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Molecular Genetics of Type 2 von Willebrand Disease

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Abstract

Type 2 von Willebrand disease (VWD) is characterized by a wide heterogeneity of functional and structural defects. These abnormalities cause either defective von Willebrand factor (VWF)-dependent platelet function in subtypes 2A, 2B, and 2M or defective VWF-factor VIII (FVIII) binding in subtype 2N. The diagnoses of types 2A, 2B, and 2M VWD may be guided by the observation of disproportionately low levels of ristocetin cofactor activity or collagen-binding capacity relative to VWF antigen. The abnormal platelet-dependent function is often associated with the absence of high molecular weight (HMW) multimers (type 2A, type 2B), but the HMW multimers may also be present (type 2M, some type 2B), and supranormal multimers may exist ("Vicenza" variant). The observation of a low FVIII-to-VWF:Ag ratio is a hallmark of type 2N VWD, in which the FVIII levels depend on the severity of the FVIII-binding defect. Today, the identification of mutations in particular domains of the pre-pro-VWF is helpful in classifying these variants and providing further insight into the structure-function relationship and the biosynthesis of VWF. Thus, mutations in the D2 domain, involved in the multimerization process, are found in patients with type 2A, formerly named IIC VWD. Mutations located in the D′ domain or in the N terminus of the D3 domain define type 2N VWD. Mutations in the D3 domain characterize Vicenza and IIE patients. Mutations in the A1 domain may modify the binding of VWF multimers to platelets, either increasing (type 2B) or decreasing (type 2M, 2A/2M) the affinity of VWF for platelets. In type 2A VWD, molecular abnormalities identified in the A2 domain, which contains a specific proteolytic site, are associated with alterations in folding, impairing VWF secretion or increasing its susceptibility to proteolysis. Finally, a mutation localized in the carboxy-terminus CK domain, which is crucial for the dimerization of the VWF subunit, has been identified in a rare subtype 2A, formerly named IID. *Int J Hematol.* 2002;75:9-18.

Key words: von Willebrand disease; Type 2 von Willebrand disease; von Willebrand factor genetics

1. Introduction

von Willebrand disease (VWD) type 2 refers to molecular variants with a qualitative defect of von Willebrand factor (VWF) [1]. This glycoprotein has 2 essential functions required for the arrest of bleeding at wound sites: it promotes platelet adhesion to the subendothelium and platelet-to-platelet cohesion during thrombi growth, and it serves as carrier for factor VIII (FVIII) in the circulation. Thus, one can distinguish variants exhibiting an abnormal platelet-dependent function (types 2A, 2B, and 2M) from those with a defective binding of VWF to FVIII (type 2N).

Although no accurate incidence figure is available, type 2 variants are generally thought to account for 20% to 30% of all patients with VWD. Interim data from French patients included in the INSERM Network on ''Molecular abnormalities in von Willebrand disease'' indicate a nearly equal distribution among the 4 subtypes (unpublished data).

This article first describes the variants related to abnormal VWF-dependent platelet function, ie, types 2A, 2B, and 2M. Despite the latter characteristic, these type 2 variants are extremely heterogeneous in terms of clinical severity, inheritance pattern, laboratory presentation, location of mutations, and choice of appropriate treatment. Moreover, some of these variants are still extremely difficult to subclassify.

We also emphasize the relative frequency of type 2N VWD. This type is well defined by the FVIII-binding

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defect and by the underlying mutations; it may still be misclassified, however, as mild hemophilia A if the VWF-FVIII–binding defect is not systematically searched. The differential biological diagnosis is of major importance for providing the optimal treatment and relevant genetic counseling.

2. Clinical Manifestations and Inheritance

It is generally admitted that the severity of bleeding in type 2 (2A, 2B, 2M) variants is intermediate between that of type 1 VWD, characterized by mild to moderate bleeding, and that of type 3 VWD, associated with profound, life-threatening bleeding. Bleeding symptoms reflect the defect in primary hemostasis and include mucosal bleeding, especially epistaxis and menorrhagia, but also bruising, bleeding from the oral cavity, and postoperative bleeding. Gastrointestinal bleeding that may be associated with angiodysplasia [2] appears to be relatively frequent in type 2A and 2B patients, probably because the high molecular weight (HMW) VWF multimers (which are lacking in these subtypes) are essential to prevent or arrest the bleeding under conditions of high shear stress [3].

Although the autosomal inheritance of VWD predicts an equal frequency of both genders, females comprise approximately 60% of patients with VWD because of the recurrent challenges of bleeding with menstruation and childbirth. The registry of type 2 VWD in the French INSERM Network, which comprises more than 200 unrelated patients, includes 63% females (unpublished data). Women with type 2 VWD are at risk of hemorrhagic complications during delivery because even if VWF levels rise during pregnancy, the protein remains dysfunctional; in a recent study, the reported incidence of primary postpartum hemorrhage was approximately 18% [4]. Moreover, severe thrombocytopenia frequently occurs during pregnancy in patients with type 2B [5], complicating their management; in addition, infants with type 2B VWD often have neonatal thrombocytopenia.

Inheritance of type 2 VWD causing defective VWF-dependent platelet function is generally dominant, although some of these variants are apparently transmitted in a recessive manner; the penetrance is more complete than in type 1.

Type 2N VWD is recessively inherited and clinically similar to mild hemophilia A. The clinical expression depends primarily on the procoagulant FVIII (FVIII:C) levels. Although some cases of FVIII:C deficiency with levels of approximately 1 to 2 IU/dL (but never less than 1 IU/dL) have been described, most patients have FVIII levels of more than 5 IU/dL and relatively moderate hemorrhagic expression, comparable with that observed in moderate and minor hemophilia A. The bleeding is mainly posttraumatic or related to surgery. The spontaneous hemorrhagic syndrome is often not serious, being characterized by excessive bruising, epistaxis, muscular hematomas, and, rarely, hemarthrosis [6]. Women with type 2N VWD may suffer from menorrhagia, and hemorrhage is also frequently noted in the postpartum period.

3. Laboratory Diagnosis

Diagnosis of VWD in patients with type 2 (excluding type 2N) is generally easier than in type 1 patients because of the usually prolonged bleeding time or closure time [7] and the markedly decreased ristocetin-cofactor activity (VWF:RCo). However, the partial thromboplastin time (PTT) is frequently normal because the FVIII levels are usually not decreased; the VWF antigen (VWF:Ag) levels are variably decreased. Finally, what are striking are the disproportionately low levels of VWF:RCo relative to VWF:Ag, a characteristic of most patients with type 2 VWD [8]. The evaluation of the ability of VWF to bind to immobilized collagen (collagen-binding assay: VWF:CB) may also be very useful. A low VWF:CB/VWF:Ag ratio is characteristic of most type 2 variants, as VWF:CB clearly reflects the presence of HMW VWF multimers; thus, this ratio is low in type 2A or 2B and is in the normal range in type 2M. Unfortunately, most assays for VWF activity are not well standardized. In type 2B VWD, thrombocytopenia may exist: it can be intermittent and exacerbated by surgery, pregnancy, or administration of desmopressin and tends to increase with age.

The diagnosis of subtype is often difficult because the discriminating tests that are necessary to distinguish among specific subtypes are not easy to perform in all laboratories [9]; their relevance is crucial for appropriate treatment of patients. These tests are ristocetin-induced platelet aggregation (RIPA) at low concentrations of ristocetin and multimeric analysis of plasma VWF using both low and high resolution gels. Low resolution gels may show 3 different profiles: (1) normal distribution, (2) absence of HMW multimers and predominance of the protomer, or (3) presence of supranormal multimers. High resolution gels may show different abnormalities such as the modification in the intensity or the position of the satellite subbands of each multimeric unit. Other tests are also helpful: assays based on ristocetin- or botrocetin-induced binding of VWF to platelets, assays based on binding of VWF to collagen, and studies of VWF levels and multimeric structure in platelet lysates. Table 1 summarizes the main results of the discriminating tests in variants with abnormal VWF-dependent platelet function. Finally, the identification of mutations is also helpful, particularly if already described in the database (accessible on the Internet at http://mmg2.im.med.umich.edu/vWF). However, it appears that some candidate mutations responsible for an atypic phenotypic pattern are impossible to subclassify at the present time. Therefore, it is important to assess the effect of a mutation on the structure and function of VWF by expression of the mutated protein, but this assessment can be done only in some research laboratories.

FVIII deficiency is a hallmark of type 2N VWD. The FVIII levels, measured by 1-stage, 2-stage, chromogenic, or immunological assays, range from 1 to 40 IU/dL. The FVIII levels depend on the severity of the FVIII-binding defect. Indeed, in patients with completely absent FVIII-binding function, the FVIII:C levels are significantly lower than in patients in whom this function is only markedly decreased [10]. The phenotypic diagnosis of type 2N VWD is based on the in vitro measurement of the capacity of plasma VWF to bind exogeneous FVIII (VWF:FVIIIB). The reference VWF:FVIIIB assay is a solid-phase immu-

Table 1.
Phenotypic Characterization of Most Variants (Type 2) of von Willebrand Disease*

New terminology	2A	2A	2A	2M	2M	2B	2B	2N
Old terminology	IIA	IIC	IID		I "Vicenza"	IIB†	I New York, I Malmö	Normandy
Inheritance	Dominant	Recessive	Dominant	Dominant	Dominant	Dominant	Dominant	Recessive
VWF:RCo-to-VWF:Ag ratio	Decreased	Decreased	Decreased	Decreased	Normal or nearly normal	Decreased (or normal)	Normal	Normal
VWF:RCo-to-VWF:CB ratio	Decreased	Decreased	Decreased	Normal	Normal	Decreased	Normal	Normal
VWF:FVIIIB*	Normal	Normal	Normal	Normal	Normal	Normal	Normal	Decreased
Plasma multimers	Loss of large and intermediate	Loss of large	Loss of large	Normal	Presence of ultralarge	Loss of large	Normal	Normal (or rarely decrease of large)
Platelet multimers	Normal or loss of large and intermediate	Loss of large	Loss of large	Normal	Normal	Normal	Normal	Normal
RIPA	Decreased (or absent)	Decreased (or absent)	Decreased	Decreased	Normal	Enhanced sensitivity at low doses	Enhanced sensitivity at low doses	Normal
Ristocetin-induced VWF binding to platelets	Decreased	Decreased	Probably decreased	Decreased	Normal	Enhanced sensitivity at low doses	Enhanced sensitivity at low doses	Normal
Botrocetin-induced VWF binding to platelets	Decreased	Decreased	Probably decreased	Normal or decreased	Normal	Enhanced sensitivity at low doses	Enhanced sensitivity at low doses	Normal

*VWF indicates von Willebrand factor; VWF:FVIIIB, factor VIII–binding activity; RIPA, ristocetin-induced platelet aggregation.
†Patients exhibit intermittent or chronic thrombocytopenia.

noassay [11] in which bound FVIII is measured using either a chromogenic assay or an immunological assay. This test clearly differentiates real type 2N VWD patients (markedly decreased VWF:FVIIIB) from type 2N heterozygotes with intermediate VWF:FVIIIB, who synthesize both normal and defective VWF (and do not exhibit FVIII deficiency).

The VWF levels in type 2N VWD are usually normal or subnormal when the ranges established in normal individuals within a given ABO blood group are taken into account. The FVIII:C-to-VWF:Ag ratio has been measured in French type 2N VWD patients (homozygotes or compound heterozygotes) [12]; in all cases, it is lower than 0.5, being lower than 0.3 in patients with total lack of FVIII-binding capacity. Thus, the phenotype of type 2N patients differs from that of other VWD phenotypes. In contrast to other variants, type 2N VWD is characterized by defective coagulation with generally normal primary hemostasis.

4. Pathophysiology and Molecular Basis

An understanding of the pathogenesis of VWD variants requires some knowledge of VWF biochemistry and function. The VWF gene is located at the tip of the short arm of chromosome 12. VWF is first synthesized in megakaryocytes and endothelial cells as a large precursor (pre-pro-VWF) that undergoes dimerization and then multimerization and processing. The precursor, composed of 2813 amino acid (aa) residues, contains a signal peptide of 22 aa, a large propeptide of 741 aa, and a mature subunit of 2050 aa. This precursor contains 4 types of homologous domains: 3 A domains, 3 B domains, 2 C domains, and 5 D domains, which are arranged in the following order: D1-D2-D′-D3-A1-A2-A3-D4-B1-B2-B3-C1-C2-CK (Figure 1). The disulfide bridges involved in the dimerization are located at the carboxy-terminus of the CK domain (Figure 1). Additional disulfide bonds are then formed between the D3 domains of the pro-VWF dimers, yielding multimers that may exceed 20 million daltons. Posttranscriptional modifications, including proteolytic removal of the propeptide, glycosylation, and sulfation, occur before the storage or the secretion of VWF multimers. Two remarkable disulfide loops are formed, 1 in the A1 domain between cysteine residues 1272 and 1458 and the other in the A3 domain between cysteine residues 1686 and 1872 (Figure 1); there is a specific proteolytic site within the A2 domain, between aa Y1605 and M1606 (Tyr 842 and Met 843 in the mature subunit).

The dimerization and the degree of multimerization of VWF are essential for its role in platelet adhesion and aggregation. This function of VWF is also related to binding sites of the subunit for specific receptors or ligands (Figure 1). In certain conditions or in the presence of modulators, VWF is able to bind to platelet glycoprotein Ib (GPIb) and to connective tissue. In particular, the A1 domain contains binding sites for platelet GPIb [13], snake venom protein botrocetin [14], and minor binding sites for collagens [15]. The major binding site for fibrillar collagens is in the A3 domain [16]. Finally, a binding site for the GPIIb/IIIa complex, another

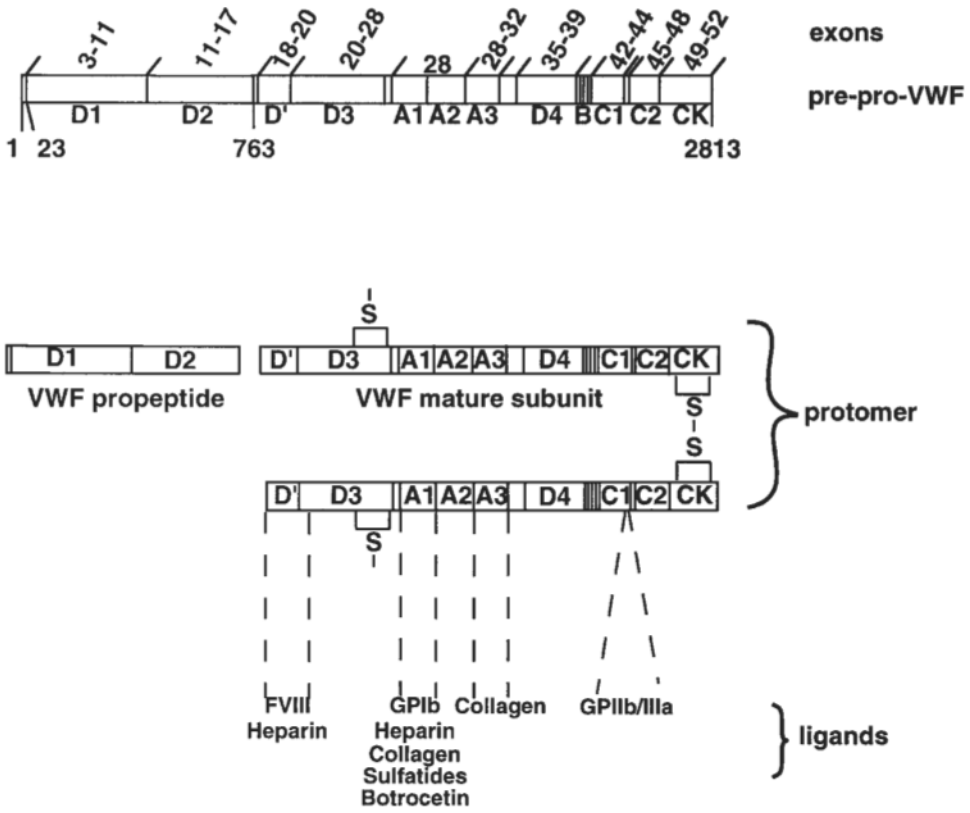

Figure 1. Schematic representation of pre-pro-VWF (4 types of repeating domains, A to D), encoded by the VWF gene (52 exons); VWF propeptide; and the protomer of VWF composed of 2 disulfide-linked mature VWF subunits and functional domains binding to specific ligands or receptors (Mazurier et al, *Annales de Genetique*. 1998;41:34-43).

platelet receptor, has been localized in the C1 domain, including the 2507-2509 RGD sequence [17,18]

Plasma VWF is the carrier for FVIII in the blood circulation. The binding of FVIII to VWF in a tight but noncovalent plasma FVIII/VWF complex is essential for the survival of FVIII in vivo. The FVIII-binding site has been located on the 272-aa N-terminal part of the mature VWF subunit, corresponding to the D′ domain and to the N-terminal part of the D3 domain [19,20].

Thus, mutations within these different homologous domains may interfere with VWF processing and multimer assembly or secretion, render the multimers more sensitive to proteolysis in plasma, or modify the binding of VWF to GPIb or to collagen. The presence of a mutated VWF with an altered FVIII-binding site results in diminished or no FVIII-VWF interaction. Thus, noncomplexed FVIII is not protected from proteolytic degradation and, in addition, it is catabolized more rapidly.

In order to improve understanding of type 2 VWD, we will analyze the impact of mutations in the different domains (Tables 2 and 3). We will use the new nomenclature proposed for VWF aa residue numbering: aa 1 to 2813 in the pre-pro-VWF (whereas the old nomenclature numbered aa of mature VWF from 1 to 2050). We have chosen to report only well-characterized mutations published in full articles.

4.1. Mutations in the D2 Domain

Several gene defects (3 missense mutations, 2 insertions, and 1 deletion) have been found in the D2 domain encoded by exons 11 to 17 [21-25]. These gene defects were identified in patients with a 2A phenotype showing 2 uncommon characteristics: (1) a peculiar multimeric pattern with a lack of

Table 2.
Localization of Molecular Defects in the von Willebrand Disease (VWD) Variants

Localization of Molecular Defect	VWD Variants
D2 domain	2A (formerly IIC)
D′ domain	2N
D3 domain	2M (formerly type I "Vicenza")
	2A (formerly IIE)
	2N
A1 domain	2B (formerly IIB)
	2M
	2A/2M or unclassified
	2A-like
A2 domain	2A (formerly IIA)
A3 domain	New variant with decreased binding of VWF to collagen
CK domain	2A (formerly IID)

Table 3.
Molecular Defects in the A1 Domain Identified in Patients With Type 2B, 2M, 2A-Like, or Still Unclassified von Willebrand Disease

Type 2B		Type 2M		Type 2A-Like		Unclassified (2A/2M)	
Mutation	Reference	Mutation	Reference	Mutation	Reference	Mutation	Reference
P1266L	42	G1324S	65	R1315C	70	L1276P	40
H1268D	43	G1324A	40			R1374C	71
C1272R	45	E1359K	40			R1374H	71
M1304insM	46	F1369I	66			R1374L	40
R1306Q	47	I1425F	66			C1458Y	40
R1306L	48	Q1191del11	67				
R1306W	49	K1408delK	68				
R1308C	50						
R1308P	47						
I1309V	51						
S1310F	52						
W1313C	53						
V1314F	54						
V1314L	55						
V1316M	56						
P1337L	57						
R1341L	47						
P1341Q	58						
R1341W	59						
L1460V	60						
A1461V	61						

large multimers, a concordant increased proportion of protomer, and an absence or decrease of satellite subbands and (2) a recessive inheritance. The phenotype of these rare VWD patients was previously named IIC. These mutations localized in exons coding for the propeptide presumably interfere with VWF processing and multimer assembly. The patients are either homozygous for 1 of these defects or compound heterozygous with a IIC mutation on 1 allele and another defect inducing the absence of VWF synthesis (stop codon or deletion) on the other allele. The expression of recombinant VWF (rVWF) harboring these IIC mutations has confirmed the involvement of the D2 domain in the multimerization process [22]. Indeed, the essential role of the propeptide in promoting multimer formation has been established. A mutant lacking the propeptide fails to assemble into multimers beyond the dimer stage [26,27]. The propeptide is directly involved in the multimerization process, possibly by catalyzing disulfide interchanges resulting in the formation of interdimeric disulfide bonds [28]. The aa sequence CGLC found in both D1 and D2 domains of VWF propeptide (aa 159-162 and 521-524, respectively), homologous to the catalytic site sequence CGXC of the endoplasmic reticulum disulfide isomerase, appears to be of particular importance in multimer assembly because the addition, by mutagenesis, of a second Gly residue in the CGLC sequence of either domain has been found to prevent the multimerization of recombinant mutated VWF [29].

4.2. Mutations in the D′ Domain

A few months after the reports of the index cases of type 2N VWD [11,30], a missense VWF gene mutation (T791M) located in the D′ domain was characterized in the homozygous state in 1 of these patients, who was from Normandy [31]. Since then, numerous VWF gene missense mutations have been characterized, most of which have been confirmed by expression studies indicating that these mutations do indeed cause the FVIII-binding defect.

These mutations are located in exons 18-20, affecting aa residues in the FVIII domain (aa 764-1035 of pre-pro-VWF): R782W, G785E, E787K, C788R and C788Y, T791M, Y795C, M800V, R816W and R816Q, H817Q, R854Q and R854W, and C858F [12]. The R854Q substitution is extremely frequent.

Moreover, expression studies have shown that some type 2N VWD mutations (C788Y, C788R, D879N, C858F) not only dramatically decrease FVIII binding, but also induce a defect in expression (ie, a quantitative VWF deficiency) and a decrease in the HMW forms of VWF [32-34].

Patients with type 2N VWD are either homozygous or hemizygous for a specific type 2N mutation; some are compound heterozygotes, harboring a mutation on 1 allele and another type 2N mutation on the other. Other patients are type 2N/type 3 compound heterozygotes. All patients exhibit a markedly decreased (or nil) FVIII binding responsible for the FVIII deficiency.

In contrast, the heterozygous presence of a type 2N mutation on 1 allele, with no abnormality of the second allele, is responsible for an intermediate VWF:FVIIIB, inducing no FVIII deficiency and no abnormality of the FVIII:C-to-VWF:Ag ratio.

4.3. Mutations in the D3 Domain

A missense mutation has been identified in exon 27 encoding the D3 domain [35]. This mutation (R1205H) has been found in a particular group of 2M VWD, named

"Vicenza." Patients have moderate to mild bleeding symptoms, slightly prolonged or normal bleeding time, and very low VWF antigen and activity levels in plasma but normal VWF parameters in platelets. Interestingly, these patients display a unique multimer pattern in plasma, with the presence of ultra-HMW multimers, so-called supranormal multimers [36,37]. The inheritance is autosomal dominant. The response to desmopressin is satisfactory.

The R1205H mutation has been identified in Italian and German patients; haplotype identity in the 7 Italian families suggests a common genetic origin of this mutation [35]. In our experience with the French INSERM Network, Vicenza patients are not rare (9 families) and may be misclassified among type 1 VWD as VWF:RCo and VWF:Ag levels are not discrepant and because the visualization of the ultralarge multimers is only possible with low resolution gels with very large pore size. Recently, after review of multimer analysis in a large number of VWD patients, another group estimated that Vicenza VWD is more prevalent than previously appreciated [38]; however, no DNA analysis was performed to confirm the diagnosis.

Today, the pathogenesis of Vicenza VWD is not elucidated. It has been speculated that the low plasma VWF levels are due to an impaired constitutive secretion and that the presence of supranormal multimers in low concentrations is merely the result of a low level of stimulated secretion [35]. Our group carried out expression studies of R1205H in Cos cells and found a decreased expression suggesting an impaired secretion (unpublished results). Elucidation of the pathological mechanism would require expression in cells with adequate storage granules. It is noteworthy that the functional studies that we performed on rVWF and the purified plasma VWF of Vicenza patients showed normal ristocetin- and botrocetin-induced binding of VWF to platelets. This finding could lead to classification of these patients among type 1 VWD.

Recently, 3 other candidate mutations located in exon 26 have been described [39]. One of them (Y1146C) leads to a Vicenza phenotype. The 2 others are cysteine mutations (C1143Y and C1173R) found in patients with type 2A VWD (formerly IIE); expression studies have not yet been published.

These mutations affect cysteine residues that are potentially participating in intermolecular disulfide bonding in the D3 domain essential for VWF multimerization. These mutations are probably rather common.

Of note is the identification, in exons 20, 24, and 27, of 4 type 2N VWD missense mutations responsible for abnormal VWF binding to FVIII (D879N, Q1053H, C1060R, and C1225G) [12,33,40]. Three of these mutations located outside of the FVIII-binding domain probably indirectly affect this site in changing its conformation.

4.4. Mutations in the A1 Domain

The first 5′ part of a large exon (exon 28) encodes the A1 domain. This domain contains the GPIb-binding sites and, therefore, mutations in this exon may modify the binding of VWF multimers to platelets, either increasing or decreasing the affinity of VWF for platelet GPIb, thus causing gain- or loss-of-function of VWF.

4.4.1. Increased Affinity of VWF for Platelet GPIb

A large spectrum of mutations (approximately 20), which are all located in the same region of the A1 domain, mostly in the C1272-C1458 loop, cause a gain-of-function of VWF, which is characteristic of type 2B VWD [41-61]. Inheritance is usually dominant. Patients mostly exhibit low VWF:RCo-to-VWF:Ag ratios and a strikingly enhanced platelet aggregation and binding of plasma VWF to normal platelets in the presence of low ristocetin concentrations. In all patients, platelet VWF multimers are normal. The highest MW forms of 2B VWF spontaneously bind to platelets in vivo, and patients often exhibit thrombocytopenia due to the clearance of VWF-platelet complexes. Thus, plasma VWF generally displays a loss in HMW multimers. However, in some cases (formerly called "type I New York" or "Malmö" phenotypes), plasma VWF multimer distribution is normal [62,63]. Among patients, the bleeding diathesis is variable and may be due to the loss of HMW multimers and/or to the inability of VWF that is adsorbed on platelets to bind to the subendothelium. The administration of desmopressin, which can exacerbate thrombocytopenia, is controversial. Four mutations (R1306W, R1308C, V1316M,and R1341Q) account for approximately 90% of type 2B VWD. In France, among 55 unrelated patients with type 2B phenotype, these mutations are also particularly frequent and so are 2 other mutations: R1306Q and P1337L [40]. Most New York and Malmö phenotypes are due to the same substitution, P1266L [42]. It is interesting to note that some mutations inducing type 2B VWD have also been found in patients with apparent type 2A [40,44,45,54,64]. However, functional studies of the rVWFs harboring these mutations clearly showed that the latter induce an increased affinity of rVWF for GPIb. Thus, the apparent 2A phenotype of the corresponding patients might be explained by the spontaneous binding of plasma high- and intermediate-MW multimers to platelets and by the persistence in the circulation of low-MW multimers unable to bind to platelets. These patients have an extremely severe form of type 2B VWD in contrast to patients with the New York or Malmö phenotypes, who have a mild form. In fact, all type 2B mutations appear to inactivate a regulatory function of the A1 domain and allow binding to platelets in the absence of vascular injury, thereby causing this gain-of-function phenotype.

4.4.2. Decreased Affinity of VWF for Platelet GPIb

Mutations also located in the VWF A1 domain may induce decreased platelet-dependent function [40,65-70]. Some of these mutations cause type 2M (multimer) VWD associated with normal VWF multimer distribution. Other mutations induce a more (or less) pronounced loss of HMW multimers, and patients with these mutations may be classified as type 2A (or 2M), or they may still be unclassified. Studies of mutated rVWFs have helped to enhance understanding of the molecular basis of these variants.

Patients with typical type 2M VWD are characterized by markedly decreased VWF:RCo levels despite the presence of HMW multimers. Binding of plasma VWF to GPIb is decreased in the presence of ristocetin but is normal or sub-

normal with botrocetin. Two unrelated patients have a missense mutation at position 1324 (G1324S and G1324A), characterizing the formerly named "type B" [40,65]. Other missense mutations and a small deletion have been described [40,66,67]. Two other missense mutations, with Phe-Ile changes (F1369I and I1425F), and the E1359K mutation have been described [40,66] and induce a similar phenotype. Two small in-frame deletions, Q1191del11 and K1408delK [67,68], were also reported in type 2M patients, but they both cause decreased ristocetin- and botrocetin-induced binding of VWF to platelets. In all cases, the 2M mutations impair VWF binding to GPIb without affecting multimer assembly [66-69].

Other patients exhibit disproportionately low VWF:RCo levels and a more pronounced decrease of HMW multimers and are thus classified as type 2A rather than type 2M VWD. However, some cases remain unclassified, and the expression of the corresponding rVWF may be helpful. The decrease of HMW multimers and botrocetin-induced binding to GPIb are in favor of type 2A. One mutation, R1315C, which was identified in 10 unrelated French patients, results in an almost typical 2A phenotype that we named "2A-like." The corresponding rVWF exhibits decreased expression, lack of high- and intermediate-MW multimers, and markedly decreased ristocetin- and botrocetin-induced binding to GPIb [70]. It is noteworthy that 3 different mutations at the same aa position (R1374) are also responsible for abnormal multimerization and platelet-dependent function [40,71].

Thus, the existence within the A1 domain of mutations inducing either gain (type 2B) or loss (type 2M or type 2A) of function further underlines that this domain has an important regulatory role in the binding of VWF to platelet GPIb, probably through conformational changes. The distinction between type 2M and 2A tends to be difficult in some cases.

4.5. Mutations in the A2 Domain

The 3′ part of exon 28 encodes the A2 domain, which contains the important proteolytic site between Y1605 and M1606. Around this site 25 missense mutations [40,41] clustered within a 168-aa segment (aa 1504 to 1672) induce type 2A (formerly IIA) VWD with a dominant inheritance [40,72-86]. Two mutations, R1597W and I1628T, are extremely frequent, being identified in one third of French patients [40]. It is probably the easiest variant to identify. Patients display low VWF:RCo-to-VWF:Ag ratios, loss of large and intermediate plasma VWF multimers with concomitant increase of the intensity of the satellite bands, decreased RIPA, and reduced ristocetin- or botrocetin-induced plasma VWF binding to platelets. In 1983, a subdivision of these patients had already been proposed, based on the presence or absence of large VWF multimers in platelets [87]. The latter may account for the variability of desmopressin response in patients with type 2A. In fact, 2 molecular mechanisms responsible for the type 2A phenotype are now recognized after expression of corresponding mutated rVWF. In the first, referred to as group 1, mutations induce defective intracellular transport of VWF, leading to the impaired secretion of VWF multimers in both plasma and platelets [72]. Mutations of group 2 do not interfere with VWF secretion, but render the circulating protein more sensitive to proteolysis in plasma [88]. Recently, the protease responsible for cleavage of the peptide bond between Y1605 (Tyr842) and M1606 (Met843) has been identified [89]; mutations of group 2 may induce a conformational change of VWF, making it more susceptible to cleavage by this protease [90]. The V1665E mutation represents a distinctive category of type 2A mutations that cause extensive intracellular proteolysis of the mutant subunits [91]. Indeed, the mutated protein was shown to be degraded intracellularly to generate a fragment, suggesting that a similar protease is responsible or that the Y1656-M1606 peptide bond is selectively sensitive to a variety of intracellular and extracellular proteases. However, it is not well understood why mutations lead to one or the other causative mechanism.

4.6. Mutation in the A3 Domain

Recently, our group studied 2 related patients with mild bleeding symptoms and a slightly prolonged bleeding time; VWF levels were moderately decreased; the plasma VWF multimeric pattern as well as ristocetin- or botrocetin-induced binding of VWF to platelets were normal. In contrast, both patients repeatedly showed decreased binding of VWF to collagen. A new mutation S1731T within exon 30 of VWF gene was identified, and expression studies confirmed that this mutation was responsible for the abnormal binding of VWF to collagen [92].

4.7. Mutations in the CK Domain

The last exon (exon 52 of the VWF gene) encodes the "cysteine knot" (CK) domain. In the endoplasmic reticulum, pro-VWF species form disulfide-linked dimers through some cysteine residues located in the 90-aa CK domain. Thus, the structure of this domain is crucial for the dimerization of VWF subunits and therefore for its subsequent multimerization [93]. A rare dominant form of type 2A VWD (formerly named type IID) was described in 1984 and is characterized by an absence of large multimers in both plasma and platelets, with abnormalities of the internal structure of each multimeric unit detected using high-resolution gels [94]. More recently, a missense mutation (C2773R), which disrupts a disulfide bond required for dimer formation, has been described in patients who cannot assemble large multimers [95].

4.8. Research of Mutations in the C1 Domain

To date no gene defects causing type 2 VWD have been described in exons encoding for the C1 domain containing the platelet GP IIb/IIIa-binding site.

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