletal bleedings. Carriers of one null allele are usually asymptomatic at VWF levels between 15% and 90% of normal. Bloodgroup O has a minor influence (-10%) on FVIII:C and VWF levels.



**TABLE 7.** The Antwerp Classification and Characterization of Quantitative Type 1 and Type 3 von Willebrand Diseases (VWD)

Category VWD	BT	FVIII:C (%)	VWF (%) Ag	RCo	RIPA	Bleeding Type	VWF Gene Mutation
Severe type 3 Recessive	↑↑↑	1–9	Zero	Zero	Zero	Severe Hemophilia	Double Non-sense
Severe type 1 Recessive	↑↑↑	9–40	1–10	0–6	Zero	Moderate Severe	Double Missense
Bloodgroup O (30–32) (Pseudo-VWD)	N	35–150	35–150	35–150	N	Asymp Very mild	None
Carrier type 3	N↑	30–140	15–90	15–90	N	Asymp	Single
Minor influence (-10%) of bloodgroup O						Very mild	Non-sense (null allele)
Carrier type 1 (polymorphism)	N	N	N	N	N	Asymp	Single Missense
Mild type 1 Recessive or Variable penetrance and multigenetic background	N↑	20–80	20–50	20–50	N	Mild	Mis/Non-sense or Y1584C/ Bloodgroup O (19,50)
Dominant type 1	N↑	20–80	10–40	0–30	N	Mild	Single
Secretion defect	↑/↑↑	5–20	5–20	5–20		Moderate	Missense
Dominant Type 1 Vicenza	N/↑	<15	<15	<15		Moderate	Single <sup>R1205H</sup> Missense

Designed by Michiels, 2004.

N = normal, asymp = asymptomatic.

Recessive type 1 VWD differs from type 3 VWD by the presence of detectable VWF:Ag and FVIII:C levels between 0.09 to 0.40 U/mL. Recessive severe type 1 VWD is caused by homozygous or double heterozygous missense mutation in the VWF gene resulting in the reduced production of abnormal VWF protein. Patients with recessive type 1 VWD indeed show an abnormal VWF multimeric pattern in plasma and/or platelets consistent with severe type 2 VWD. Carriers of a missense mutation may have mild bleeding and mild VWF deficiency and can be diagnosed by a double VWF peak on CIE. There will be cases of mild and moderate recessive laboratory phenotype type 1 VWD due to double heterozygosity of two identical or different missense or one missense and one non-sense mutation and such cases are in fact variants of type 2 VWD.

Bloodgroup O itself is a cause of congenital VWF deficiency (30,31). Recently, it has become evident that the combination of a missense mu-

tation Y1584C and bloodgroup O is associated with symptomatic mild VWD (32,33). Mild deficiency of VWF in the range of 0.20 to 0.60 U/mL, with normal ratios of VWF:RCo/Ag and VWF:CB/Ag, normal VWF multimers, and a completely normal response to DDAVP with VWF level rising from below to above 1.00 U/mL are very likely cases of pseudo-VWD in individuals with normal VWF protein and gene. Autosomal dominant type 1 VWD variants are in fact type 2 variants caused by a heterozygous missense mutation in the VWF gene that produces a mutant VWF protein, which has a dominant effect on normal VWF with regard to the synthesis, processing, storage, secretion, and/or proteolysis of VWF in endothelial cells.

VWD Vicenza represents a specific example with a normal secretion of ultralarge VWF multimers followed by a fivefold more rapid clearance of the FVIII-VWF complex, resulting in FVIII:C and VWF levels below 15 U/mL and therefore to be categorized as type 1 VWD (49).

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