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Molecular and Cellular Biology of Von Willebrand Factor

Susan E. Lyons and David Ginsburg

Von Willebrand factor (vWF), a central protein in the regulation of blood coagulation, serves as a major adhesive link between platelets and the blood vessel wall and also functions as a carrier in plasma for factor VIII. Abnormalities of vWF result in von Willebrand disease (vWD), a common inherited human bleeding disorder. Deficient von Willebrand factor function has been proposed as potentially protective against the development of coronary vascular disease and several recent investigational therapies are directed at the vWF–platelet interaction. This review summarizes the current state of knowledge regarding the biosynthesis and processing of vWF and the relationship of vWF structure to function. Finally, recent progress in identifying specific genetic mutations responsible for the many variants of vWD is discussed. (Trends Cardiovasc Med 1994;4;34–39)

Von Willebrand factor (vWF), a multimeric plasma glycoprotein, plays a central role in hemostasis. vWF mediates initial platelet adhesion to the subendothelium after vascular injury. In addition, vWF carries factor VIII in the circulation and is required for factor VIII stability in plasma (Figure 1). vWF is synthesized from an 8.7-kilobase (kb) mRNA and is expressed exclusively in endothelial cells and megakaryocytes. The vWF gene contains 52 exons, spanning 178 kb on chromosome 12. A partial unprocessed pseudogene, duplicating exons 23–34, is present on chromosome 22 (Sadler 1991, Ginsburg and Bowie 1992) (Figure 2).

In 1926, Erik von Willebrand first described a severe bleeding disorder in a family on an island of the Åland archipelago. The autosomal dominant inheritance pattern of the disease, later named von Willebrand disease (vWD), clearly showed that it was distinct from hemophilia A, an X-linked bleeding disorder caused by defects in factor VIII. In 1971, vWF was immunologically distinguished from the factor VIII and, in 1985, the vWF cDNA was cloned from endothelial cell cDNA libraries and confirmed by amino acid sequencing (Ginsburg and Bowie 1992). Since that time, a great deal has been learned about vWF's structure, function, and the defects that result in vWD.

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• Von Willebrand Factor Biosynthesis

Synthesis of vWF is a complex multistep process. The vWF primary translation product is a 2813-amino-acid pre-propolypeptide with a 22-amino-acid classic signal sequence that is cleaved cotranslationally (Figure 2). The 741-amino-acid propeptide is removed at a later step, leaving a 2050-amino-acid mature vWF protein. Posttranslational processing of vWF includes dimerization, glycosylation, sulfation, propeptide cleavage, and multimerization, followed by storage or secretion (Figure 3). Characterization of these biosynthetic steps in megakaryocytes and cultured endothelial cells

has shown that N-linked carbohydrates are added in high-mannose form in the endoplasmic reticulum (ER) and are further processed to complex forms in the Golgi apparatus (Wagner 1990). O-glycosylation and sulfation of some N-linked oligosaccharides also occur in Golgi compartments. vWF molecules dimerize within the ER via disulfide bond formation at the carboxyl-terminal end. The propeptide is cleaved in Golgi and post-Golgi compartments and disulfide linkages are formed at the amino termini to generate high-molecular-weight (HMW) multimers consisting of up to 100 subunits. vWF is the only protein known to form disulfide-linked multimers in a compartment past the ER. In cultured endothelial cells, weak base inhibits multimerization of dimers, and acidic pH is required in cell-free synthesis, suggesting that multimerization occurs in an acidic cellular environment. These data suggest that an active catalytic process may be involved in disulfide bonding of dimers, since spontaneous oxidation of free sulfhydryls would be optimal at basic, not acidic pH (Wagner 1990).

vWF is secreted via constitutive and regulated pathways. In cultured endothelial cells, 95% of vWF synthesized is secreted constitutively, while the remaining 5% is packaged in specialized storage granules termed Weibel–Palade bodies (Wagner 1990). Similar granules observed in platelets are termed α granules. In electron-microscopic studies, these granules appear as rodlike structures 0.1 μm wide and up to 4 μm long. Tubular structures seen inside the granules are hypothesized to be vWF multimers. Recently, a transmembrane receptor, P selectin, also known as GMP140, PADGEM, or CD62, was found to colocalize with vWF to Weibel–Palade bodies and α granules (Bonfanti et al. 1989).

In vivo, plasma vWF levels rise rapidly after administration of epinephrine, vasopressin, nicotinic acid, or the vasopressin analogue, 1-desamino-8-D-arginine-vasopressin (DDAVP). These agents appear to cause release of vWF from storage pools, though they do not stimulate vWF secretion from endothelial cells in vitro. In cultured endothelial cells, the release of vWF from storage granules can be stimulated with various secretagogues, including the calcium ionophore A23187, phorbol myristate acetate (PMA), thrombin,

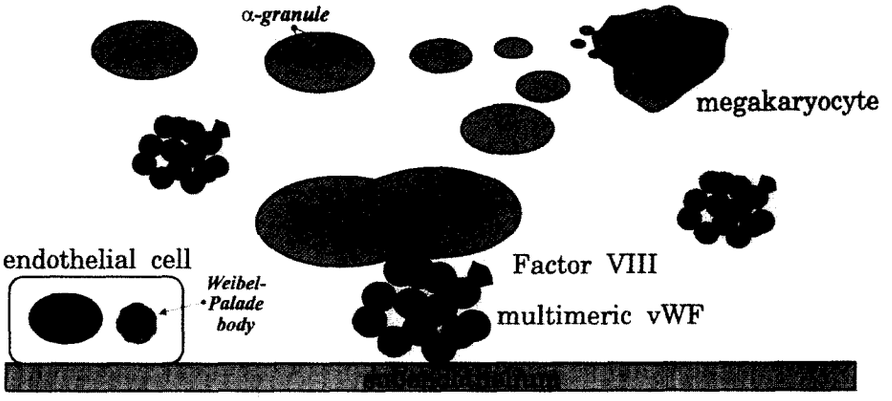


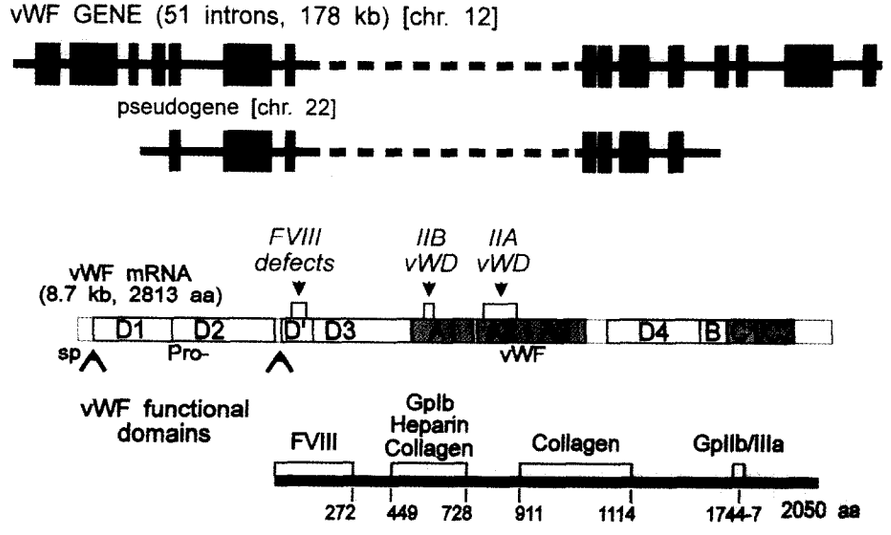
Figure 1. Von Willebrand factor (vWF) function; vWF monomers are indicated as *red circles*. Multimeric vWF is seen to be stored within the Weibel–Palade body of the endothelial cell and in the granule precursor of the megakaryocyte, subsequently giving rise to the platelet α granule. vWF is shown bound to the platelet surface and to the subendothelium, serving a critical adhesive bridging function. The plasma complex of multimeric vWF with factor VIII also acts to localize factor VIII to the site of clot formation.

fibrin, histamine, and complement proteins C5b–9 (Wagner 1990). Thrombin and fibrin are present at sites of vascular injury, while histamine and complement factors are localized to areas of inflammation. The storage granules have been shown to contain larger multimeric vWF species than the constitutively secreted pool (Sporn et al. 1986). Release of these larger multimers, which are most effective in mediating platelet-binding functions, may play a role in the rapid response to vascular injury.

- **Von Willebrand Factor Expression in Heterologous Cells**

Cloning of the cDNA for vWF has enabled study of vWF biosynthesis in heterologous cells. vWF has been expressed by

Figure 2. Schematic of the human von Willebrand factor (vWF) gene, structural features of pre-pro-vWF, and functional domains within the mature vWF protein: the vWF gene and pseudogene are shown at the **top**, with *blue boxes* representing exons and the *line* representing introns. The vWF primary translation product, pre-pro-vWF, is depicted in the **middle**. The location of signal peptide (sp) and propeptide (pro) cleavage sites are indicated by *arrowheads*, and repeated domains are labeled with letters, *A–D*. Regions containing clustered mutations responsible for factor-VIII (FVIII)-binding defects, type-IIB von Willebrand disease (vWD), and type-IIA vWD are indicated. At the **bottom** of the panel, the locations of vWF functional domains within the mature vWF protein are shown, with amino acid numbers for mature vWF indicated below. Adapted from Ginsburg and Bowie (1992), with permission.



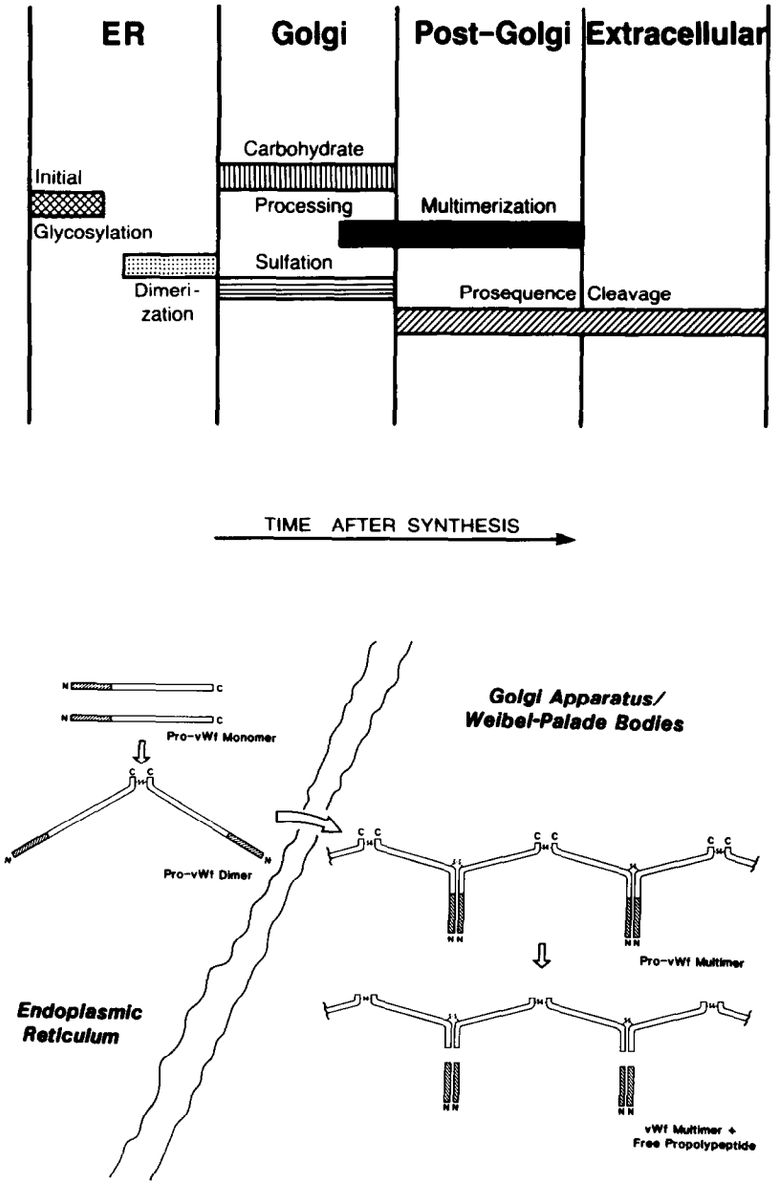


Figure 3. Posttranslational processing of von Willebrand factor (vWF): the processing and assembly of vWF is illustrated schematically here. The sequential occurrence of each step within discrete intracellular compartments is summarized at the **top** of the figure. Dimerization of pro-vWF monomers via disulfide formation at the C termini is shown at the **bottom**, with subsequent multimerization via interactions at the N termini within the Golgi and later compartments. From Ruggeri et al. (1994), with permission.

transient and stable transfection in a variety of cell types. In most cases, vWF is secreted as HMW multimers, pro-vWF is correctly cleaved to the mature form though less efficiently than in endothelial cells, and the recombinant vWF appears to function comparably to endothelial vWF in platelet- and collagen-binding assays (Bonthron et al. 1986, Meulien et al. 1992, Verweij et al. 1987, Wise et al. 1988). Although the vWF molecule itself appears to specify the information needed for most of the steps in vWF synthesis in heterologous cells, only secretory cell lines exhibit regulated secretion of vWF, suggesting that the specialized cellular machinery for storage is required for vWF transport to secretory granules (Wagner et al. 1991).

The 741-amino-acid vWF propolypeptide has been shown to be required for multimer assembly. When COS cells were transfected with vWF cDNA lacking the sequence for the propolypeptide, only dimers were formed (Verweij et al. 1987, Wise et al. 1988). Therefore, the propeptide is not required for dimerization, but is essential for further multimerization. Studies by Verwiej et al. (1988) and by Wise et al. (1988) showed that an uncleavable pro-vWF species formed normal multimers in COS cells. Surprisingly, the propolypeptide could be present in *trans* and still direct multimer assembly (Wise et al. 1988). Cotransfection of the cDNA for the mature vWF protein with the cDNA for the propeptide on a separate plasmid resulted in secretion of vWF multimers indistinguishable from multimers secreted by cells transfected with the full-length vWF cDNA. In a cell-free system, multimerization of dimers also required the propeptide, consistent with results in COS cells. In this system, however, the propeptide was required in *cis* with the mature vWF sequence in order for multimer assembly to proceed (Mayadas and Wagner 1989). Recently, Mayadas and Wagner (1992) proposed a disulfide isomerase activity for the propolypeptide. Protein disulfide isomerases are found in the ER, where folding and oligomerization typically take place; thus, the presence of a disulfide isomerase activity within vWF would provide a mechanism for vWF multimerization in a post-ER compartment. In addition, a role for the vWF propeptide in targeting pro-vWF to the storage granule has been proposed (Wagner et al. 1991). A proprotein-processing enzyme termed PACE or furin, that cleaves pro-vWF at the correct cleavage site, has recently been identified (Wise et al. 1990).

• Von Willebrand Factor Function

vWF–Factor VIII Interaction

vWF forms a complex with factor VIII in plasma through electrostatic and hydrophobic interactions. vWF protects factor VIII from degradation and may also serve to localize factor VIII to the site of a clot. Activation of factor VIII by thrombin results in dissociation of the vWF–factor VIII complex, allowing activated factor VIII to perform as a cofactor in activation of factor X by factor IXa, and also rendering it susceptible to degradation by activated protein C (Kaufman

1992). The domain(s) on vWF responsible for binding to factor VIII has been localized to the first 272 amino acids of vWF (Sadler 1991, Ruggeri et al. 1994). Propeptide cleavage appears to be required for factor VIII binding, though the exact role of the propeptide in formation of the factor-VIII-binding site is controversial (Leyte et al. 1991, Wise et al. 1991, Kaufman 1992).

While the concentration of vWF in plasma is 10 μg/mL and factor VIII in plasma is only 0.2 μg/mL, vWF appears to be saturated with factor VIII at a molar ratio of approximately 50:1 (vWF to factor VIII). This saturation of vWF with factor VIII may explain the observation that decreases in plasma vWF are generally accompanied by corresponding decreases in factor VIII levels. Thus, in severe forms of vWD, the clinical picture often has characteristics both of a platelet disorder and of hemophilia A (Sadler 1991, Ginsburg and Bowie 1992).

Role of vWF in Adhesion to the Subendothelium

vWF is required for platelet adhesion at sites of vascular injury. When vessel wall subendothelium is exposed, vWF binds to the subendothelial matrix and to platelets, promoting platelet aggregation and formation of a platelet plug. Constitutively secreted vWF is found both distributed in the basement membrane and free in plasma. An additional pool of higher-molecular-weight vWF is present in the storage granules of the platelet and endothelial cell and can be released in response to vessel wall injury. The largest vWF multimers have higher affinity for binding to cell matrix than do the smaller vWF multimeric species (Ruggeri et al. 1994).

The components of the subendothelial matrix involved in the binding of vWF have not been conclusively identified. In vitro, vWF has been shown to bind collagen types I, III, and VI, heparin, and sulfatides. However, antibody studies and treatment of endothelial matrix with collagenase, or growth of extracellular matrix in the presence of the collagen synthesis inhibitor, α,α′-dipyridyl, suggest that the fibrillar collagens (types I and III) are not necessary for vWF subendothelial binding (Ruggeri et al. 1994). Collagen VI, which is resistant to collagenase and α,α′-dipyridyl, remains a potential ligand for vWF in the matrix (Rand et al. 1991).

Role of vWF in Platelet Adhesion and Aggregation

Platelets adhere to vWF through two distinct receptors, GpIb/IX and GpIIb/IIIa. Unlike the factor VIII interaction with vWF, platelet binding depends on multimeric size. HMW vWF multimers bind platelets at both receptors with much higher affinity than the lower-molecular-weight forms (Gralnick et al. 1981). In vitro, platelet adhesion mediated by vWF is highly shear rate dependent. At low shear rates, platelet adhesion to subendothelium can take place even in the absence of vWF. However, at high shear rates, simulating the environment in the microvasculature, vWF function becomes essential (Weiss et al. 1989, Ruggeri et al. 1994).

Circulating vWF does not bind to resting platelets in normal hemostasis. Upon exposure of vessel subendothelial matrix, vWF binds matrix components and then gains the ability to bind the platelet receptor GpIb (Wagner 1990, Ruggeri et al. 1994). These results suggest either that vWF undergoes a conformational change after matrix binding that enables it to bind to GpIb, or that, alternatively, a high local concentration of vWF is required for platelet GpIb binding to occur. Experimentally, plasma vWF can bind to platelet GpIb after removal of sialic acid residues from the vWF molecule (Gralnick et al. 1985), or by the addition of the antibiotic ristocetin or the snake-venom protein, botrocetin (Ruggeri et al. 1994). These alterations may in some ways imitate the changes that occur through vWF binding to subendothelium in vivo, but to date these effects are not well understood. Since the botrocetin-binding site on vWF is in the same region as binding sites for the subendothelial matrix components, collagen and heparin, and the GpIb binding site, it is hypothesized that the formation of the botrocetin–vWF complex mimics the conformational changes that are normally brought about through vWF interaction with the subendothelium.

The platelet GpIIb/IIIa receptor only becomes available for vWF binding after platelet activation. Platelets are activated at sites of vascular injury by a variety of agonists. In addition, binding of vWF to the platelet GpIb receptor appears to activate platelets, making the GpIIb/IIIa receptor available for ligand binding (Savage et al. 1992). GpIIb/IIIa is a member of the integrin family of adhesive proteins, which bind the consensus site RGD. An RGD sequence near the C terminus of vWF appears to be responsible for vWF binding to GpIIb/IIIa (Beacham et al. 1992). Other adhesive proteins, including fibrinogen, thrombospondin, fibronectin, and vitronectin, also contain RGD sites and may compete with vWF for binding to the GpIIb/IIIa receptor. The RGD site on subendothelial matrix vWF may also contribute to endothelial cell attachment to the vessel wall.

The Central Role of vWF in Thrombosis and Hemostasis

The central functional role of vWF in hemostasis is illustrated in Figure 1. Through distinct binding domains for discrete receptors on the platelet surface and other receptors within the subendothelium, vWF performs a bridging function, binding the platelet to the vessel wall at sites of vascular injury. This appears to be one of the earliest events triggering formation of the initial platelet plug. The unique multimeric structure of vWF may be particularly important for its adhesive function by enabling multivalent interactions. By serving as the carrier for factor VIII, vWF may also coordinate formation of the fibrin blood clot at the site of a growing platelet thrombus. The critical role of vWF in coagulation is evident from the profound bleeding disorder that results from complete deficiency of vWF (see below). Indeed, patients with severe vWD suffer from both the immediate type of bleeding associated with loss of platelet function as well as the often delayed visceral bleeding resulting from deficient fibrin clot formation, characteristic of classic hemophilia. The central position of vWF in clotting function has made it a target for a number of novel antithrombotic therapies centered on antibodies, peptides, or other compounds that inhibit vWF function (Ruggeri 1992).

• Domain Structure of von Willebrand Factor

vWF has a repeated structure, consisting of four types of domains A–D, shown in Figure 2 (Sadler 1991, Ginsburg and Bowie 1992). Each domain is repeated

2–5 times within the vWF polypeptide, accounting for ~90% of the protein sequence. The A1 domain contains a number of important functional regions, including binding sites for collagen, heparin, sulfatides, GpIb, and botrocetin. The A3 domain contains an additional collagen binding site. The C1 domain contains the RGD(S) sequence recognized by the integrins GpIIb/IIIa and the vitronectin receptor. Another RGD sequence is present in the propeptide, but no role in integrin binding has been observed. Cysteine is the most common amino acid in vWF, comprising 8.3% of the vWF polypeptide. A total of 52 disulfide bridges have been defined, all involved in intra- or intersubunit disulfide bonds (Sadler 1991, Ginsburg and Bowie 1992, Ruggeri et al. 1994).

• Von Willebrand Disease

vWD encompasses a heterogeneous group of inherited defects in vWF. In epidemiologic studies in which populations are examined for laboratory abnormalities in vWF, a prevalence of vWD as high as 1% has been reported (Rodeghiero et al. 1987), though clinically significant vWD is probably observed less frequently in the population. Symptoms vary from mild to severe bleeding, and inheritance is generally autosomal dominant. A large number of vWD subtypes have been described, distinguished by subtle variations in vWF activity and/or multimer pattern (Ginsburg and Bowie 1992, Bloom 1991). The vast majority of vWD subtypes can be broadly placed into two types. Type-I vWD is characterized by a quantitative defect in plasma vWF, with normal vWF multimer structure. Type-III vWD is the severe quantitative form of vWD with very low or undetectable plasma vWF. Type-II vWD variants are associated with qualitatively abnormal vWF. The molecular genetics of vWD has recently been reviewed (Ginsburg and Bowie 1992).

Type-I/III vWD

Type-I vWD is the most common form, accounting for approximately 70%–80% of all vWD. Type-III vWD is quite rare, with a prevalence of 0.5–3 per million (Weiss et al. 1982). Though parents of type-III vWD patients are generally asymptomatic (recessive inheritance), in some cases parents have been observed to have mild type-I vWD. Whether type-I vWD represents the heterozygous form of type-III vWD or a distinct genetic disorder is currently unknown. Gross gene deletions, nonsense mutations, and a frameshift mutation [reviewed by Ginsburg and Bowie (1992) and Ginsburg and Sadler (1993)] have been identified as the molecular defects in a limited number of type-III vWD patients.

Type-IIA vWD

The most common type-II vWD subtype, type IIA, is characterized by selective loss of intermediate and HMW multimers. The bleeding diathesis in type-IIA vWD is thought to result from the deficiency of these larger vWF multimeric species, which appear to be most potent in mediating platelet adhesion. Recently, specific single amino acid substitutions have been identified as the molecular basis for type-IIA vWD in a large number of patients (Ginsburg and Bowie 1992, Ginsburg and Sadler 1993). With two possible exceptions, all of these mutations are located within a 134-amino-acid segment of the vWF A2 domain (Figure 2). Expression of recombinant vWF containing type-IIA vWD mutations in heterologous cells suggests that type-IIA vWD arises via two distinct molecular mechanisms. For one set of mutations, referred to as group 1, the single amino acid substitution results in a defect in intracellular transport at the level of the ER. Since type-IIA vWD is autosomal dominant, the mutant allele is coexpressed with a normal allele. The larger the multimer form, the greater is the probability of containing one or more mutant subunits and the greater is the likelihood that it will be retained within the ER. Thus, only the smallest multimeric species are secreted from the cell, accounting for the classic type-IIA vWD phenotype (Lyons et al. 1992). In group-2 type-IIA vWD, normal vWF multimeric species are secreted in vitro, but are presumably cleaved by one or more plasma proteases in vivo, resulting in the characteristic loss of large multimers (Lyons et al. 1992, Dent et al. 1991).

Type-IIB vWD

Type-IIB vWD is also characterized by loss of large vWF multimers, but through a unique "gain of function" mechanism involving increased affinity for platelet GpIb. Patients have thrombocytopenia, absence of HMW plasma vWF multimers, and significant clinical bleeding. Spontaneous binding of the most active large type-IIB vWF multimers to platelets, followed by clearance of these complexes from the circulation, is thought to result in the characteristic type-IIB phenotype. A number of mutations have been identified in type-IIB vWD patients, all located within a disulfide bond loop between Cys509 and Cys695 in the GpIb-binding region of vWF (Figure 2). Four common mutations account for >90% of the reported type-IIB vWD patients (Ginsburg and Bowie 1992, Ginsburg and Sadler 1993).

Factor-VIII-Binding Defects

A rare group of patients have recently been reported with reduced factor VIII levels, normal vWF antigen levels and activity, normal bleeding time, and a bleeding phenotype similar to mild to moderate hemophilia A, but with apparent autosomal recessive inheritance. This variant has been termed vWD "Normandy" after the birthplace of one of the first patients. Mutations within the factor-VIII-binding domain of vWF, which abolish or reduce binding of factor VIII to vWF, have now been identified in a number of these patients (Mazurier 1992, Ginsburg and Sadler 1993, Ginsburg and Bowie 1992). These mutations are all located within the factor-VIII-binding domain at the N terminus of vWF (Figure 2).

• Future Directions

A great deal has been learned about the unique cellular and molecular biology of vWF since the first distinction of this protein from factor VIII ~20 years ago. Recently, mutations responsible for many of the unique vWD variants have been identified. With the characterization of additional mutants, it should become possible to diagnose and classify vWD by using a DNA diagnostic approach. Further understanding of the complex structure and function of vWF also has important implications for the understanding of the regulation of blood coagulation. vWF presents a particularly attractive target for the development of new anticoagulant therapies. Elevated vWF levels may be an independent risk factor for coronary-artery disease, and vWD has been postulated to

provide a protective effect (Ginsburg and Bowie 1992). Since the moderate quantitative decrease in vWF associated with the common type-I variant of vWD is generally associated with only a mild bleeding disorder, therapies designed to disrupt vWF function could be anticipated to have a relatively large therapeutic index and relatively small risks of unwanted hemorrhage. Indeed, a number of antibody and recombinant peptide reagents are currently under development as potential new anticoagulants (Ruggeri 1992). Thus, the recent explosion in knowledge about vWF should have important clinical impact, not only for the care of vWD patients, but for the much larger population at risk for pathologic thrombosis and hemorrhage.

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