



HAL
open science

Role of the factor VIII-binding capacity of endogenous von Willebrand factor on the development of factor VIII inhibitors in patients with severe hemophilia A

Yohann Repessé, Catherine Costa, Roberta Palla, Erika Farrokhi Moshai, Annie Borel-Derlon, Roseline d'Oiron, Chantal Rothschild, Amal El-Beshlawy, Mohsen Elalfy, Vijay Ramanan, et al.

► To cite this version:

Yohann Repessé, Catherine Costa, Roberta Palla, Erika Farrokhi Moshai, Annie Borel-Derlon, et al.. Role of the factor VIII-binding capacity of endogenous von Willebrand factor on the development of factor VIII inhibitors in patients with severe hemophilia A. *Haematologica*, In press, 10.3324/haematol.2018.212001 . hal-02017457

HAL Id: hal-02017457

<https://hal.sorbonne-universite.fr/hal-02017457>

Submitted on 13 Feb 2019

HAL is a multi-disciplinary open access archive for the deposit and dissemination of scientific research documents, whether they are published or not. The documents may come from teaching and research institutions in France or abroad, or from public or private research centers.

L'archive ouverte pluridisciplinaire **HAL**, est destinée au dépôt et à la diffusion de documents scientifiques de niveau recherche, publiés ou non, émanant des établissements d'enseignement et de recherche français ou étrangers, des laboratoires publics ou privés.

Title: Role of the factor VIII-binding capacity of endogenous von Willebrand factor on the development of factor VIII inhibitors in patients with severe hemophilia A

Running title: Immune-protective role of endogenous VWF towards therapeutic FVIII

Yohann Repessé^{1,2,3}, Catherine Costa^{4†}, Roberta Palla⁵, Erika Farrokhi Moshai^{6,7,8}, Annie Borel-Derlon^{1,2,3}, Roseline D'Oiron⁹, Chantal Rothschild¹⁰, Amal El-Beshlawy¹¹, Mohsen Elalfy¹², Vijay Ramanan¹³, Peyman Eshghi¹⁴, Johannes Oldenburg¹⁵, Anna Pavlova¹⁵, Frits R Rosendaal¹⁷, Flora Peyvandi^{5,16}, Srinivas V Kaveri^{6,7,8}, Sébastien Lacroix-Desmazes^{6,7,8}

¹CHU de Caen, Haematology laboratory, Caen, France; ²INSERM, U1237, GIP Cyceron, Caen; ³Normandie Univ, UNICAEN, UFR Santé, Caen; ⁴Service de Génétique et Biologie Moléculaires, APHP, Groupe Hospitalier Cochin Broca, Hôtel-Dieu, Site Cochin, Paris, France; ⁵Department of Pathophysiology and Transplantation, Università degli Studi di Milano; ⁶Sorbonne Universités, UPMC Univ Paris 06, UMR_S 1138, Centre de Recherche des Cordeliers, Paris; ⁷INSERM, UMR_S 1138, Centre de Recherche des Cordeliers, Paris; ⁸Université Paris Descartes, Sorbonne Paris Cité, UMR_S 1138, Centre de Recherche des Cordeliers, Paris; ⁹Centres de traitement de l'hémophilie, APHP, Le Kremlin-Bicêtre, France; ¹⁰Centres de traitement de l'hémophilie, APHP, Hôpital Necker, Paris France; ¹¹Pediatric Hematology Department, Cairo University Pediatric Hospital, Cairo, Egypt; ¹²Faculty of Medicine, Ain Shams Center, University - Department Pediatrics, Cairo, Egypt; ¹³Jehangir Clinical Development Centre, Department of Hematology, Jehangir Hospital Premises, Pune, India; ¹⁴Pediatric Congenital Hematologic Disorders Research Center, Shahid Beheshti University of Medical Sciences, Tehran, Iran; ¹⁵Institute of Experimental Haematology and Transfusion Medicine, University Clinic Bonn, Germany ; ¹⁶Fondazione IRCCS Ca' Granda Ospedale Maggiore Policlinico, Angelo Bianchi Bonomi Hemophilia and Thrombosis Center; ¹⁷Department of Clinical Epidemiology, Leiden University Medical Center, Leiden, The Netherlands;

Corresponding author: Sébastien Lacroix-Desmazes at INSERM UMR S 1237, Equipe 16, Centre de Recherche des Cordeliers, Paris, F-75006 France - Tel: 01 44 27 81 93 - Fax: 01 44 27 81 94. Email: Sebastien.Lacroix-Desmazes@crc.jussieu.fr

Words: 1496; B&W Figure: 1; Table: 1; References: 16

Supplemental file: Methods, Supplementary results, 2 Figures, 2 Tables

Keywords: hemophilia A, factor VIII inhibitors, von Willebrand factor, immunogenicity

The development of factor VIII (FVIII) inhibitors is the major complication of replacement therapy in patients with severe hemophilia A (HA). Experimental and clinical evidence suggest that the presence of exogenous von Willebrand factor (VWF) in FVIII products reduces the immunogenicity of therapeutic FVIII.¹⁻³ However, a direct immuno-protective effect of endogenous VWF remains unclear.^{4, 5} The binding of VWF to FVIII implicates the first 272 amino acids of the mature VWF (D'-D3 region) encoded by the exons 18-23 of the *VWF* gene.⁶ Mutations in the *VWF* gene that result in quantitative or qualitative defects in VWF lead to von Willebrand disease (VWD). Polymorphisms in the *VWF* gene were studied in the context of venous thrombosis and VWD,^{7, 8} but, to our knowledge, not in that of HA. Here, we investigated whether the capacity of endogenous VWF in patients with severe HA to modulate inhibitor development depends on its capacity to bind to therapeutic FVIII. Our working hypothesis was that gene variations in the D'-D3 region result in qualitative changes in the capacity of circulating endogenous VWF to bind FVIII. While such polymorphisms do not translate into coagulation abnormalities, they might have an impact on the stabilization of the therapeutically administered exogenous FVIII in patient with HA. The consequence would be an increased ratio of free versus bound FVIII molecules and a potentially reduced immuno-protection of FVIII by VWF. Our result show that the relative binding of endogenous VWF to therapeutic FVIII is a poor predictor of inhibitor development, probably reflecting the multi-causal nature of the inhibitor risk.^{9, 10}

We first evaluated the capacity of endogenous VWF in the plasma of 48 randomly selected patients with severe HA to bind rFVIII *in vitro*. VWF:Ag levels were 89.8% (standard error mean (SEM) 10.4) and 91.9% (SEM 13.0) for inhibitor-positive and inhibitor-negative patients, respectively (95% CI -30.9 to 35.0). The relative VWF binding to FVIII (referred to as VWF:FVIII:B) was determined in each sample using an immuno-assay initially validated for the diagnosis of type 2N VWD (See Methods in supplement). VWF:FVIII:B was normally distributed and ranged between 41.1% and 158.9%. Interestingly, the distribution of VWF:FVIII:B was different for inhibitor-positive and negative patients (Figure 1A) with means of 86.4% (SEM 5.1) for inhibitor-positive patients as opposed to 103.6% (SEM 5.8) for inhibitor-negative patients (95% CI 1.3-33.2). The ROC curve of VWF:FVIII:B as a predictor of inhibitor development in patients with severe HA yielded an area under the curve of 0.668 (95% CI 0.513-0.821, Figure 1B). Upon examination of the coordinates of the ROC curve, we chose a potential VWF:FVIII:B cut-off value of 95% that yielded the best relation between sensitivity and specificity. Using this cut-off value, a VWF:FVIII:B below 95% was more frequent among inhibitor-positive patients than inhibitor-negative patients (71% versus

37%), and a value below this cut-off was associated with an over fourfold increased risk of inhibitor development (odds ratio 4.3, 95% CI 1.3-14.5). The proposed cut-off value exhibited a sensitivity of 0.71 (95% CI 0.48-0.89) and specificity of 0.63 (95% CI 0.42-0.81). The calculated positive and negative predictive values (PPV and NPV) for the prediction of inhibitor development were 0.4 and 0.83, respectively, using an inhibitor prevalence of 30%. These data suggest the potential of the VWF:FVIII assay in the preventive identification of severe HA patients at a low risk of developing inhibitors during FVIII replacement therapy. It is noteworthy, however, that a substantial number (38%) of the inhibitor-negative patients included in the study had VWF:FVIII scores lower than the median of the whole population; conversely, 35% of the inhibitor-positive patients presented with VWF:FVIII scores greater than the median. These results highlight the multi-causal nature of inhibitor development.

Exons 18 to 23 were directly sequenced in order to characterize Single Nucleotide Polymorphisms (SNP) in the *VWF* gene from the 48 patients previously tested for VWF:FVIII (See Supplementary Methods). Four SNPs were identified with a prevalence equivalent to that previously described in different non-hemophilic populations⁸: c.2365 A>G, p.Thr789Ala (rs1063856); c.2385 T>C, p.Tyr795Tyr (rs1063857); c.2555 G>A, p.Arg852Gln (rs216321) and c.2880 G>A, p.Arg960Arg (rs1800380). The association between VWF:FVIII and SNP genotypes was assessed. The two silent SNPs (p.Tyr795Tyr and p.Arg960Arg) (data not shown) and the p.Thr789Ala had no impact on VWF:FVIII (Figure 1C). However, c.2555 G>A SNP, corresponding to the substitution of an arginine with a glutamine at position 852, was associated with a statistically significant reduction in VWF:FVIII in the case of plasma from the heterozygous G/A patients as compared to plasma from patients with the homozygous frequent G/G genotype ($P < 0.001$, 95% CI 11.87-42.51) (Figure 1D). No patient with the rare A/A genotype was detected. Two patients carried either one of the p.Arg854Gln and p.Arg924Gln mutations associated with VWD. The transition p.Arg854Gln, described as a type 2N VWD causative mutation,¹¹ was found in one patients without inhibitor. Previously reported to be a polymorphism in a study of type 2N VWD mutations,¹² the p.Arg924Gln, which represents a non-conservative amino acid substitution in exon 21, was observed in one patient with inhibitor. These missense mutations were associated with normal VWF:Ag levels and reduced VWF:FVIII, 41% and 42% in one inhibitor-negative patient and one inhibitor-positive patient, respectively (lower 2 points in Figure 1D). A previous study by Nesbitt *et al.* had identified the c.2555 G>A SNP in 16 of 148 screened alleles.¹³ In contrast to our findings, their results had suggested that the

VWF:FVIIIIB was not affected by the p.Arg852Gln polymorphism in VWF, possibly owing to the relatively low number of patients with the c.2555 G>A SNP included in their study.

In an attempt to determine whether the c.2555 G>A SNP in exon 18 of the *VWF* gene is associated with the occurrence of FVIII inhibitors in the patients, we searched for the SNP in 235 patients enrolled in the SIPPET study.² The cohort included 163 inhibitor-negative patients and 72 inhibitor-positive patients, encompassing 14 low-responder and 48 high-responder patients. Genotype frequencies of the polymorphism are summarized in Table 1. The distribution of the c.2555 G>A genotypes did not deviate from the Hardy-Weinberg equilibrium in both inhibitor-negative and inhibitor-positive patients. No clear association between the c.2555 G>A SNP genotypes and the development of inhibitors was observed (Table 1, OR 0.61, 95% CI 0.28-1.32). These data are in line with a similar analysis performed in parallel using biological samples from a multicentric retrospective cohort of 281 patients with severe HA¹⁴ (supplementary Tables S1 and S2), suggesting that the different ethnic origin of patients in the SIPPET cohort² does not account for the results. Genotypes and alleles frequencies in both cohorts were identical to results from the 1000 Genomes Project (1111G).⁸

If our working hypothesis is correct, the nature of the VWF variant should play a role predominantly for patients receiving rFVIII products, but not for patients receiving exogenous VWF with the pdFVIII products. Among the 235 SIPPET patients included in the present study, 118 patients were treated with rFVIII concentrates and 117 patients received pdFVIII products following randomization (1:1). Associations between the genotype distribution and development of FVIII inhibitors were addressed in the two groups of patients (Table 1). There was again no clear association between the presence of A allele of the c.2555 G>A SNP and the presence of a FVIII inhibitor, both in the case of the rFVIII-treated group (OR 1.16, 95% CI 0.41-3.30) and of the pdFVIII-treated patients (OR 0.12, 95% CI 0.09-1.24). A genome-wide association study evaluated 13331 SNPs from 1,081 genes using the Illumina iSelect platform for associations with inhibitor development in patients with HA. The study group included 833 subjects from three independent cohorts. The authors identified 53 SNPs as significant predictors of the inhibitor status, thus highlighting the complexity of the anti-FVIII immune response.¹⁵ However, the genome-wide association study did not find associations of SNPs in the *VWF* gene with the inhibitor status of the patients, which comforts the present findings.

A major limitation of the study is the discrepancy between our observations: i) an overall reduced relative endogenous VWF binding endogenous VWF in the plasma from inhibitor-

positive severe HA patients, ii) a reduced relative endogenous VWF binding with the c.2555 G>A SNP and iii) the lack of association of the 2555 G>A SNP with the inhibitory status of the patients. Recently, Muczynski et al. developed a recombinant FVIII (FVIII-KB013bv) that contains two VWF-specific nanobodies in place of the B domain.¹⁶ FVIII-KB013bv has a 25-fold increased affinity for VWF as compared to B domain-deleted FVIII, and exhibits a prolonged blood residence time in FVIII-deficient mice. Interestingly, FVIII-KB013bv demonstrated an almost complete lack of immunogenicity *in vivo* in FVIII-deficient mice. In view of the latter information, the discrepancy between our observations may be explained by the fact that, owing to the multi-causal nature of the inhibitor risk, an affinity of the endogenous VWF for therapeutic FVIII in the high physiological range does not systematically play a major protective role. Instead, a stabilization of the complex beyond the physiological equilibrium affinity is required to exert blatant immune-protective functions.

Acknowledgements

The work was supported by Institut National de la Santé et de la Recherche Médicale (INSERM), Centre National de la Recherche Scientifique (CNRS), Université Paris Sorbonne, and Agence Nationale de la Recherche (ANR-07-MRAR-028-01).

†This work is dedicated to the memory of our late colleague and friend Catherine Costa, an extraordinary geneticist and a wonderful human being.

Authors contribution

Designed research: YR, SVK, SLD

Performed research: YR, CC, RP, EFM, AP, SLD

Participated to cohorts: CC, ABD, Rd'O, CR, AEB, ME, VR, PE, JO, PMM, FRR, FP

Analyzed data: YR, RP, FP, SLD

Wrote the paper: YR, RP, FP, SLD

The authors declare no conflict of interest

Appendix

SIPPET Study Group. S. Hanagavadi, Davangere, India; R. Varadarajan, Chennai, India; M. Karimi, Shiraz, Iran; M. V. Manglani, Mumbai, India; C. Ross, Bangalore, India; G. Young, Los Angeles, USA; T. Seth, New Delhi, S. Apte, Pune, India; D. M. Nayak, Karnataka, India;

E. Santagostino, M. Elisa Mancuso, Milan, Italy; A. C. Sandoval Gonzalez, Monterrey, Mexico; J. N. Mahlangu, Johannesburg, South Africa; S. Bonanad Boix, Valencia, Spain; M. Cerqueira, Rio de Janeiro, Brazil; N. P. Ewing, Duarte, USA; C. Male, Vienna, Austria; T. Owaidah, Riyadh, Saudi Arabia; V. Soto Arellano, Fargo, USA; N. L. Kobrinsky, Jackson, USA; S. Majumdar, R. Perez Garrido, Sevilla, Spain; A. Sachdeva, New Delhi, India; M. Simpson, Chicago, USA; M. Thomas, Kerala, India; E. Zanon, Padova, Italy; B. Antmen, Adana, Turkey; K. Kavakl, Izmir, Turkey; M. J. Manco-Johnson, Aurora, USA; M. Martinez, Buenos Aires, Argentina; E. Marzouka, Santiago, Chile; M. G. Mazzucconi, Rome, Italy; D. Neme, Buenos Aires, Argentina; A. Palomo Bravo, Malaga, Spain; R. Paredes Aguilera, Mexico City, Mexico; A. Prezotti, Vitoria, Brazil; K. Schmitt, Linz, Austria; B. M. Wicklund, Kansas City, USA; B. Zulfikar, Istanbul, Turkey.

References

1. Lai J, Hough C, Tarrant J, Lillicrap D. Biological considerations of plasma-derived and recombinant factor VIII immunogenicity. *Blood*. 2017;129(24):3147-3154.
2. Peyvandi F, Mannucci PM, Garagiola I, et al. A Randomized Trial of Factor VIII and Neutralizing Antibodies in Hemophilia A. *N Engl J Med*. 2016;374(21):2054-2064.
3. Calvez T, Chambost H, d'Oiron R, et al. Analyses of the FranceCoag cohort support differences in immunogenicity among one plasma-derived and two recombinant factor VIII brands in boys with severe hemophilia A. *Haematologica*. 2018;103(1):179-189.
4. Gangadharan B, Ing M, Delignat S, et al. The C1 and C2 domains of blood coagulation factor VIII mediate its endocytosis by dendritic cells. *Haematologica*. 2017;102(2):271-281.
5. Meeks SL, Cox CL, Healey JF, et al. A major determinant of the immunogenicity of factor VIII in a murine model is independent of its procoagulant function. *Blood*. 2012;120(12):2512-2520.
6. Leyte A, Verbeet MP, Brodniewicz-Proba T, van Mourik JA, Mertens K. The interaction between human blood-coagulation factor VIII and von Willebrand factor. *Biochem J*. 1989;257:679-683.
7. Bittar LF, de Paula EV, Mello TB, et al. Polymorphisms and mutations in vWF and ADAMTS13 genes and their correlation with plasma levels of FVIII and vWF in patients with deep venous thrombosis. *Clin Appl Thromb Hemost*. 2011;17(5):514-518.
8. Wang QY, Song J, Gibbs RA, et al. Characterizing polymorphisms and allelic diversity of von Willebrand factor gene in the 1000 Genomes. *J Thromb Haemost*. 2013;11(2):261-269.
9. Gouw SC, van den Berg HM. The multifactorial etiology of inhibitor development in hemophilia: genetics and environment. *Seminars in thrombosis and hemostasis*. 2009;35(8):723-734.
10. Eckhardt CL, van Velzen AS, Fijnvandraat CJ, van der Bom JG. Dissecting intensive treatment as risk factor for inhibitor development in haemophilia. *Haemophilia*. 2016;22(3):e241-244.
11. Gaucher C, Mercier B, Jorieux S, Oufkir D, Mazurier C. Identification of two point mutations in the von Willebrand factor gene of three families with the 'Normandy' variant of von Willebrand disease. *Br J Haematol*. 1991;78(4):506-514.

12. Hilbert L, Jorieux S, Proulle V, et al. Two novel mutations, Q1053H and C1060R, located in the D3 domain of von Willebrand factor, are responsible for decreased FVIII-binding capacity. *Br J Haematol.* 2003;120(4):627-632.
13. Nesbitt IM, Goodeve AC, Preston FE, Peake IR. von Willebrand factor/factor VIII binding is not affected by the Arg89Gln polymorphism in von Willebrand factor. *Thromb Haemost.* 1996;76(5):820-821.
14. Repesse Y, Peyron I, Dimitrov JD, et al. Development of inhibitory antibodies to therapeutic factor VIII in severe hemophilia A is associated with microsatellite polymorphisms in the HMOX1 promoter. *Haematologica.* 2013;98(10):1650-1655.
15. Astermark J, Donfield SM, Gomperts ED, et al. The polygenic nature of inhibitors in hemophilia A: results from the Hemophilia Inhibitor Genetics Study (HIGS) Combined Cohort. *Blood.* 2013;121(8):1446-1454.
16. Muczynski V, Casari C, Moreau F, et al. A factor VIII-nanobody fusion protein forming an ultrastable complex with VWF: effect on clearance and antibody formation. *Blood.* 2018;132(11):1193-1197.

Table 1. c.2555 G>A genotypes distribution and association with the development of FVIII inhibitor in 235 patients with severe HA from the SIPPET study²

rFVIII + pdFVIII	Inh-negative (n=163)	Inh-positive (n=72)			OR	95% CI
		LR (n=24)	HR (n=48)	LR+HR (n=72)		
G/G	129 (79%)	22	40	62 (86%)	0.61	0.28-1.32
G/A + A/A	34 (21%)	2	8	10 (14%)		

rFVIII-treated group	Inh-negative (n=73)	Inh-positive (n=45)			OR	95% CI
		LR (n=16)	HR (n=29)	LR+HR (n=45)		
G/G	63 (86%)	15	23	38 (84%)	1.16	0.41-3.30
G/A + A/A	10 (14%)	1	6	7 (16%)		

pdFVIII-treated group	Inh-negative (n=90)	Inh-positive (n=27)			OR	95% CI
		LR (n=8)	HR (n=19)	LR+HR (n=27)		
G/G	66 (73%)	7	17	24 (89%)	0.35	0.09-1.24
G/A + A/A	24 (27%)	1	2	3 (11%)		

LR: Low Responder; HR: High Responder; CI: confidence interval; OR: Odds Ratio.

Figure Legend

Figure 1. Relative endogenous VWF binding and inhibitory status in patients with severe HA. Panel A. Association between relative VWF binding (VWF:FVIII_B) and inhibitor status in severe HA patients (n=48). The X axis represents the inhibitor status: patients with HA without FVIII inhibitor (Inh-neg) and with FVIII inhibitor (Inh-pos). The Y axis represents the relative binding of recombinant FVIII to the endogenous VWF in the plasma of patients with severe HA measured by ELISA (expressed in %). The 95% confidence intervals (CI) was constructed with the standard errors derived from the Student's t distribution. Panel B. Receiver Operating Characteristic (ROC) curve for predicting inhibitor development in patients with severe HA by measurement of VWF:FVIII_B. The true positive rate (sensitivity) is plotted as a function of the false positive rate (100-specificity). AUC, Area Under Curve. Panels C and D. Association between VWF:FVIII_B and the p.Thr789Ala (c.2365 A>G) polymorphism (C) or the p.Arg852Gln (c.2555 G>A) polymorphism (D) in the exon 18 of the *VWF* gene. Statistical difference were determined using the Student's t test.