Role of von Willebrand factor in the haemostasis

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von Willebrand factor (VWF) is an adhesive and multimeric glycoprotein that found its historical origin in 1924, when the Finnish physician Erik von Willebrand first reported a family with a serious hereditary bleeding affecting consanguineous families[1]. The proband was a five years old girl with severe bleeding since birth. Three sisters had died before the age of four, one living sister, aged three, also was severely affected. von Willebrand had thought that it was a disorder of platelet function or a vascular defect as a possible cause of the bleeding.

Since the original observations by Erik von Willebrand, the disease has been extensively studied and it was shown in the mid 1950s that impaired haemostasis was because of lack or an abnormality of a plasmatic factor - the von Willebrand factor - necessary for normal hemostasis[2,3].

Molecular biology of von Willebrand factor

The advent of modern molecular techniques led to the cloning of the *VWF* gene in 1985 using endothelial cell cDNA libraries[4-6]. The gene is located on the short arm of chromosome 12 at the locus 12p13.3[7] and spans 178 kilobases. The human *VWF* gene contains 52 exons and the exon 28 is the largest, its length is 1.4 kb[8]. The *VWF* gene is transcribed into a 9 kb mRNA which is translated into a protein of 2813 amino acids with an estimated M_r of 310,000 daltons. Comparison of the available protein sequence of the plasma VWF polypeptide subunit with the redicted sequence from the cDNA established the pre-propolypeptide nature of VWF. Pre-propolypeptide VWF contains a 22 amino acids signal peptide, a 741 amino acids pro-polypeptide and the mature subunit[9-10]. Cleavage of the 741 amino acids propolypeptide from the aminoterminus results in the mature VWF subunit of 2050 amino acids. A partial, highly homologous, VWF pseudogene, encompassing exons 23-34 of the functional gene, resides on chromosome 22[11]. Ninety-seven percent sequence homology with the authentic *VWF* gene on chromosome 12 suggests a recent evolutionary origin of the pseudogene[12]. The homologous nature of the pseudogene may complicate analysis of the *VWF* gene when examining genomic DNA.

Analysis of the amino acid sequence shows extensive repetition which defines four distinct domains that are repeated from two to four times each. There are three A-domains, three B-domains, two C-domains and four D-domains. The A-domains correspond to the Cysteine-poor region of VWF. The B-domains are small and contain 25 to 35 amino acid residues, while the duplicated C-domains contain 116 to 119 residues. The four D-domains contain 351 to 376 residues present in four copies. These are arrange in the sequence: D1-D2-D'-D3-A1-A2-A3-D4-B1-B2-B3-C1-C2-CK. The protein is remarkably rich in cysteine, which comprises 234 of the 2813 residues in pre-pro-VWF. In the secreted protein, all cysteine residues appear to be paired in disulfide bonds. The VWF subunit comprises domains of which specific functions have been identified. The D'-D3 domains exhibit a binding site for factor VIII (FVIII) and for heparin. In addition, the D'-D3 domains are possible binding sites for P-selectin, which has been found to anchor newly released ultra-large VWF to the surface of activated endothelial cells and thus present the VWF cleavage site to ADAMTS-13, a disintegrin-like and metalloprotease with thrombospondin type 1 (TSP1) motif[13-14]. The A1 domain is the only known binding site for the platelet receptor glycoprotein (GP) Ibα, and contains additional binding site for heparin, sulphated glycolipids and the snake venum botrocetin. Although controversially discussed, the A1 domain also seems to provide a binding site for collagen. The A2 domain contains the cleavage site for the metalloprotease ADAMTS-13. The A3 domain is the binding site for fibrillar collagen type I and III. The C1 domain which comprises the RGD sequence, is the binding site for the integrin αIIbβ3.

In vivo biosynthesis of VWF is limited to

endothelial cells and megakaryocytes[15-16]. Endothelial cells synthesize VWF as a pre-pro-VWF, a signal peptide, the pro-peptide and the mature VWF subunit. After synthesis into the endoplasmic reticulum, the signal peptide is cleaved, and 12 *N*-linked high-mannose containing oligosaccharide chains are added to each VWF molecule. *N*-linked glycosylation refers to the linkage of carbohydrate structures to the terminal amide-group of Asn-residues present in the motif Asn-XXX-Ser/Thr/Cys, where X is any amino acid except Pro. Following glycosylation, dimerisation of pro-VWF occurs through inter-subunit carboxyl termini disulphide bond formation[17]. This "tail-to-tail" dimerisation function requires only sequences within the last 150 residues. The carboxyl-terminal 90 residues comprise the "CK" domain that is homologous to the "cystine knot" superfamily of protein. These family members share a tendency to dimerise, through disulfide bonds. Alignment of the VWF "CK" domain with its nearest homologous shows the cysteine that forms an interchain disulfide and suggests that Cys2010 of the mature VWF subunit may contribute to dimerisation of pro-VWF in the endoplasmic reticulum. This conclusion is supported by the phenotype of patients with Cys2010Arg mutations, who cannot form large VWF multimers[18]. In the Golgi apparatus, the *N*-linked oligosaccharide chains of VWF are further modified by a series of glycosidases and glycosyltransferases to produce complex type carbohydrates. In addition, 10 *O*-linked oligosaccharide chains are also added to each monomer. *O*-linked glycosylation involves the attachment of *N*-acetyl-galactosamine moieties to Serine and Threonine residues, a process that occurs at a later stage during synthesis. Finally, multimerisation of pro-VWF dimers takes place in the post-Golgi involving another round of disulphide bond formation near the amino-termini of the subunits. As a result, mature VWF exits in the plasma as a series of oligomers containing a variable numbers of subunits, ranging from a minimum of two to a maximum 40, with the largest multimers having molecular weights in excess of 20,000 kDa[19]. Additional modifications in the trans-Golgi network include the proteolytic removal of the large VWF propeptide and multimer formation follow sulfatation. The VWF propeptide plays an essential role in the assembly of multimers. Deletion of the propeptide abolished the multimerisation. The propeptide structure has suggested a possible mechanism for its function. The D1-D2 domains of the VWF propeptide contain CXXC sequences, where C is cysteine residues, that resemble the functional sites of thiol: disulfide oxidoreductases. Insertion of an extra glycine into these sequence was compatible with dimmer formation in the endoplasmic reticulum, but the dimers were transported to the Golgi and secreted without forming multimers[19].

However, the VWF synthesised within endothelial cells is either directly released into the plasma through a constitutive secretory pathway, or tubulised and stored into organelles known as Weibel-Palade bodies (WPBs), rod-shaped organelles unique to endothelial cells. VWF is not only stored in WPBs but it also drives the formation of these vesicles. Although the expression of VWF is necessary, it is not sufficient to drive the formation of WPBs. Expression of VWF in some cell lines, including COS (monkey kidney cells) does not result in the formation of pseudo-WPBs. And the porcine aortic endothelial cells lack WPBs although they do express VWF. The tubular storage is supposed to compact VWF by 100-fold and determine the unique shape of WPBs. The elongated shape of WPBs may be essential to the physiological function of VWF because the haemostatic function of this protein relies on its storage format as highly compacted tubules[20]. The twisted tubular striations observed by tomographic analysis indicate these tubules may be assembled by a "spring-loading mechanism". Upon exocytosis, it allows a rapid unfolding of VWF tubules into ultralong strings (up to 100 μm) docking on the endothelial cells to adhere the platelets[21]. It is generally assumed that VWF stored within WPBs is composed of the largest multimeric species[17,22], ultra-large VWF, usually not observed in the blood of normal individuals[23]. In response to several agonists of physiological relevance, such as histamine, oestrogens, thrombin and fibrin, regulated secretion of stored VWF from endothelial cells occurs[24]. VWF multimers and the VWF propeptide are secreted together in 1:1 stoichiometric amounts, but subsequently have different fates. After secretion, the propeptide dissociates from VWF multimers and circulates independently as a non-covalent homodimer with a very short half life of ~2 hours. The plasma level of VWF propeptide is ~1 μg/mL. In contrast,

VWF multimers are cleared more slowly with a half life of ~12 hours, and the plasma concentration averages ~10 μg/mL.

The second storage site for VWF is within the platelet α-granules, which may contain as much as 20% of the total VWF present in blood. The VWF of platelet α-granules characteristically also consists of the ultra-large VWF multimers[25]. The release platelet VWF from α-granules can be triggered by a variety of different agonists, including adenosine diphosphate (ADP), collagen and thrombin and this ensures ultra-large VWF multimers availability at site of vascular injury.

VWF functions

VWF is an adhesive plasma glycoprotein which performs its haemostatic functions through binding to FVIII, to platelets surface glycoproteins, and to constituents of connective tissue. VWF acts as a stabilizer of FVIII in the circulation. This is obtained by the formation of a non-covalently bound VWF-FVIII complex that protects FVIII from degradation by activated protein C, and localises FVIII to sites of platelet plug and subsequent clot formation[26,27]. The FVIII binding site on VWF is within the amino-terminal 272 amino acid residues of the mature subunit. Amino acid residues in the FVIII protein involved in the binding of VWF are located in the segment between residues 1669-1689, and optimal binding requires sulfatation of Tyr1680. When FVIII is activated during blood coagulation, thrombin cleaves it after Arg1689. This cleavage destroys the VWF binding site and release FVIIIa. In addition, VWF blocks the interaction of FVIII with lipoprotein-related receptors and thereby increases the half life of FVIII in the circulation. VWF for FVIII has an important protective role both under normal physiological conditions and in patients with haemophilia[19,28]. In haemophilia patients who have developed FVIII inhibitors following replacement therapy, VWF may protect exogenous FVIII from the binding of inhibitory antibodies[29]. The major inhibitor epitopes in FVIII are located in the A2 domain (residues 373-740) or in the C2 domain (residues 2173-2332) of FVIII. Some epitopes are also located in the A3 domain. The most controversial risk factor for the development of inhibitors in haemophilia A patients comes from the type of the therapeutic FVIII molecule used. A systematic review on the epidemiology of inhibitors in haemophilia A investigated the influence of different FVIII products, plasma-derived (pd) or recombinant FVIII (rFVIII)[30]. Plasma-derived products may vary in purity and VWF content. Recombinant products produced in mammalian tissue culture are of high purity and do not contain VWF multimers. Some studies had claimed a higher propensity of recombinant products to cause inhibitor formation, which, however, lack prospective studies. For example, a recent report documents a higher success rate using a plasma-derived, VWF containing product compared with recombinant FVIII in elimination of inhibitors by immunotolerance[31]. It has been hypothesised that VWF could prevent binding of inhibitors by masking epitopes on the C2 domain, probably due to the presence within C2 of overlapping binding sites for the two ligand or by competition for processing by antigen-presenting cells[32].

VWF has a central role in primary haemostasis where it mediates platelet adhesion to damaged vascular subendothelium and subsequently platelet aggregation. Following a vascular injury, VWF binds specifically to fibrillar collagen type I and III. Binding sites for fibrillar collagen have been identified within VWF domains A1 and A3, although mutagenesis studies suggest that the major site in VWF domain A3 and the minor site in domain A1 interact with different targets on collagen. Recently has been reported that the A3 domain is necessary and sufficient to support binding to fibrillar collagen type I and III, while A1 domain is involved in binding to collagen type VI[33,34]. Once VWF is immobilised in subendothelial connective tissue, its main function is to mediate adhesive interactions of platelets exposed to rapid blood flow. Two distinct platelets receptor for VWF, the glycoprotein GPIbα in the GPIb-IX-V complex and the integrin αIIbβ3 (GPIIb-IIIa complex) are localised on platelet membrane. VWF-dependent platelet adhesion occurs optimally under conditions of high fluid shear stress, but such conditions are cumbersome to duplicate *in vitro* and several more convenient surrogate assays are commonly used, as a bacterial glycopeptide antibiotic ristocetin or botrocetin, protein derived from the viper *Bothrops jararaca* that cause platelets agglutination through mechanism dependent on VWF and GPIbα. Thus, only

the use of experimental models that approximate the hemodynamic conditions in the circulation has elucidated the distinct physiological significance of the different ligand-receptor interactions that mediate VWF binding to platelets. The binding of GPIbα to the A1 domain of VWF can tether platelets to a surface when the flow velocity is elevated, such as the high shear stress (>5,000 s^{-1}) found in arterial circulation[37]. Interaction of the platelet GpIb receptor with the A1-domain of immobilised VWF results in initial adhesion, characterised by a continuous surface translocation of the platelets. The process ultimately leads to stable platelet adhesion by means of interaction of the platelet collagen receptors GpVI and GpIa/IIa38, activation of the platelet αIIbβ3 receptor complex[39], and finally platelet aggregation.

Plasma VWF exists as a multimers of various sizes, the largest VWF multimers are usually contained in storage granules and are not seen in the blood of normal people. Ultra-large VWF multimers can be detected in normal plasma only transiently, after induction of secretion from endothelial cells with the therapeutic agent I-deamino-δ-D-arginine vasopressin (DDAVP)[40] or in diseases such as thrombotic thrombocytopenic purpura (TTP)/haemolytic uraemic syndrome. Ultra-large VWF multimers are hyperactive in binding the platelet receptor GPIb-IX-V complex, which results in spontaneous platelet aggregation[41]. Because of this prothrombotic property, ultra-large VWF should be quickly removed from the plasma of healthy individuals[23]. The regulation of plasma VWF size occurs by specific proteolytic process by the metalloprotease ADAMTS-13[42]. VWF is cleaved within the A2 domain at its Tyr1605-Met1606 bond and separated into two smaller species, each presenting a cleaved subunit at the amino or carboxyl terminal end[43]. Recently studies show that arterial fluid shear stress promotes proteolysis of plasma VWF[21,44]. Ultra-large VWF has been shown to undergo conformational changes upon application of hydrodynamic shear making it exposed to enzymatic cleavage. An elegant study using force atomic microscopy demonstrated that VWF undergoes a shear stress-induced structural transition from a globular state to an extended or stretched chain conformation exposing individual globular domains[45]. Recently, the study of Schneider and colleagues has shown that shear flow is able to stretch VWF, and tensile force exerted on the multimer could cause conformational change in the A2 domains that enables the cleavage of the scissile bond that is buried in the unsheared state of VWF[46]. More recently, other authors confirmed these original data by using different and sophisticated microfluidic devices[47]. The stretching of VWF molecules has been directly visualised under flow conditions and the appropriate shear force has been determined to be in the range between 3,000 and 5,000 s^{-1} (>30 dyn/cm^2)[46]. The VWF conformational changes induced by high shear stress are of particular relevance, since the different VWF conformers have a very different efficiency both in performing the haemostatic functions of the protein and in favouring its proteolytic processing by the metalloprotease ADAMTS-13.

Haemostasis depends on the balanced participation of VWF, and this balance reflects a competition between the biosynthesis of large VWF multimers and their degradation by the ADAMTS-13 metalloprotease. Severe deficiency of ADAMTS-13 activity may cause thrombotic thrombocytopenic purpura (TTP), a life-threatening haematological disease associated with extensive platelet- and VWF-rich thrombus formation. On the other hand, mutation in the A2 domain may increase the susceptibility of VWF to cleavage and lead to von Willebrand Disease (VWD) type 2A, which is characterised by selective depletion of large VWF multimers.

The pathophysiologic importance of VWF is not limited to the phenotypes of VWD and TTP. In fact, VWF level also correlates with thrombosis risk and inversely with bleeding risk within the apparently healthy population[48]. Furthermore, bleeding risk and thrombosis risk appear to vary continuously and reciprocally across the normal range of VWF levels, and there is no clear boundary between a normal and a pathological level of risk for these adverse events.

Keywords: von Willebrand factor, molecular biology, haemostasis, platelets adhesion, ADAMTS-13.

Conflicts of interest disclosure

The Authors stated that they had no interests which might be perceived as posing a conflict or bias.

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