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Review Article

Von Willebrand disease mutation spectrum and associated mutation mechanisms

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ABSTRACT

Von Willebrand disease (VWD) is a bleeding disorder that is mainly caused by mutations in the multimeric protein von Willebrand factor (VWF). These mutations may lead to deficiencies in plasma VWF or dysfunctional VWF. VWF is a heterogeneous protein and over the past three decades, hundreds of VWF mutations have been identified. In this review we have organized all reported mutations, spanning a timeline from the late eighties until early 2017. This resulted in an overview of 750 unique mutations that are divided over the VWD types 1, 2A, 2B, 2M, 2N and 3. For many of these mutations the disease-causing effects have been characterized *in vitro* through expression studies, *ex vivo* by analysis of patient-derived endothelial cells, as well as in animal or (bio) physical models. Here we describe the mechanisms associated with the VWF mutations per VWD type.

1. Introduction

Von Willebrand factor (VWF) is a multimeric hemostatic protein produced solely by endothelial cells and megakaryocytes [1,2]. *VWF* is transcribed from the small arm of chromosome 12 (12p13.31) and translates into a 2813 amino acid protein. Newly synthesized VWF consists of 16 domains: a 22 amino acid signal peptide at the N-terminal end of the protein, a propeptide comprising the D1 and D2 domains, and mature VWF comprising the D′-D3-A1-A2-A3-D4-C1-C2-C3-C4-C5-C6-CK domains (Fig. 1A) [3]. The signal peptide is cleaved off after synthesis in the endoplasmic reticulum (ER). Then, proVWF undergoes several post-translational modifications, like glycosylation and C-terminal dimerization [4,5]. In the trans-Golgi network (TGN), the propeptide is cleaved by furin, but remains non-covalently linked to mature VWF and helps to chaperone mature VWF in the multimerization process [6]. VWF multimers can contain up to 80 subunits, which are translocated from the TGN to the alpha granules in megakaryocytes and to the cigar-shaped vesicles called Weibel-Palade bodies in endothelium [7,8]. Upon vascular damage, VWF multimers are released from the endothelial cells and through the aid of vascular flow form ultra-large VWF strings attached to the exposed collagen [9]. The unwinding of VWF into large strings exposes the binding site for platelet glycoprotein Ib (GPIb) in the A1 domain, thereby attracting platelets to sites of vascular damage and starting primary hemostasis [10]. Opening up the A2 domain exposes the cleavage site of the metalloprotease ADAMTS13 (A Disintegrin And Metalloprotease with ThromboSpondin motif repeats 13), which cleaves VWF between amino acid positions 1605 and 1606 [9]. This leads to the release of smaller and larger VWF multimers into the circulation. In the circulation factor VIII (FVIII) is bound to VWF, which extends the half-life of FVIII [11]. VWF has a half-life of 8–12 hours and remains in the circulation until it is cleared from the system by macrophages in the liver and spleen [12,13].

Malfunction in one of the processes described above may lead to von Willebrand disease (VWD), the most common inherited bleeding disorder that is mainly associated with mucocutaneous and surgical bleeding [14]. Based on the plasma phenotype, VWD patients are categorized into one of the VWD types: 1, 2A, 2B, 2M, 2N and 3 [15]. VWD type 1 and 3 patients are typified by a partial or complete deficiency in plasma VWF respectively. These deficiencies are caused by loss of production, reduced secretion from endothelial cells and platelets, or increased clearance of VWF from the circulation. Four different qualitative defects lead to VWD type 2, and include a defect in plasma multimers in type 2A, increased binding to GPIb in type 2B, decreased

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binding to GPIb or collagen in type 2M and defective binding to coagulation FVIII in type 2N.

In 1985 four independent groups succeeded to clone and sequence the *VWF* gene [16–19]. From this moment on many groups sequenced *VWF* of VWD patients and this led to the identification of hundreds of variations in *VWF*. To prove that the identified variations are indeed disease-causing, VWF constructs containing many of these variants have been overexpressed in heterologous cell systems. For VWF, studies have been performed in stable Furin producing BHK, COS-7, AtT-20, HEK293, HEK293T and HEK293 EBNA cells, with only HEK293 and AtT-20 cells being able to store VWF in pseudo-Weibel-Palade bodies [20]. Expression studies led to the identification of secretion and multimerization defects and binding defects to FVIII and GPIb. Recent advances also allow for the culture of endothelial cells from VWD patients directly and have been helpful in unraveling mutation mechanisms in a patient-specific environment [21,22]. Although the *in vitro* systems have been useful, the effects of flow on VWF and clearance of the protein are difficult to determine. However, by the use of several plasma parameters and VWD mouse models, clearance defects and the *in vivo* effect of ADAMTS13 on VWF have also been investigated [23–26].

In this review we endeavored to organize all published *VWF* mutations and their disease causing mechanism based on an extensive literature search (search term in supplementary data). Throughout this review the mutations and mechanisms will be explained per VWD type. Some mutations have been assigned in literature to different VWD types, which could be explained in various ways: a patient could have been misdiagnosed, the mutation may truly result in different phenotypes or a mutation may have combined phenotypic characteristics of multiple VWD types. We have depicted mutations in this review as they were assigned in literature. Furthermore, it is important to note that if more than one mutation was reported for a single patient and the disease-causing mutation was not certain, the mutations were not included in this review. Also for many candidate mutations the disease causing effect has not been proven yet, however for simplicity we call them mutations throughout this review.

2. VWD type 1

VWD type 1 is the most common type comprising about 60–70% of the patient population [27]. Type 1 patients show a partial deficiency in VWF with a comparable decrease of VWF antigen (VWF:Ag) and VWF activity (VWF ristocetin cofactor activity, VWF:RCo). A VWF:Ag below a cut-off of 30 IU/dL in combination with a VWF:RCo/VWF:Ag ratio $>$ 0.6 and a normal VWF collagen binding is diagnostic for type 1 VWD, thereby accepting minor abnormalities in VWF multimers [14,15,28]. VWF:Ag levels between 30 and 50 IU/dL may be considered as VWD depending on the bleeding phenotype. Although VWD type 1 is the most frequent type of VWD, extensive investigations to the mutation mechanisms of VWD type 1 started only between 2000 and 2010 with three large studies conducted in the United Kingdom, Europe and Canada [29–31]. In these studies mutations were identified in 53–70% of the patients. Later studies in Sweden and Canada confirmed these numbers [32,33]. Importantly, some type 1 patients included in the European MCMDM-1VWD study showed minor multimer abnormalities and were later reassessed to other VWD types. Those cases with minor multimer abnormalities showed a higher proportion of mutations, whereas in individuals with completely normal VWF multimers mutations were identified in approximately 50% of the individuals [34]. More recently, large VWD population studies increased the number of identified type 1 mutations, with the most recent studies reporting identification of mutations in about 90% of the patients [27,35,36]. These studies, however, had strict inclusion criteria increasing the chance to identify mutations. Overall, a little more than 250 unique mutations have been assigned to VWD type 1 patients (Table S1A–D). However, the disease-causing effect was only proven by *in vitro* investigations for about one quarter of these mutations.

Mutations associated with VWD type 1 are dispersed throughout the whole VWF protein (Fig. 1A–C). Most mutations identified are heterozygous missense mutations, however also (small) deletions/insertions, splice site and nonsense mutations have been reported. The mutation mechanisms can roughly be divided in three groups: decreased VWF production, decreased secretion and increased clearance.

2.1. Decreased VWF production

A lower VWF production has mostly been reported for patients heterozygous for a null allele. These heterozygous null alleles can be the result of nonsense mutations, frameshift mutations caused by (small) deletions or insertions and by splice site mutations. Heterozygosity for a null allele leads to production of protein from the non-mutated allele only, resulting in an expected production of only 50% of the normal VWF production. Since normal levels of VWF in plasma range between ~50 and 200 IU/dL, heterozygosity for a null allele will lead to VWF levels ranging between ~25 and 100 IU/dL [37]. Therefore, some people at the lower end of this distribution will be diagnosed as VWD type 1 and some people will be considered unaffected and are probably asymptomatic.

Small deletions and insertions often lead to a frameshift, which generates a premature stop codon usually within a few amino acids (indicated as for example p.Pro812Arg fs*31). Premature stop codons caused by frameshift or nonsense mutations lead to an mRNA product which is mostly degraded by nonsense-mediated decay. Splice site mutations are also found in VWD type 1 patients and may lead to exon skipping or intron retention. Depending on the reading frame of an exon, this could lead either to the production of a truncated protein or to a premature stop codon. Whether exon skipping forms a truncated or nonsense allele is partly predicted by the reading frame (Fig. 1B), however this can only be proven by investigating RNA products in platelets or patient-derived endothelial cells [38–41]. For example, in the case of c.1534-3C > A and for c.5842 + 1G > C, this results in three different mRNA products [39,41]. Although not frequently reported, some mutations have been identified in the promoter region of *VWF* as well [29–31,42]. These may lead to altered binding of transcription factors to the *VWF* promoter region [42].

2.2. Decreased secretion

Some patients have normal VWF production, but a decreased secretion of VWF from the endothelial cells. Existence of secretion defects from platelets is likely but has so far not been studied. Secretion defects encompass retention of VWF in the ER or Golgi, decreased Weibel-Palade body exocytosis and possibly degradation of mutant VWF by the proteasome. Secretion defects have been studied on a cellular level by *in vitro* overexpression of mutant VWF constructs in heterologous cell systems, or by studies on patient-derived endothelial cells. Decreased secretion from mutant VWF producing cells compared to normal VWF producing cells has been identified for many of the VWD type 1 mutations tested *in vitro* and decreased secretion is often seen in combination with an *in vitro* multimerization defect (Fig. 1A, Table S1A–D). Whether decreased secretion of VWF is caused by retention of VWF in the ER or Golgi can easily be studied by co-staining of VWF with an ER or Golgi marker and this defect has been