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A mechanistic model to quantify von Willebrand factor release, survival and proteolysis in patients with von Willebrand disease

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Running title: A mechanistic model for diagnosing VWD

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ABSTRACT

A reduced von Willebrand factor (VWF) synthesis or survival, or its increased proteolysis, alone or in combination, contributes to the development of von Willebrand disease (VWD).

We describe a new, simple mechanistic model for exploring how VWF behaves in well-defined forms of VWD after its DDAVP-induced release from endothelial cells. We aimed to ascertain whether the model can consistently predict VWF kinetic changes. The study involved 9 patients with VWD types Vicenza (a paradigmatic form with a reduced VWF survival), 8 type 2B, 2 type 2A-I, 1 type 2A-II (associated with an increased VWF proteolysis), and 42 normal controls, whose VWF levels were measured after a 24h-long DDAVP test. The rate constants considered were: k_0, associated with the VWF release phase; k_1, illustrating the phase of conversion from high- to low-molecular-weight VWF multimers; k_e, associated with the VWF elimination phase. The amount of VWF released (*D)* was also measured.

k_e and D were significantly higher in O than in non-O blood group controls; k_1 was also higher, but less markedly so. All the parameters were accelerated in type Vicenza, especially k_e ($p<0.0001$), which explains the significant reduction in VWF half-life. In types 2B and 2A-II, k_1 was one order of magnitude higher than in controls, which explains their loss of large VWF multimers. All parameters except k_e were lower in type 2A-I.

The proposed mechanistic model clearly describes the altered biochemical pathways in well-characterised VWD, prompting us to suggest that it might help clarify elusive forms of VWD too.

Key words: VWF, VWD, DDAVP, VWF survival, VWF clearance.

INTRODUCTION

Von Willebrand factor (VWF) contributes to haemostasis by mediating platelet adhesion and platelet aggregation at the site of vascular injury (1,2). VWF is a polymeric glycoprotein synthesised and stored in the form of ultra-large (UL) multimers in megakaryocytes (alpha granules) and endothelial cells (Weibel-Palade bodies) (3). VWF may be released from alpha granules on platelet activation, and from Weibel Palade bodies on vascular injury, or under various physiological (4,5) and pharmacological stimuli (e.g. desmopressin) (6-8). Endothelial cells secrete VWF continuously to maintain basal plasma VWF levels (5). VWF function is modulated by its multimer organisation: large and ultra-large VWF multimers have the greatest haemostatic effect, mainly due to their capacity to bind to platelets and sub-endothelial collagen during platelet plug formation; their absence is associated with a severe bleeding tendency (9-12). Low-molecular-weight (LMW) multimers have a weaker haemostatic capacity, their main function being to carry FVIII, which does not require a full multimer organisation (13). VWF released from endothelial cells undergoes extensive cleavage by circulating ADAMTS-13 protease, and this process gives rise to a heterogeneous VWF multimer pattern comprising high-molecular-weight (HMW) and LMW oligomers (14,15).

Quantitative and qualitative VWF defects lead to von Willebrand disease (VWD) (16,17), the most common inherited bleeding disorder. Quantitative VWF defects are associated with VWD types 1 and 3, and qualitative defects with types 2A, 2B, 2M and 2N. These various disease types are characterised by a different pathophysiology and bleeding risk (18,19). Several laboratory tests have been developed to identify and classify the different types of VWD (20). VWF levels are quantified by means of an antigen assay (VWF:Ag), which is unable to distinguish between HMW and LMW multimers. VWF collagen binding (VWF:CB) (21) and VWF ristocetin cofactor activity (VWF:RCo) (22) are measured mainly to explore the haemostatically more efficient large VWF multimers and VWF binding to collagen in the former case, and platelet GPIb in the latter.

Quantitative and functional tests can be used in combination to classify VWD patients by type (1, 2, 3) and subtype (2A, 2B, 2M, 2N) (23, 24).

From a kinetic standpoint, plasma VWF levels and patterns of HMW and LMW multimers depend on the balance between three determinants: 1) the amount and rate of VWF release; 2) ADAMTS-13 proteolytic activity; and 3) VWF clearance from the plasma. These processes can be explored in detail by administering DDAVP, which induces an acute release of the VWF stored in the Weibel Palade bodies of endothelial cells, followed by proteolysis of the UL multimers and VWF clearance. This approach is currently used to explore VWF half-life (25), and the simplest model employed to date consists of a bi-exponential function describing the rise and fall of plasma VWF:Ag and VWF:CB levels following desmopressin stimulation with the aid of a physiology-based mathematical model. This method can provide an estimate of the amount and rate of VWF release, in stimulated and basal conditions, and its clearance and half-life. It also enables us to characterise the VWF kinetics in normal individuals with the O and non-O blood groups (26), and in some types of VWD (type 1, type 2B, type 3) (27-30). On the other hand, this method cannot quantify the proteolytic activity of ADAMTS-13, which is known to be higher in type 2A and 2B VWD than in normal subjects, and also in individuals in the O vis-à-vis the non-O blood groups.

Our team recently developed a more sophisticated pharmacokinetic (PK) model that can also account for proteolysis of the super ultra-large (SUL) VWF multimers adhering to the vessel wall, their conversion into smaller circulating molecules (SUL→HMW and SUL→LMW), and the biotransformation of HMW to LMW oligomers (31, 32). The structural complexity of this model made it necessary to analyse pooled data on a homogeneous VWD population, however, or to obtain a large set of experimental data for each patient, in order to obtain a statistically reliable estimate of the pharmacokinetic parameters. We consequently simplified the original model and used it to analyse the time courses of VWF:Ag and VWF:CB plasma concentrations after DDAVP challenge in normal subjects and VWD patients known to have a short VWF half-life (type Vicenza) or abnormal proteolysis (types 2A and 2B). The aim of the present study was to ascertain

whether our new model can consistently predict kinetic changes in paradigmatic VWD types, with a view to characterising the VWF kinetics of other VWD types with less clear kinetic features.

MATERIALS AND METHODS

Patients and normal subjects were studied in accordance with the Helsinki Declaration, after obtaining their written informed consent, and our ethical board's approval of the study.

Haemostatic analysis. The main haemostatic findings in the patients involved were as reported elsewhere (33). VWF antigen (VWF:Ag) was measured by enzyme-linked immunosorbent assay (ELISA) using horseradish peroxidase (HRP)-conjugated anti-VWF polyclonal antibody (Dako, Glostrup, Denmark). VWF collagen-binding (VWF:CB) activity was assessed with an ELISA test using type III collagen (Sigma, Milan, Italy). DDAVP (1-desamino-8-D-arginine vasopressin; Emosint, Sclavo, Italy) was administered subcutaneously at a dose of 0.3 μg/kg. Blood samples were collected before and 15, 30, 60, 120, 180, 240, 360 and 480 min, and 24 hours after administering DDAVP. The time courses of the VWF:Ag and VWF:CB plasma concentrations after the DDAVP challenge were analysed using our new mathematical model, which is described below and in the Results section. VWF multimers were analysed by electrophoresis on 1.8% high-gelling-temperature agarose containing 0.1% sodium dodecyl sulphate (27). The multimers were detected by autoradiography after reaction with anti-VWF polyclonal antibody (DAKO) labelled with ^{125}-I and viewed with the DS-50000 Epson densitometer scanner.

Genetic analysis. Genomic DNA was extracted from peripheral blood leukocytes using the QIAamp DNA Blood Mini Kit (Qiagen, Hilden, Germany). The *VWF* gene exons, including the intron-exon boundaries, were amplified and sequenced using primers chosen according to the *VWF* sequence established by Mancuso et al (34). The Big Dye Terminator Sequencing kit v.1.1 (Perkin Elmer, Wellesley, MA, USA) and an ABI 3130XL Genetic Analyzer (AB) were used for DNA sequencing.

Mathematical model. This is a simplified version of the model proposed by Galvanin et al. (31, 32), a physiology-based compartmental model of VWD capable of characterising in detail the mechanisms of VWF release, proteolysis and clearance, and the multimer distribution of VWF in the plasma. The new model comprises two compartments, and was designed to investigate the time courses of plasma VWF:Ag and VWF:CB levels (Fig. 1a) after DDAVP challenge.

The model is described by a system of differential and algebraic equations where each subject is characterised using three main PK constants, namely the VWF release rate k_0 [h^{-1}], the proteolysis rate k_1 [h^{-1}], and the elimination rate k_e [h^{-1}], which is assumed to be the same for both the UL+HMW multimers and the LMW multimers. The amount of VWF released is represented by a parameter D [U/dL]. Note that, for a given subject, the k_0 parameter quantifies the rate of release, whilst D quantifies the amount of VWF released from the endothelial cells. The underlying physiological assumptions are that: (i) UL, HMW and LMW multimers are present in the basal state and/or after DDAVP; and that (ii) UL and HMW multimers can be cleaved to form LMW multimers. It is also assumed that the VWF:Ag measurements enable us to assess the quantities of UL+HMW+LMW multimers, whereas VWF:CB measures the UL+HMW multimers. The relative quantity of UL + HMW multimers and LMW multimers (derived from VWF:Ag and VWF:CB measurements) is computed in time and can be compared with the observed distribution obtained from gel electrophoresis images (see Results section). Details of the mathematical model and parameter estimation procedure are given in Appendix A.

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Statistical analyses. Data are presented as means ± SD. Normality of distribution was assessed with the D'Agostino & Pearson test. Unpaired statistical comparisons were applied: a) between O and non-O blood groups in normal subjects; b) between all normal subjects and each VWD type, except for 2A VWD (one patient). The non-parametric Mann-Whitney test was applied to all comparisons since most parameters did not pass the normality test. The significance level was set at $p<0.05$.

The procedures employed to determine the pharmacokinetic parameters in each subject are described in Appendix A.

RESULTS

Patients and healthy subjects. The study involved 9 patients with type Vicenza, 8 with type 2B, 2 with type 2A-I, and 1 with type 2A-II VWD, all genetically characterised. Their pertinent haemostatic and genetic details are given in Table 1. Forty-two normal subjects (24 with blood group O and 18 with non-O blood groups) were enrolled as controls.

Mathematical model parameters. A simplified version of the mathematical model proposed by Galvanin et al. (31,32) (see Materials and Method section) was used in this study to characterise DDAVP-induced VWF release, proteolysis and clearance mechanisms in detail for each subject. After DDAVP challenge, the model provides a mathematical description of: a) the quantity (D) of UL+HMW multimers released, the kinetics of which is explored by means of the PK parameter k_0; b) the proteolysis of UL+HMW multimers by ADAMTS13 to form LMW multimers, investigated by means of the k_1 parameter; and c) the clearance of VWF multimers from the plasma, assessed by means of the k_e parameter. Representative results of the application of the model to average data from patients with VWD (2A, 2B and Vicenza) and normal subjects after a 24-h DDAVP challenge are shown in Figure 1b for VWF:Ag and in Figure 1c for VWF:CB. As clearly shown, the model is flexible enough to capture the wide between-group variability, and the fitting is very satisfactory. In VWD patients, the accelerated proteolytic activity observed in 2B and 2A-II patients is apparent in Figure 1c (low VWF:CB levels as a result of a paucity of HMW multimers in the bloodstream). Vicenza type is characterised by a shorter half-life as a result of a faster elimination rate (VWF:Ag basal concentration is restored after 8 hours, VWF:CB after approximately 14 hours).

The mean values of the PK parameters for normal subjects and VWD patients are listed in Table 2 and Table 3, respectively. Figure 2 shows a graphical comparison between the estimated results for the different groups of subjects.

Normal subjects. The values of k_1, k_e and D (Table 2) were higher in the O than in the non-O blood group subjects, though the differences were only statistically significant for k_e ($p<0.0001$) and D ($p<0.005$), and borderline significant for k_1 ($p=0.051$). There was no difference in the k_0 value between the blood groups (Table 2). These findings suggest that blood group affects all the pathways explored except for the VWF release rate, and has a more marked effect on VWF clearance, confirming a previous report of a shorter VWF survival in subjects with the O blood group (26).

Type Vicenza VWD. All type Vicenza patients were carrying the p.R1205H mutation which, in all but two of them, was combined with the p.M740I variant. All the patients belonged to non-O blood groups except for the two carrying the p.R1205H mutation alone. All rate constants (k_0, k_1 and k_e, shown in Figure 2) were higher than in the controls, irrespective of ABO blood group. The k_e parameter showed the highest value ($p<0.00001$), which was one order of magnitude higher than in controls ($8.18+/-1.72 \times 10^{-3}$ vs $1.17+/-0.68 \times 10^{-3}$, respectively) (Tables 2, 3); the increases in k_0 and k_1 compared to controls were significant, but less pronounced (2.43-fold and 3.26-fold, respectively) than in k_e (Figure 2). The D parameter was statistically lower in patients than in controls ($p<0.005$). The most accelerated VWF pathway after DDAVP challenge in type Vicenza VWD was therefore clearance (k_e), though the other pathways were accelerated too, while the amount released (D) was lower.

Type 2B VWD. k_1 and k_e were statistically greater in these patients than in controls (Figure 2). The most pronounced increase concerned k_1, which was one order of magnitude higher than in controls ($47.1 +/-67.9 \times 10^{-4}$ vs $4.59 +/-6.40 \times 10^{-4}$); the increase in k_e was smaller (2.76-fold), but the difference was still statistically significant (Table 2-3); k_0 was statistically lower; and D was indistinguishable from that of controls. According to our model, this implies a difference in the kinetics associated with VWF release in 2B subjects, where the same amount of VWF is released but over a longer time (a slower release rate). Clearly, the most accelerated pathway in this group concerns VWF proteolysis, with the pertinent k_1 value 10.2 times higher than in normal subjects - a

finding consistent with the shortage of large VWF multimers in these patients. The post-DDAVP proteolysis of type 2B VWF was associated with a drop in platelet count from a mean 167+/-44.9x10^3/μL before to 32+/-47x10^3/μL 60 min after DDAVP. The platelet count recovered to near its pre-DDAVP value within 360 minutes (131+/-48.0x10^3/μL).

Type 2A. Type 2A-I is the VWD 2A subtype involving a defective VWF synthesis and an impaired multimer organisation (35). In this form, the k_0, k_1 and D parameters tended to be lower than normal, though not to a statistically significant degree, whereas k_e was indistinguishable from those of normal subjects. The one patient in our study with Type 2A-II - characterised by a mutation that makes VWF more sensitive to ADAMTS13 (36) – had a k_1 value 9.2 times higher than in controls and similar to the absolute value seen in type 2B VWD. The PK parameters related to the VWF release rate (k_0) or quantity (D) and clearance (k_e) did not differ significantly from those of normal subjects.

Multimer distribution. One important feature of the proposed mechanistic model is that it can be used to describe the multimer time courses in terms of UL+HMW and LMW species from variables x^{UL+HMW} and x^{LMW} (see eqs. 1-2 in Appendix A for further mathematical details). The results are given in Figure 3, where multimer patterns and mathematical simulations are compared in a representative healthy O subject (Figure 3a) and in patients with VWD types 2B (Figure 3b), 2A-II (Figure 3c) and Vicenza (Figure 3d). The relative amount of UL+HMW multimers can be calculated from the ratio VWF:CB/VWF:Ag = $x^{UL+HMW}/(x^{UL+HMW} + x^{LMW})$ in time. In normal subjects (Figure 3a) HMW + UL multimers are released soon after DDAVP administration and the proteolytic reduction to LMW is relatively slow; the clearance mechanism is also slow (after 24h some amounts of UL and increased HMW multimers are still present). In type 2B (Figure 3b) and 2A-II (Figure 3c) patients the proteolytic action is more evident and higher amounts of LMW multimers are present after DDAVP. After their DDAVP-induced release, UL and HMW multimers are removed so quickly from the circulation by the action of ADAMTS13 that they are never detectable, while LMW multimers gradually increase to very high levels.

In type Vicenza patients (Figure 3d) the model predicts a rapid disappearance of UL+HMW, just 2 hours after DDAVP administration, and of all the multimeric species within 6 hours, as a result of the accelerated clearance; and there are moderate LMW levels, as in normal subjects. These results clearly show that the multimer profiles predicted by the model are consistent with those obtained by gel electrophoresis in the subjects studied. In this regard, VWF:CB and its associated VWF:CB ratio reveal all their utility in the detection of VWF multimers, and its large components in particular (37).

DISCUSSION

A simplified version of the mechanistic model described by Galvanin et al. (31) was used in the present study to explore the abnormal biochemical pathways characterising different types of VWD, quantifying such changes on the basis of the pertinent PK parameters. Using the VWF:Ag and VWF:CB measurements obtained over a period of 24 hours after a DDAVP challenge, the mathematical model enabled us to elucidate the different pathophysiological conditions contributing to circulating VWF levels and multimer patterns. Healthy subjects were classified by O and non-O blood group and compared with type Vicenza, type 2A and type 2B VWD patients.

Three main PK parameters were studied: k_0, which explores the pathway of VWF release from endothelial cells; k_1, which elucidates the proteolytic conversion of large and ultra-large VWF multimers into LMW multimers; and k_e, which represents the clearance of VWF from the circulation and consequently its plasma half-life. The amount of VWF released after DDAVP challenge was also measured with the D parameter.

Judging from the results obtained with our model, ABO blood group mainly affects two pathways, i.e. VWF proteolysis and clearance, since the k_1 and k_e were greater for subjects in the O than in the non-O blood groups. These findings are consistent with previous reports of healthy subjects with the O blood group having a shorter VWF survival (26) and lower circulating levels than non-O

individuals (38), and a greater VWF susceptibility to ADAMTS13 (39). While the increase in k_e was statistically significant, the increase in k_1 closely approached the significance level (p=0.051). This nearly-significant difference in k_1 suggests that the influence of ABO blood group on VWF proteolysis is weaker than on VWF survival (39). If its impact on proteolysis were stronger, we would find relatively fewer large VWF multimers in O than in non-O individuals, but this has never been reported. It should be emphasised, however, that we found a marked variability in our population of normal subjects (n=42): this is hardly surprising, but might have influenced our results. Unlike our group's previous findings using a different PK model (26), the amount of VWF released after DDAVP challenge was significantly higher in O than in non-O individuals. If our present observation holds, we might surmise that more VWF is stored in the Weibel-Palade bodies of healthy individuals with the O blood group. It has been previously reported, on the other hand, that VWF platelet content (which mimics endothelial cell condition) does not differ between ABO groups (40). This goes to show that the contribution of ABO group in modulating circulating VWF in normal subjects is of interest not only per se, but also for its implication in the field of haemorrhagic and thrombotic disorders (41).

Our model identified a high elimination rate (k_e) in type Vicenza VWD, which was the highest among all the patients studied here. This is consistent with the finding that a shorter VWF half-life is responsible for the significant reduction in circulating VWF levels, despite a normal VWF synthesis (27). The p.R1205H mutation in the D3 domain of type Vicenza patients speeds up the clearance of VWF to such a degree that the circulating VWF levels are similar to those of severe type 1 VWD, i.e. often below 10 U/dL. Type Vicenza VWD often (but not always) features the presence of unusually large VWF multimers. This has been attributed by Gezsi et al (42) to a very short VWF half-life, which does not give ADAMTS13 time enough to cleave the ultra-large/large VWF multimers released by endothelial cells. Our findings confirm this hypothesis, since our patients' rate of conversion from UL+HMW to LMW VWF multimers (expressed by the k_1 parameter) seemed to be higher than normal, but less so than the increase in the k_e parameter. The

contribution of VWF proteolysis would therefore have less impact than that of its clearance, with ultra-large VWF multimers persisting as a consequence. Type Vicenza patients were also characterised by a higher rate of release, but lower amounts of VWF released (expressed by *D*) by comparison with controls. The first result is visually evident if we compare their times to peak concentrations of VWF:Ag (Figure 1b) and VWF:CB (Figure 1c) (about 0.5 h) with those obtained for the other groups of subjects (always about 2 h), and this finding has already been reported in the literature (27). The second result is novel and warrants further investigation. According to our mechanistic model, all the biochemical pathways explored by our mechanistic model, i.e. VWF release, proteolysis and clearance, in particular, appear to be accelerated in type Vicenza, confirming that the main pathological issue in this condition is a very short VWF half-life. An explanation for this picture might be that the intrinsic defect in type Vicenza VWD – the p.R1205H mutation – prompts a generalised acceleration of all the biochemical pathways, or else that a faster clearance influences the other pathways.

In type 2B VWD patients, k_1 and k_e were both significantly higher than normal, the former more so than the latter (k_1 was one order of magnitude higher than in controls), so the proteolytic pathway appears to be the prevalent one. This explains the lack of large VWF multimers in such patients. The increase in k_e confirms the shorter VWF half-life reported in patients with type 2B VWD (28). An increased proteolysis and a decreased VWF survival both contribute to the type 2B VWD phenotype (28, 43), though this is particularly true in type 2B patients lacking in large VWF multimers (44). The two aspects may be dissociated, however, as seen in type 2B patients who still have large multimers, albeit with an apparently shorter VWF half-life (44).

The picture in type 2A-II VWD patient, featuring a greater susceptibility of VWF to ADAMTS13 (36) is similar to that seen in type 2B VWD, though the underlying mechanisms are different, i.e.: a VWF more susceptible to ADAMTS13 in type 2A-II, and a greater VWF affinity for the GPIb platelet receptor (which enhances proteolysis by ADAMTS13) in type 2B. In agreement with this picture, the only patient with type 2A-II VWD had a very high k_1, similar to that of type 2B

patients. In the two type 2A-I VWD patients all PK parameters, except k_e, tended to be lower than in healthy controls, but the difference was not statistically significant. This picture is in line with these patients' defective VWF synthesis and multimerisation, as confirmed by their reduced plasma and platelet VWF levels, and shortage of large VWF multimers.

Our results can be summarised as follows:

i) the magnitude of the VWF release rate constant k_0 significantly lower than normal in type 2B and 2.43 higher in type Vicenza VWD, suggesting an accelerated VWF release pathway in this variant of the disease;

ii) k_1 (a measure of the proteolytic conversion of HMW to LMW multimers) was greater than normal in type 2B and type 2A-II (which explains the lack of large VWF multimers). It was also greater, though less markedly so, in type Vicenza patients;

iii) k_e (a measure of the VWF clearance rate) was very high in type Vicenza, consistently with the much reduced VWF half-life in this form of VWD; it was higher than normal in type 2B too (though to a lesser extent).

Our findings suggest that each of the variants of VWD explored in this study coincides with a picture in which one pathway abnormality prevails over the other(s), thereby influencing the corresponding phenotype. In type Vicenza it was the k_e parameter associated with VWF clearance that we found much greater than normal, while the other parameters were relatively less markedly altered. In type 2A-II and type 2B it was the k_1 parameter, a finding that explains the abnormal multimer pattern observed in these patients. In type 2A-I all parameters except k_e were decreased.

In conclusion, a new mechanistic model is proposed here for the quantitative description of the metabolic pathways related to VWF release, proteolysis and clearance from the bloodstream in different types of VWD and in healthy subjects. Our model proved capable of detecting the typical changes in VWF proteolysis and clearance in type 2B, type 2A-II and type Vicenza VWD. It also pointed to possible differences in VWF kinetics between normal subjects in the O and non-O blood groups. The model can be tailored to individual patients with VWD, enabling a quantitative

description of the time course of their VWF multimer distribution, and a consequently faster and more effective diagnosis of their disease. Our findings may also lay the foundations for a model-based diagnostic procedure that uses a subject's laboratory data to delineate an unknown pathophysiological condition. This would be especially useful in the case of type 1 VWD, the most common and also the most elusive form of VWD, that may stem from a reduced VWF synthesis, an abnormal proteolysis or an accelerated clearance, alone or in combination.

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LEGENDS TO THE FIGURES

Figure 1. Simplified model developed in this study. (a) Model structure; VWF:Ag and VWF:CB measurements are identified by dashed boxes; D = release of UL+HMW multimers after DDAVP administration (UL = ultra-large; HMW = high-molecular-weight; LMW = low-molecular-weight). Time profiles after DDAVP challenge of (b) VWF:Ag and (c) VWF:CB plasma concentrations for the average healthy O (HO) and non-O (HnonO) subjects and patient affected by 2A-I, 2A-II, 2B and Vicenza type VWD. Model results are shown by the lines, test samples are

indicated by squares. In the fitting procedure, measurements are assumed to be normally distributed with a standard deviation of 2 U/dL.

Figure 2. Comparison between the estimated parameter values for healthy O (HO) and non-O (HnonO) subjects and patients affected by VWD (group Vicenza, 2B, 2A-I and 2A-II). Variability for each parameter in each group is given in terms of standard deviation through error bars. Asterisks indicate parameters significantly different from control group ($p<0.01$). No statistical test was applied to 2A-II VWD type.

Figure 3. Multimer distribution in time following DDAVP challenge (left column) and as computed by the proposed model (right column) in terms of low-molecular-weight multimers (LMW, solid line) and ultra-large plus high-molecular-weight multimers (UL+HMW, dotted line); the experimental evaluation through gel electrophoresis images and the model predictions are shown for representative (a) healthy O subject; (b) type 2B patient; (c) type 2A-II patient and (d) type Vicenza patient.

Table 1. *Main hemostatic and genetic findings observed in the VWD patients and normal subjects studied.*

Subjects (No)	Age, years Range/Median	Blood group O/nonO	VWF:Ag U/dL	VWF:CB U/dL	VWF:RCo U/dL	Platelet VWF U/dL	FVIII:C U/dL	Mutations
Vicenza (9)	26-59/41	2/7	8.8±2.2	7.2±2.6	8.19±4.06	96.01±16.34	16.31±4.9	p.R1205H p.M740I + p.R1205H
2B (8)	36-65/39	5/3	41.3±9.5	8.9±4.2	22.0±7.3	119.6±24.1	52.4±8.6	p.R1308C p.V1316M p.R1306W
2A-I (2)	48-36/42	1/1	15.3±9.2	13.1±7.2	8.7±3.8	28.5±0.8	30.2±10.7	p.R1374H p.L1446P
2A-II (1)	56	0/1	41.8	5.8	6.4	86.2	57.3	p.L1562P
Normals (42)	19-52/38	17/25	96.3±46.5	99.4±45.9	-	-	101.2±40.8	
Normal range			*60-160*	*65-150*	*60-130*	*70-140*	*60-160*	

Table 2. *Mean PK and D values including statistics for model parameters obtained after parameter estimation for healthy O (HO), non-O (HnonO) and control (HO+HnonO) subjects; p-values for parameters significantly different for healthy subjects are indicated in boldface (p<0.01).*

PK parameter	**HO**		**HnonO**		**HO/HnonO p-values**	**Control (HO+HnonO)**	
	mean	**SD**	**mean**	**SD**		**mean**	**SD**
k_0 [h^{-1}]	2.64E-02	8.72E-03	2.87E-02	8.04E-03	2.17E-01	2.74E-02	8.41E-03
k_1 [h^{-1}]	6.25E-04	7.94E-04	2.37E-04	2.13E-04	1.43E-01	4.59E-04	6.40E-04
k_e [h^{-1}]	1.52E-03	6.71E-04	7.04E-04	3.57E-04	**2.32E-05**	1.17E-03	6.88E-04
D [U]	5.68E+02	1.54E+02	4.25E+02	1.01E+02	**2.93E-03**	5.07E+02	1.51E+02

Table 3. *Estimated mean PK and D values including statistics obtained after parameter estimation for VWD patients (Vicenza, 2B, 2A-I, 2A-II); standard deviation (SD) is indicated in parentheses; p-values for parameters significantly different from control group are indicated in boldface (p<0.01).*

PK param.	Vicenza		2B		2A-I		2A-II		Control (HO+HnonO)
	mean (SD)	p-values	mean (SD)	p-values	mean (SD)	p-values	mean (SD)	p-values	mean (SD)
k_0 [h^{-1}]	6.66E-02 (3.60E-02)	**9.82 E-03**	1.77E-02 (7.02E-03)	**3.76 E-03**	2.06E-02 (0.67E-02)	2.48 E-01	1.52E-02 (-)	-	2.74E-02 (8.41E-03)
k_1 [h^{-1}]	1.50E-03 (2.99E-03)	7.21 E-01	4.71E-03 (6.79E-03)	**3.17 E-03**	3.00E-04 (4.23E-04)	8.88 E-01	4.23E-03 (-)	-	4.59E-04 (6.40E-04)
k_e [h^{-1}]	8.18E-03 (1.72E-03)	**3.20 E-06**	3.23E-03 (1.18E-03)	**6.09 E-05**	1.77E-03 (0.36E-03)	1.21 E-01	1.26E-03 (-)	-	1.17E-03 (6.88E-04)
D [U]	2.71E+02 (1.80E+02)	**1.93 E-03**	5.97E+02 (3.32E+02)	9.68 E-01	3.53E+02 (5.47E+01)	7.59 E-02	6.49E+02 (-)	-	5.07E+02 (1.51E+02)

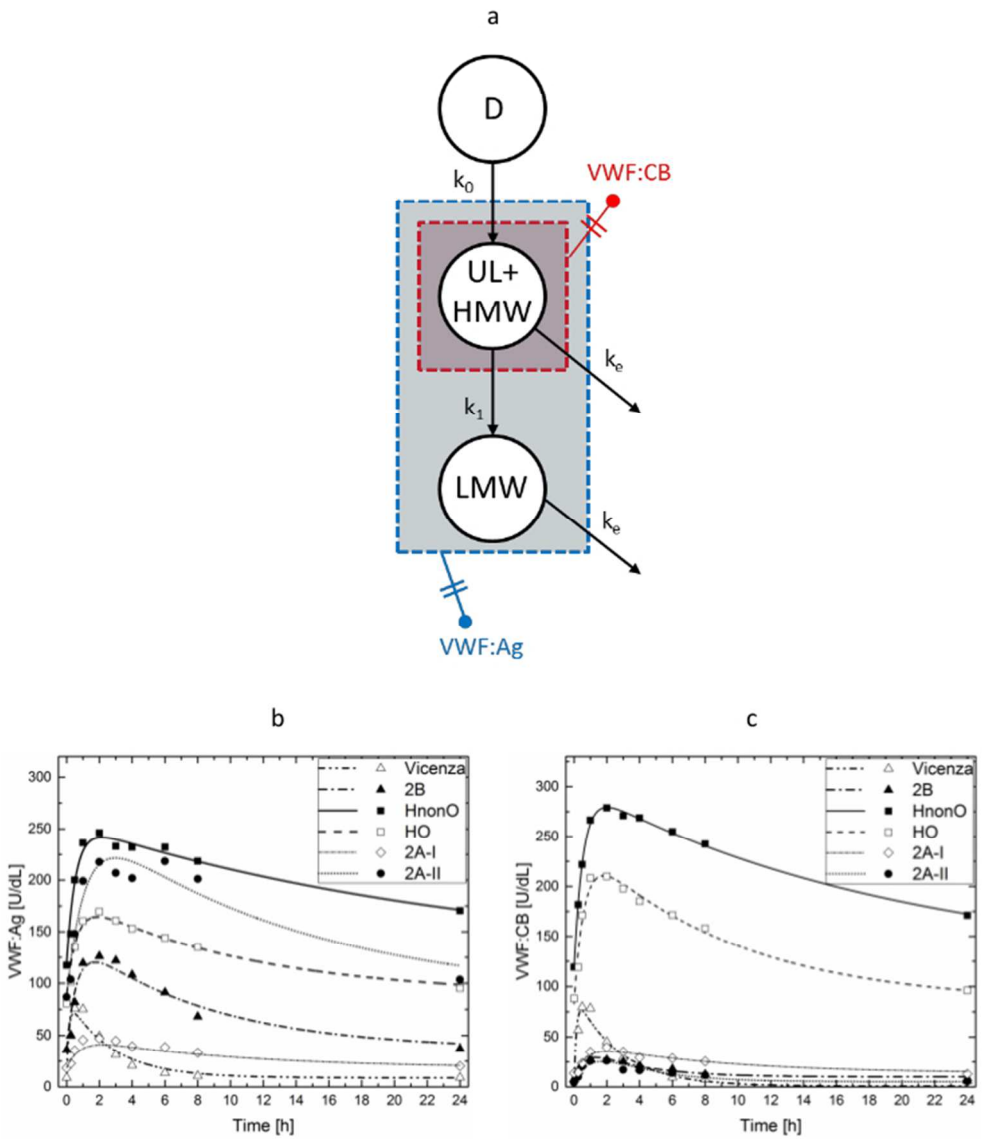

Fig.1

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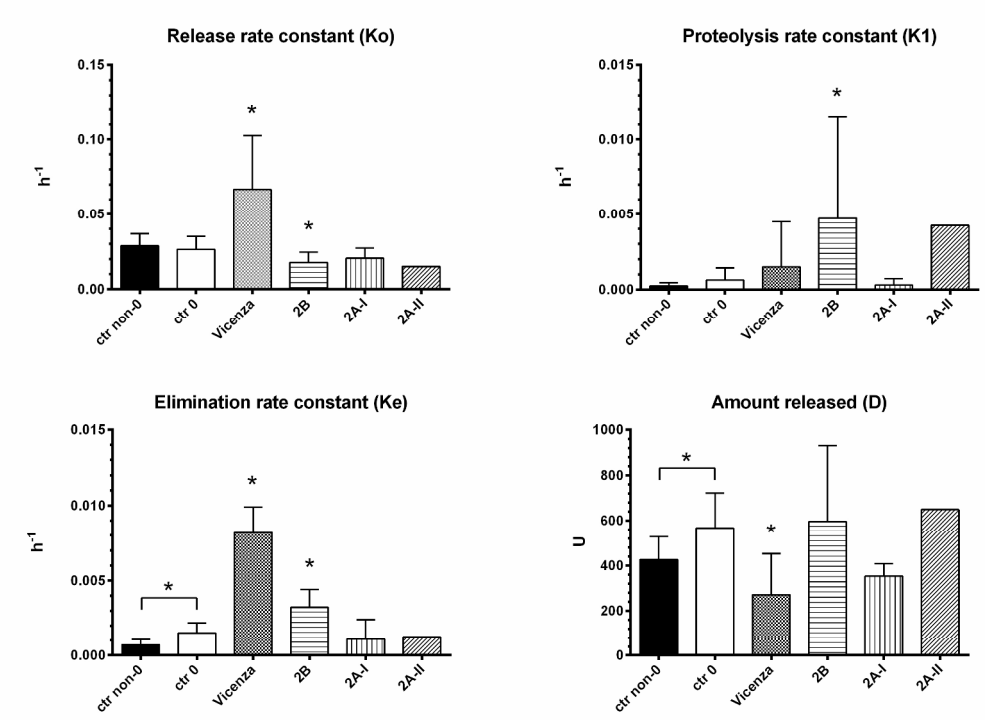

Fig.2

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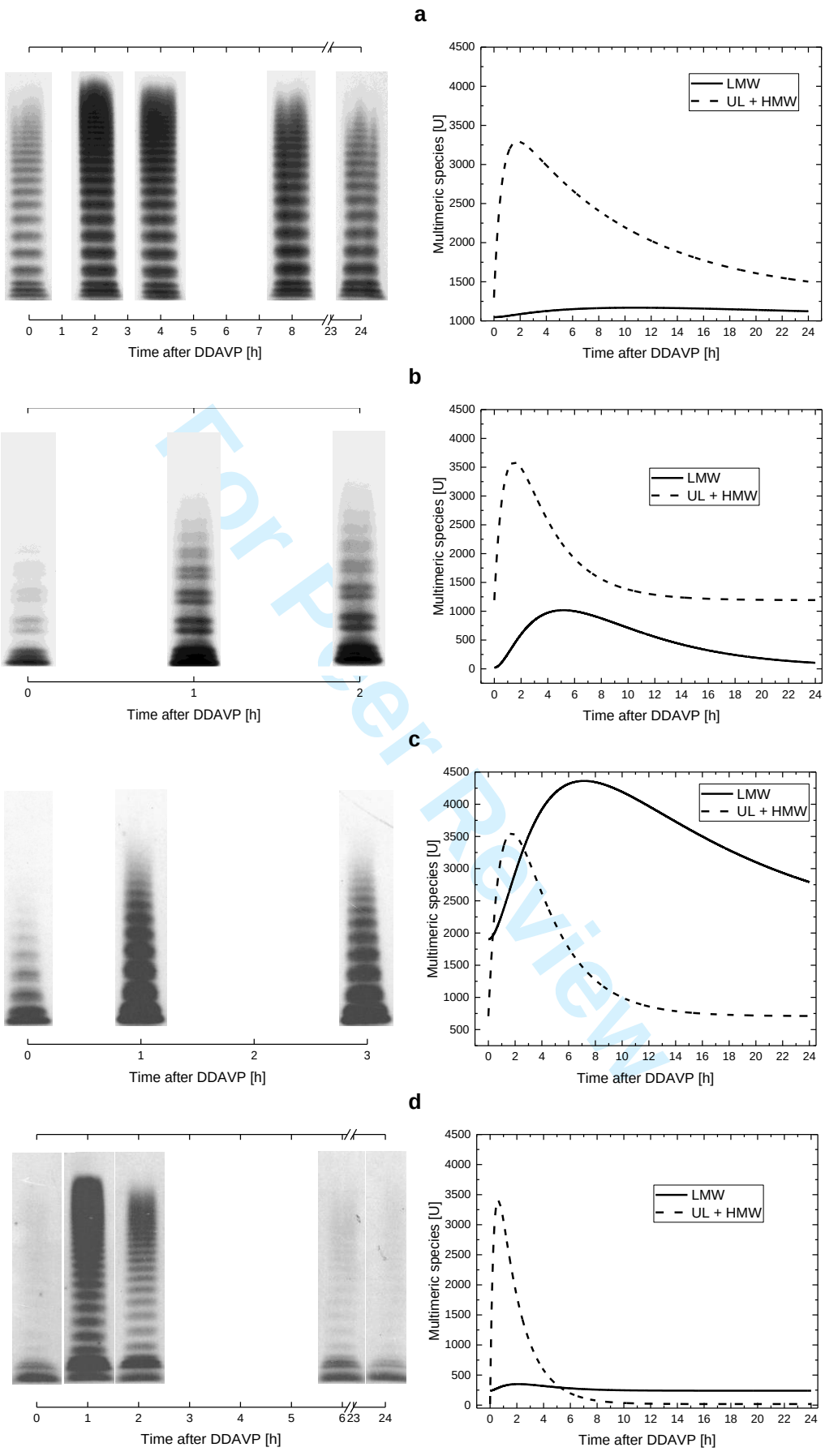

Fig. 3

Bullet points

What is known in this topic

- Reduced VWF synthesis or survival, or increased proteolysis may contribute to VWD.
- The diagnosis and characterization of VWD is often complicated, and not always successful.

What this paper adds

- A mechanistic model is proposed for exploring the release, proteolysis and elimination of VWF in patients with type 2A, 2B and type Vicenza VWD.
- In each form of VWD, the abnormality of one pathway seems to prevail over the others, thus explaining the associated phenotype.
- Our model might be helpful in the diagnosis of VWD, especially the more elusive forms.