Erasmus MC
Universitair Medisch Centrum Rotterdam

# The diagnosis of von Willebrand Disease

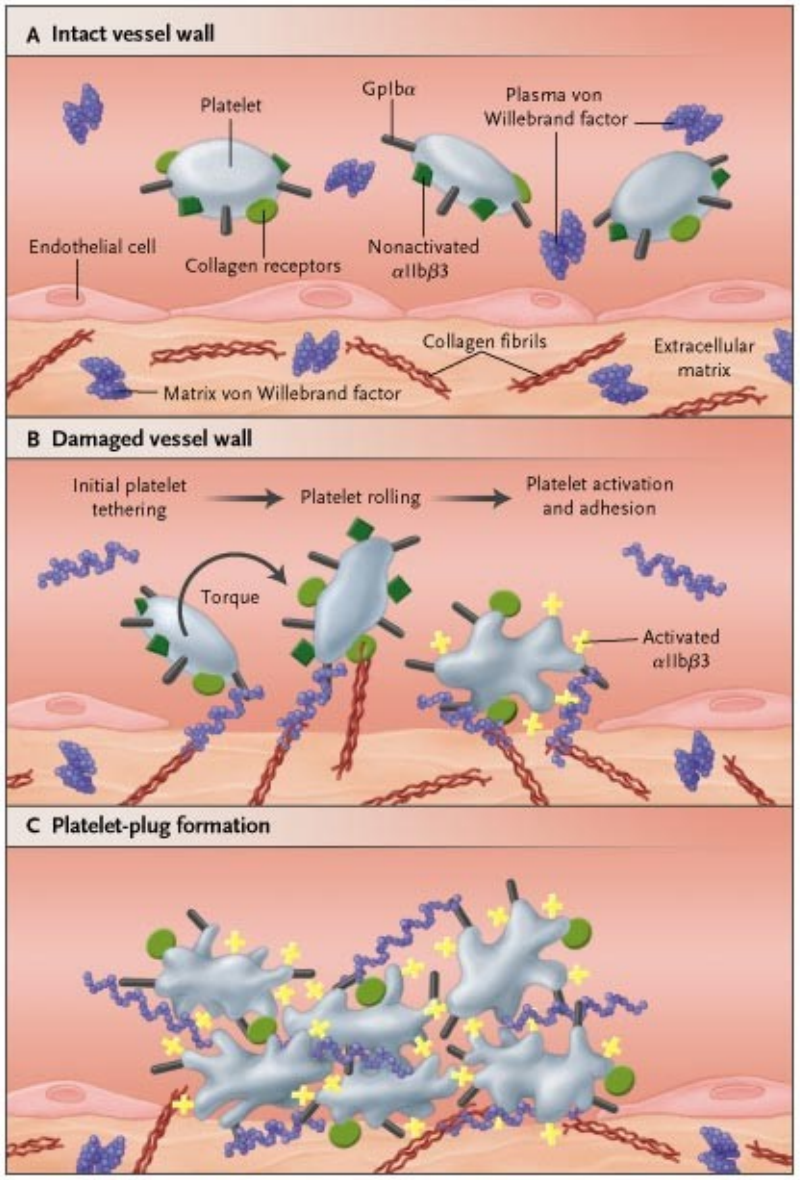
ECAT participants meeting, 10 November 2015

Leiden

Prof. Dr. Frank W.G. Leebeek

ErasmusMC, Rotterdam, The Netherlands

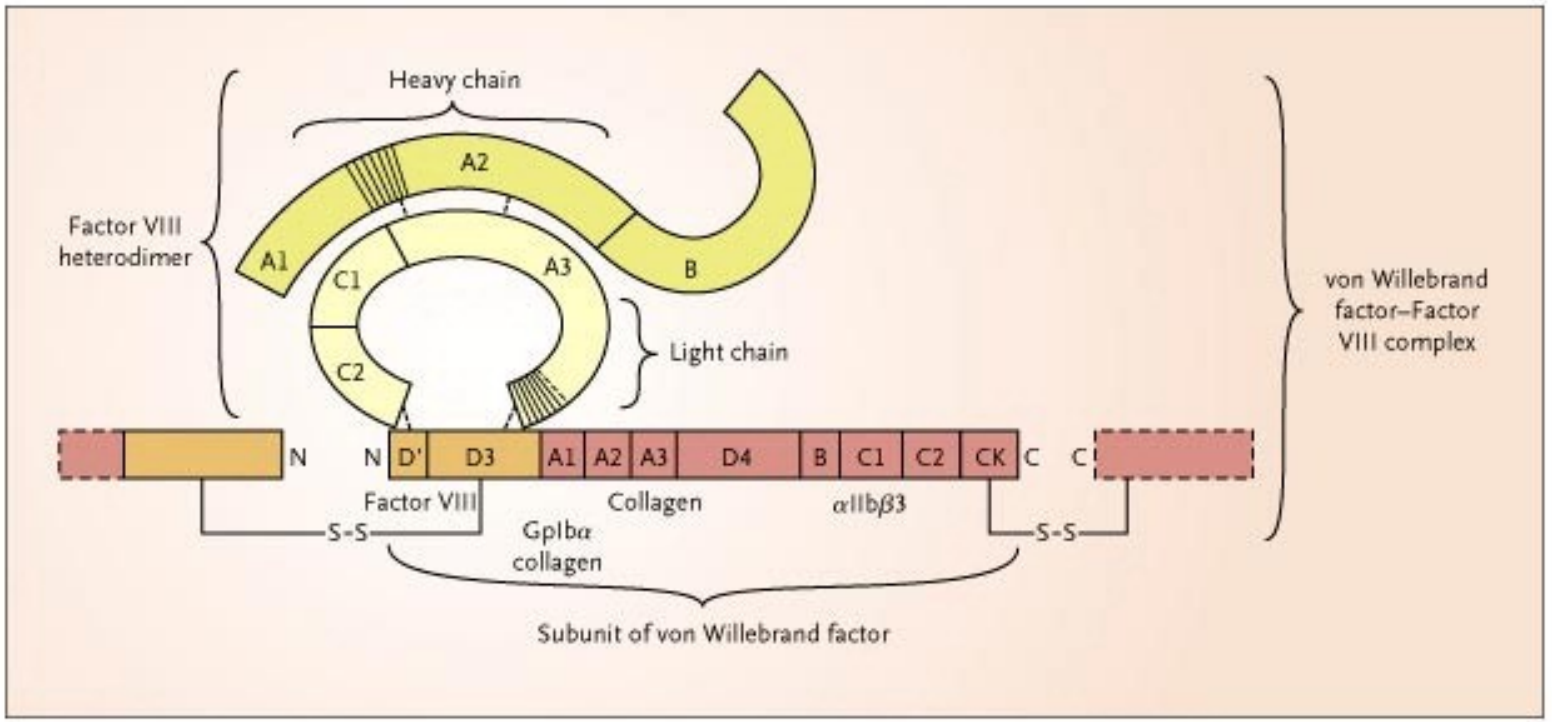
# Functions of Von Willebrand factor




1. Adhesion of platelets
2. Aggregation of platelets
3. Carrier of factor VIII

Mannucci PM, NEJM 2004

# Von Willebrand factor – carrier protein of coagulation factor VIII




Mannucci, PM. NEJM 2004

# Von Willebrand Disease (VWD)

- Most common inherited bleeding disorder
- Mucocutaneous bleeding symptoms
- Prevalence: 0.5-1%
- Autosomal dominant / recessive
- Caused by a deficiency / abnormality of von Willebrand Factor (VWF)
  - Type 1: quantitative disorder, reduced level of normal VWF
  - Type 2: qualitative disorder, abnormal VWF molecule
  - Type 3: quantitative disorder, no detectable VWF in circulation

# Von Willebrand disease (VWD)

Prevalence 1:100

- Type 1 VWD: partial quantitative deficiency of VWF
- Type 2 VWD: dysfunction of VWF
  - 2A: abnormal VWF multimer pattern
  - 2B: ↑ platelet GP1b binding, abnormal multimers
  - 2M: ↓ GP1b or collagen binding
  - 2N: ↓ FVIII binding → low FVIII concentration
- Type 3 VWD: virtually complete absence of VWF

*ISTH classification Sadler et al. JTH 2006*

# National study of moderate and severe von Willebrand disease in the Netherlands
# WiN study

- National study including children and adults from all HTCs
- Moderate or severe Von Willebrand Disease

  moderate: VWF 10-30 IU/dL or FVIII 20-40 IU/dL

  severe: VWF < 10 IU/dL or FVIII < 20 IU/dL
- All types of VWD
- Questionnaire
- Blood or saliva sample

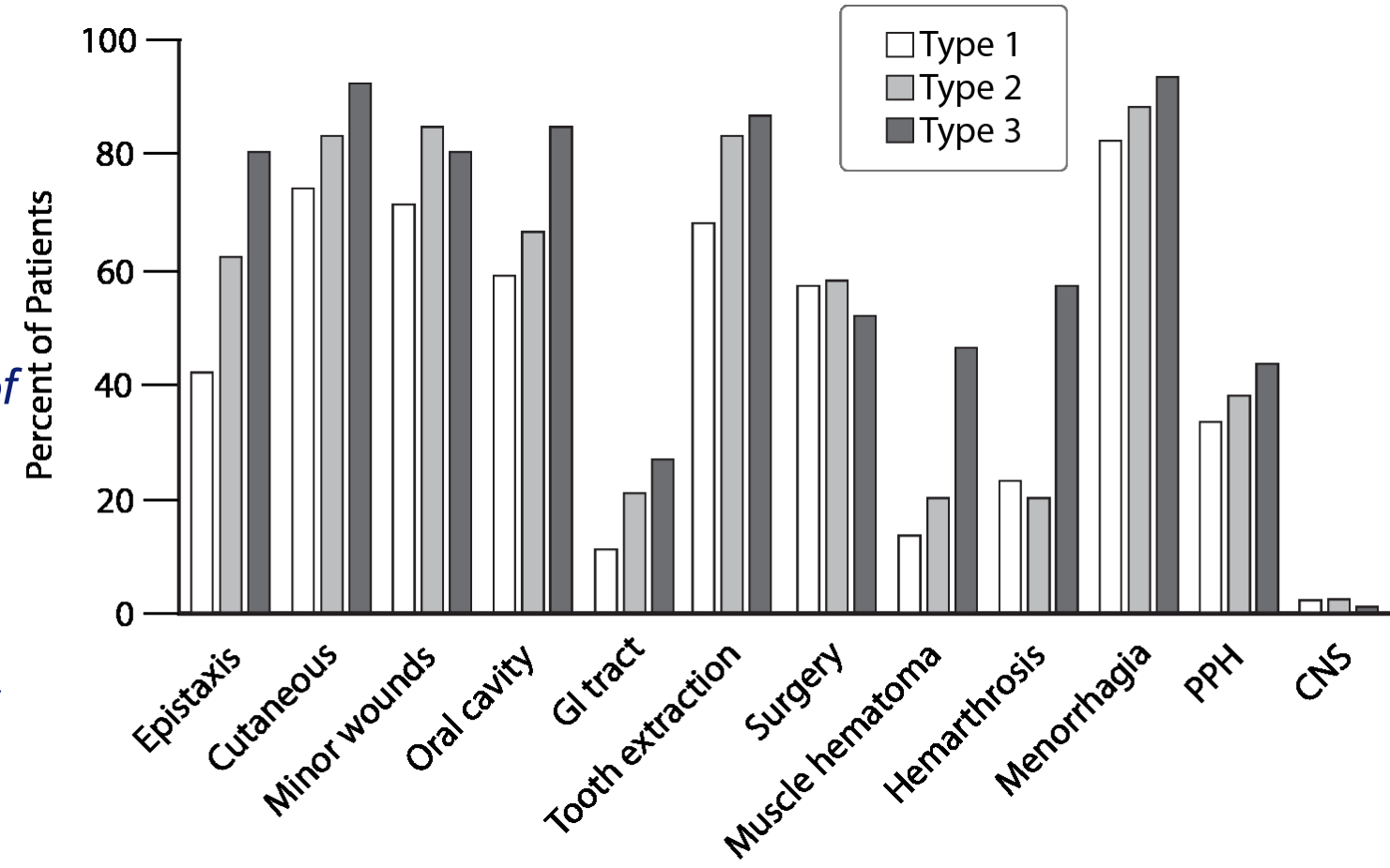
- Inclusion period 2007 - 2009
- 837 patients included

# von Willebrand disease: Symptoms

- Mucosal bleedings
  - Nose bleeds
  - Gingiva bleeds
  - Menorrhagia
- Easy bruising
- Bleeding after surgical interventions
  - dental extractions
  - tonsillectomy
- Bleeding after delivery (type 2/3)
- Joint bleeding

# Bleeding Symptoms per Type VWD (VWF < 30%)

- *Win study: > 800 patients with (moderate) severe VWD*
- *Quantitative measurement of severity by Tosetto Bleeding Score*
- *Data on bleeding, factor levels, and treatment*




*De Wee EM et al, TH 2012*

# What are the current diagnostic problems in VWD

- What is a normal and what is an abnormal VWF level ?
- Diagnosis of VWF is based on which treshold VWF level ?
- What is the best VWF activity test to be used?
- How do we distinguish between type 3 and severe type 1 ?
- Do we need genotypic analysis in VWD?

# Screening test for VWD

- Bleeding time
  - Lack of standardization, low reproducibility
  - Not sensitive enough for mild cases of VWD
  - Not predictive of bleeding tendency

- PFA-100®
  - High sensitivity for severe VWD
  - Sensitivity as low as 50% in mild VWD
  - High variability up to 20%

# Diagnosis of VWD

Based on clinical and laboratory phenotype

**Clinical phenotype**: bleeding score (ISTH BAT)

**Laboratory phenotype**:

**First line tests:**

| | |
|---|---|
| VWF:Ag | VWF antigen |
| VWF:RCo | Platelet-dependent VWF activity: VWF-to-platelet binding capacity |
| FVIII:C | Factor VIII coagulant activity |

# Diagnosis of VWD

**Second line tests:**

VWF:CB VWF collagen binding capacity

- Measures the ability of VWF to bind to collagen
- A3 domain of VWF
- High molecular weight multimers
- Results depend on type of collagen used (I or III)

VWFpp VWF propeptide

Multimer pattern

RIPA

VWF:FVIIIB

**Third line tests:**

Genetic analysis / mutation analysis

bjh guideline

# The diagnosis and management of von Willebrand disease: a United Kingdom Haemophilia Centre Doctors Organization guideline approved by the British Committee for Standards in Haematology

Mike A. Laffan,[1] Will Lester,[2] James S. O'Donnell,[3] Andrew Will,[4] Robert Campbell Tait,[5] Anne Goodeve,[6] Carolyn M. Millar[1] and David M. Keeling[7]

- We recommend against the use of reference ranges or blood group-specific ranges for the diagnosis of von Willebrand disease (VWD) (2C).
- When investigating a patient with mucocutaneous bleeding a diagnosis of VWD can be made when von Willebrand factor (VWF) activity is <0·30 iu/ml (1B).

haematologica | 2013; 98(5)

REVIEW ARTICLE

# Principles of care for the diagnosis and treatment of von Willebrand disease

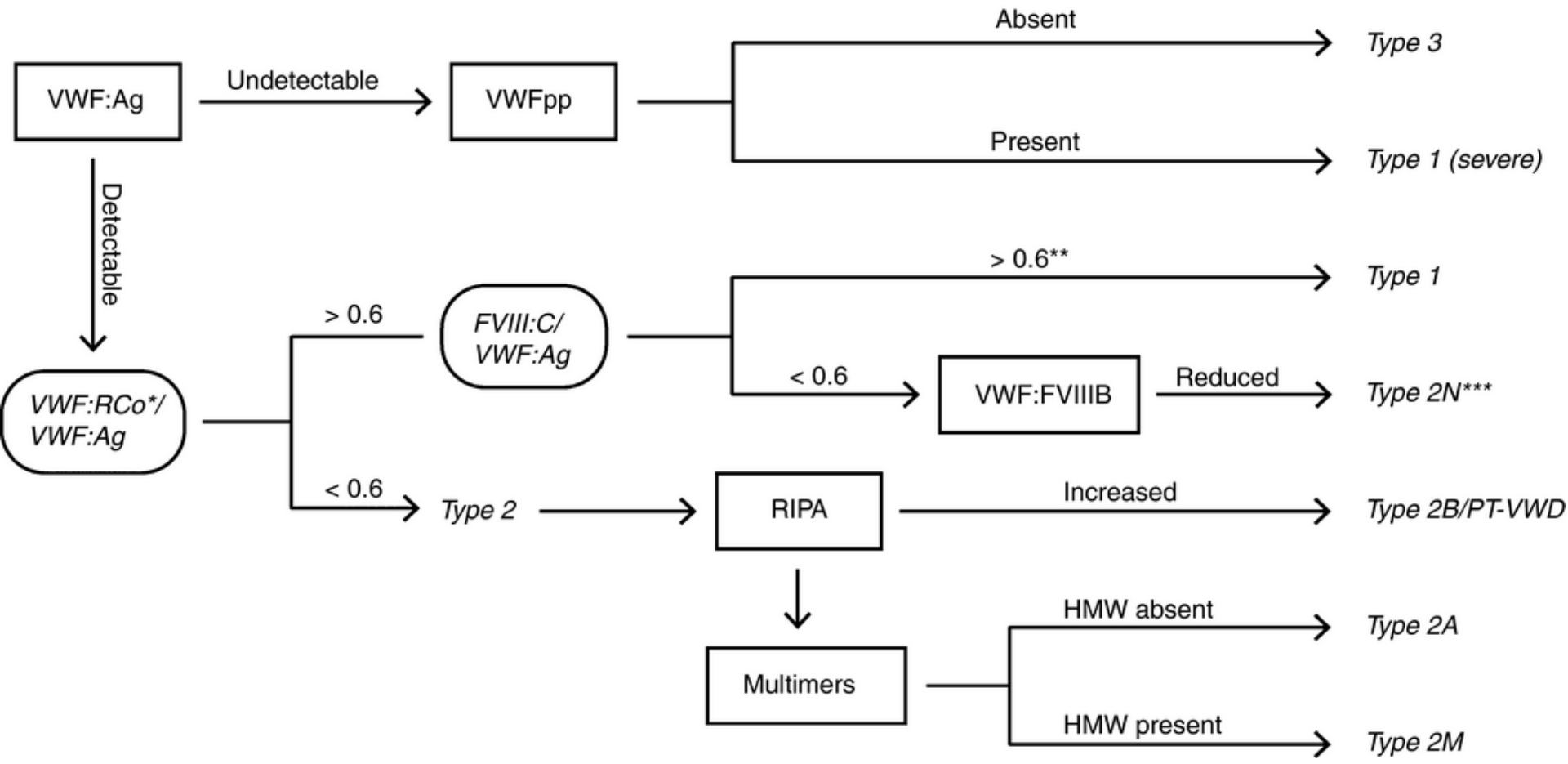
Giancarlo Castaman,[1] Anne Goodeve,[2] and Jeroen Eikenboom,[3] on behalf of the European Group on von Willebrand disease (EUVWD)

[1]Department of Cell Therapy and Hematology, San Bortolo Hospital, Vicenza, Italy; [2]Haemostasis Research Group, Department of Cardiovascular Science, University of Sheffield, United Kingdom; [3]Department of Thrombosis and Hemostasis, Leiden University Medical Center, Leiden, The Netherlands

1. VWD diagnosis should be considered within the context of an appropriate personal and/or familial bleeding history. The use of a standardized questionnaire for history collection is advisable to appreciate the severity of the bleeding tendency.
2. Other common hemostatic defects should be excluded by performing a platelet count, APTT, PT and PFA-100 (or bleeding time).
3. If personal and/or familial bleeding history is significant, VWF:RCo assay should be carried out at this stage. If not possible, VWF:Ag assay or VWF:CB assay should be performed. VWF:Ag < 3 U/dL suggests type 3 VWD. VWF:Ag and VWF:RCo and FVIII:C should be measured on the same sample to assess the presence of a reduced VWF:RCo/VWF:Ag ratio (a ratio < 0.6 suggest type 2 VWD) or FVIII:C/VWF:Ag (a ratio < 0.6 suggests type 2N VWD, to be confirmed by binding study of FVIII to patient's VWF).
4. If any of these tests is below 40 U/dL, the diagnosis of VWD should be strongly considered.

# “Low VWF”

- Patients with bleeding symptoms
- VWF levels 30-60 IU/dL
- Low heritability
- Treatment with desmopressin and / or fibrinolysis inhibitors




De Jong & Eikenboom, JTH 2016

Erasmus MC

# Platelet-dependent von Willebrand factor activity. Nomenclature and methodology: communication from the SSC of the ISTH

I. BODÓ,* J. EIKENBOOM,† R. MONTGOMERY,‡ J. PATZKE,§ R. SCHNEPPENHEIM¶ and J. DI PAOLA,** ON BEHALF OF THE SUBCOMMITTEE ON VON WILLEBRAND FACTOR

*Journal of Thrombosis and Haemostasis*, **13**: 1345–1350

| Abbreviation for VWF activity | Description |
|---|---|
| VWF:RCo | Ristocetin cofactor activity: all assays that use platelets and ristocetin |
| VWF:GPIbR | All assays that are based on the ristocetin-induced binding of VWF to a recombinant WT GPIb fragment |
| VWF:GPIbM | All assays that are based on the spontaneous binding of VWF to a gain-of-function mutant GPIb fragment |
| VWF:Ab | All assays that are based on the binding of a monoclonal antibody (m*Ab*) to a VWF A1 domain epitope |

# Platelet-dependent VWF activity assays

## VWF:RCo

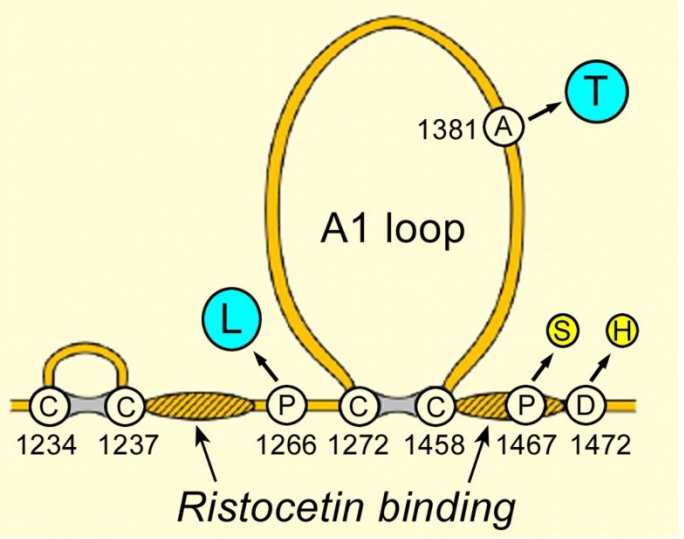
Measures the capacity of VWF to interact with GP1b/IX complex in the presence of ristocetin

Plasma VWF agglutinates formalin fixed reagent platelets

- Several commercial kits

However:

- Poor sensitivity (loss of detection 10 IU/dL)
- High coëfficiënt of variation
- Older assays were cumbersome
- Genetic variations affecting binding of ristocetin may lead to falsely abnormal values (p.D1472H)




Sadler, E Blood 2010; *Bodó et al, JTH 2015*

# Other functional VWF assays

## VWF:Ab

Measures binding of VWF A1 domain to a monoclonal antibody

Good correlation with VWF:RCo

Not really an activity assay

May not detect some type 2M mutations

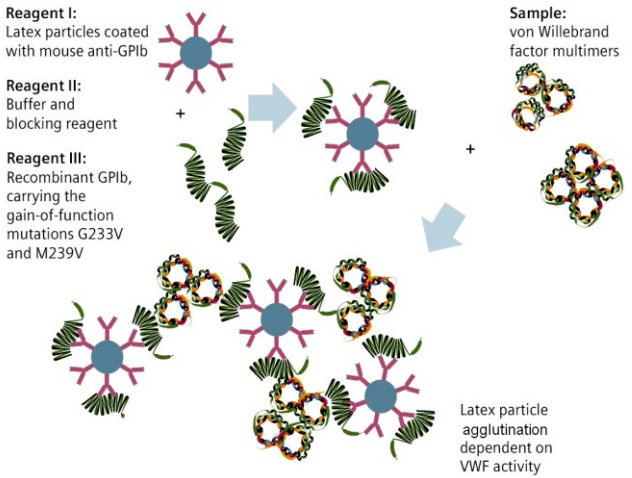
## VWF:GP1bM

Uses gain-of-function GPIb fragments

No ristocetin required

Sensitive and precise

May be affected by human-to-animal-antibodies




*Bodó et al JTH 2015; Bowyer et al Haemophilia 2016*

# Laboratory measurements

**VWF:Ab** (HemosIL$^{TM}$ von Willebrand Factor Activity)

Assay based on latex particles conjugated to MAb directed against VWF GPIb binding site

Centrally measured at inclusion in the WiN study

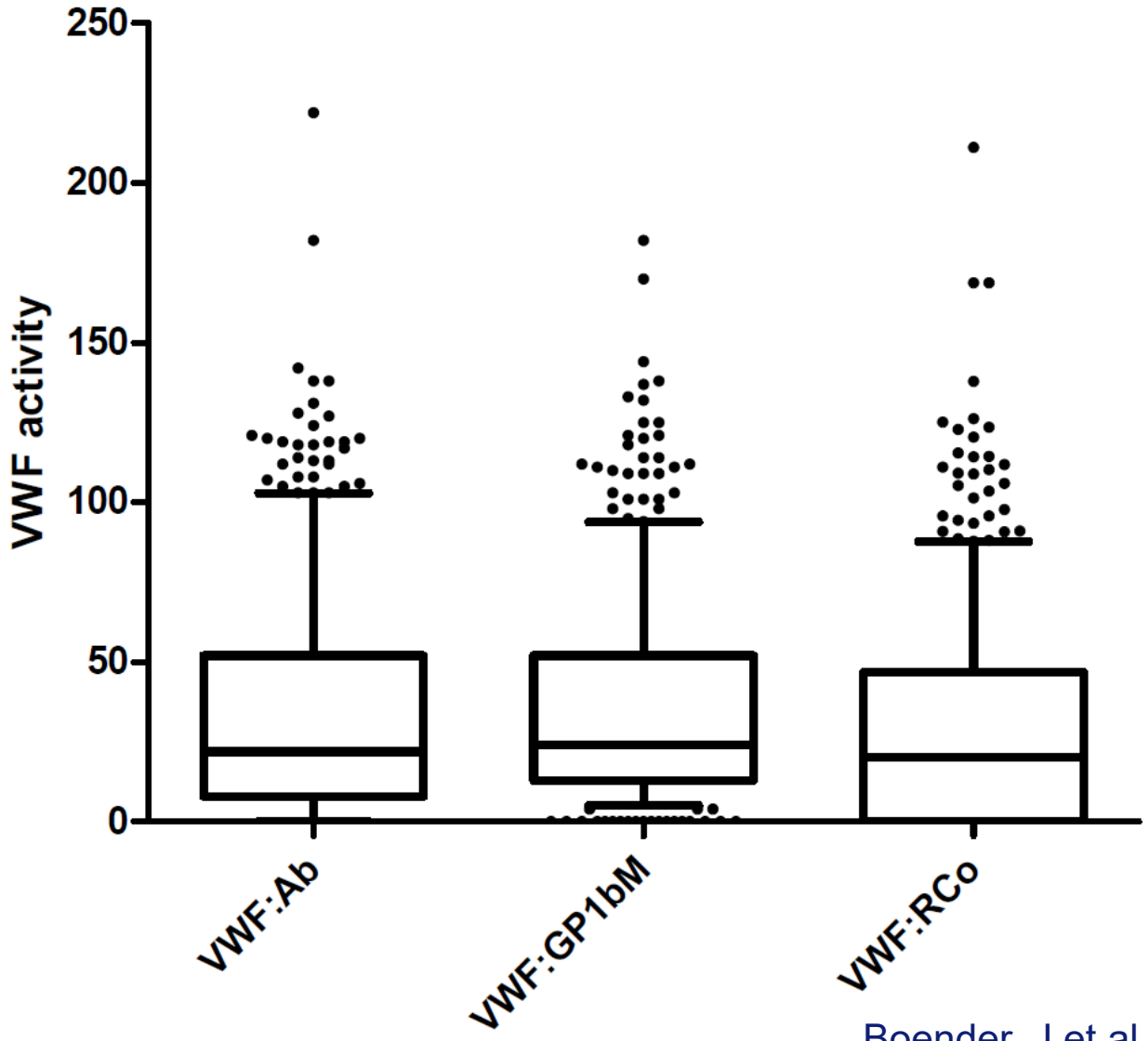
**VWF:RCo** (Siemens BC von Willebrand reagent)

**VWF:GP1bM** (Innovance VWF Ac)

# Patient characteristics (WiN study; n=658)

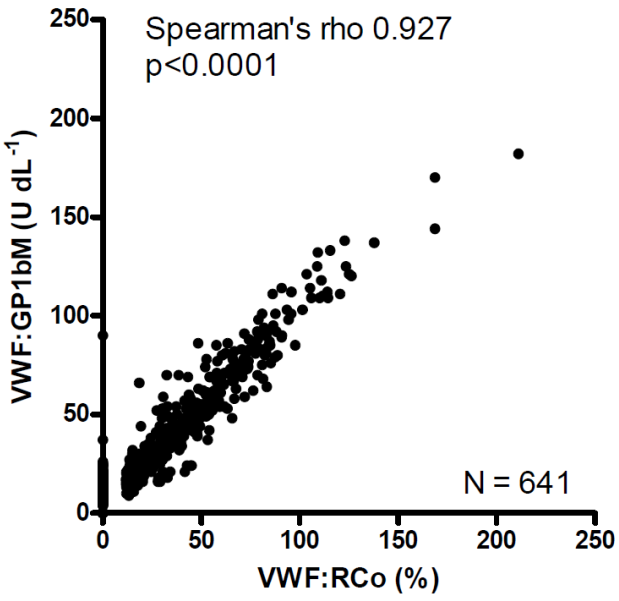
| | | |
|---|---|---|
| age, median (IQR) | 44 | 29-57 |
| male sex, n (%) | 251 | 38.1 |
| VWF:Ag [U/dL], median (IQR) | 29 | 18-45 |
| VWF:CB [U/dL], median (IQR) | 22 | 7-51 |
| FVIII:C [U/dL], median (IQR) | 51 | 32-73 |
| Bleeding score, median (IQR) | 11 | 6-16 |

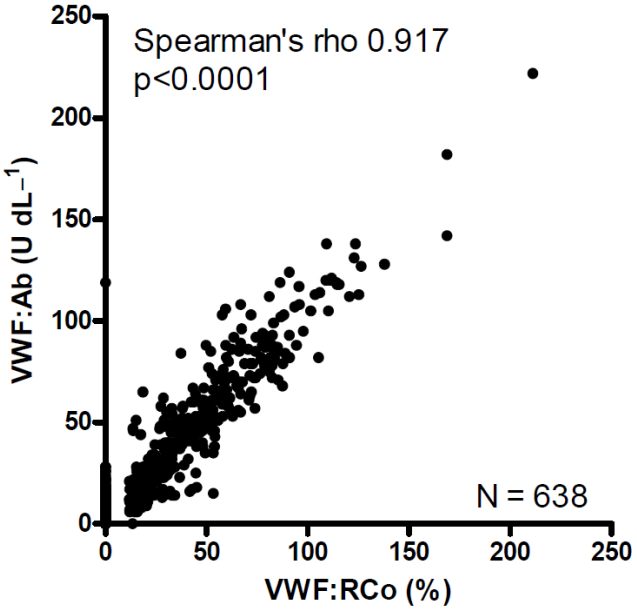
# Results - measurements

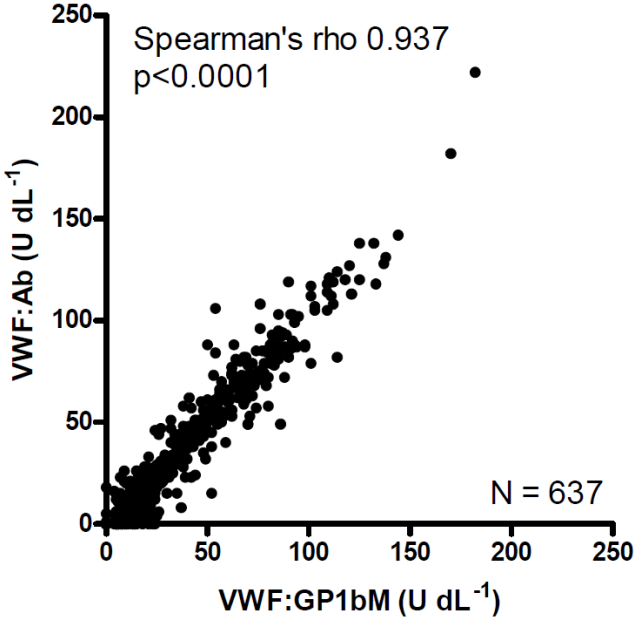



Boender, J.et al. In preparation

# Correlation between functional VWF assays



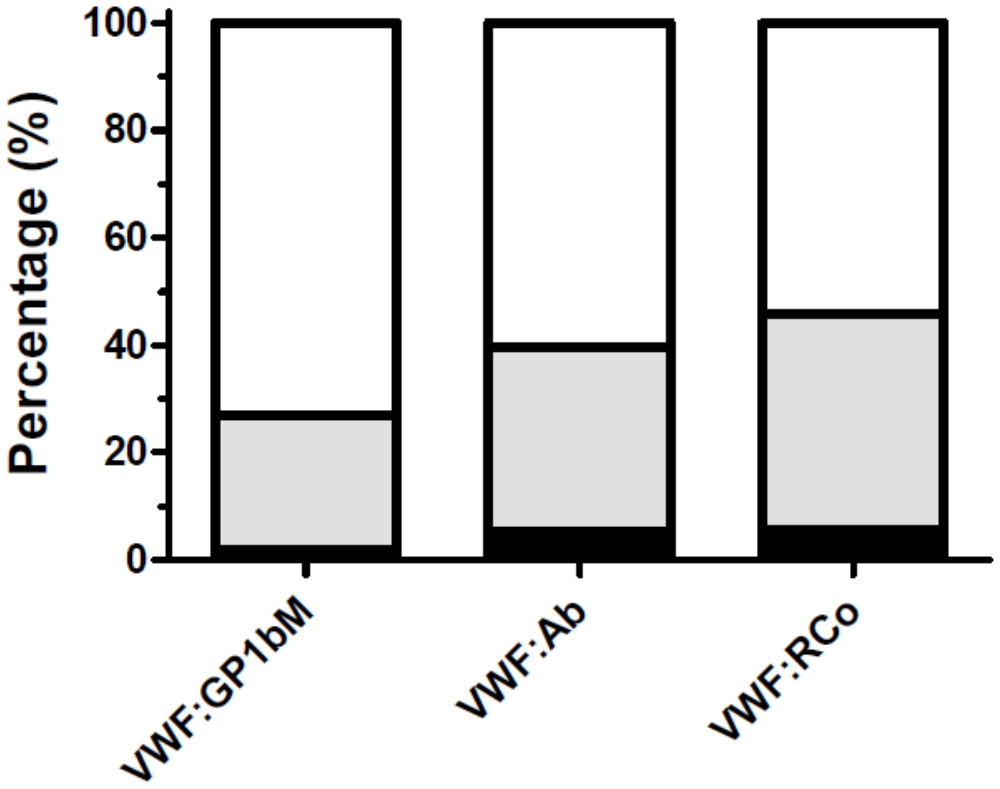




The different assays are highly correlated with each other

Lower detection limit is highest for VWF:RCo

| | |
|---|---|
| VWF:RCo | 11.9% |
| VWF:GP1bM | 4 U/dL |
| VWF:Ab | 1 U/dL |

# Effect on diagnosis of type VWD

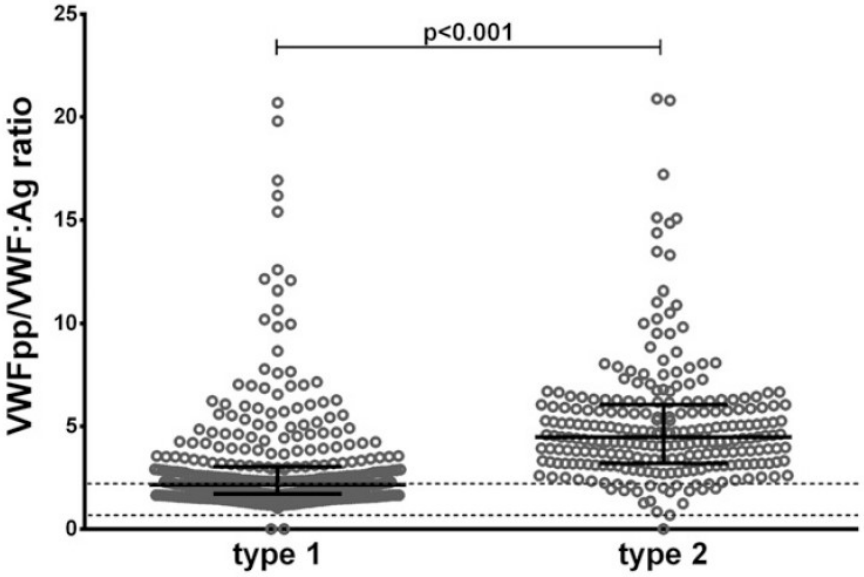


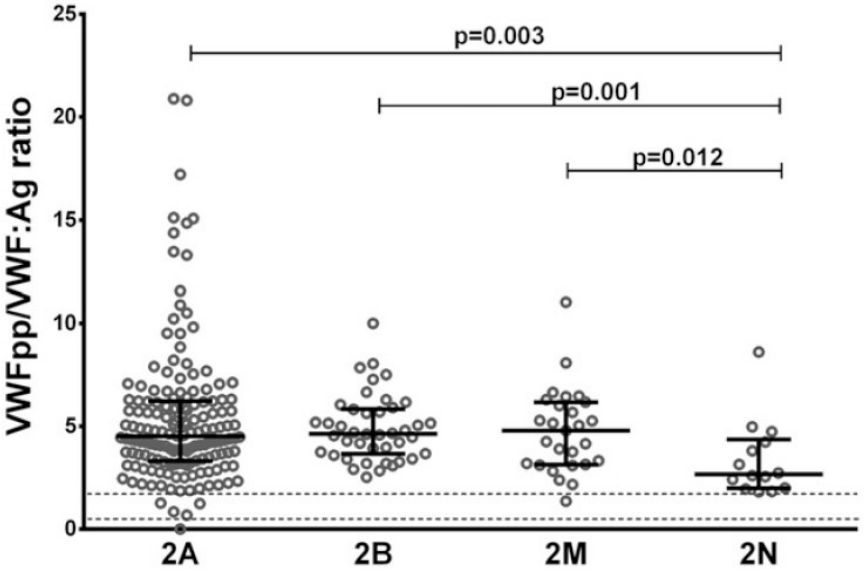

Ratio
VWF activity / VWF:Ag <0.7

| N = 641 | N = 653 | N = 642 | |
|---|---|---|---|
| 467 | 394 | 347 | Type 1 or 2N VWD |
| 161 | 224 | 259 | Type 2 A/B/M VWD |
| 13 | 35 | 36 | Type 3 VWD |

# von Willebrand factor propeptide and the phenotypic classification of von Willebrand disease

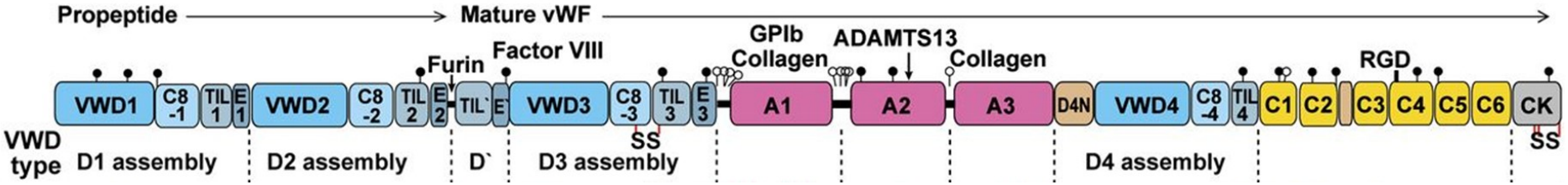
Yvonne V. Sanders,[1] Dafna Groeneveld,[2] Karina Meijer,[3] Karin Fijnvandraat,[4] Marjon H. Cnossen,[5] Johanna G. van der Bom,[6,7] M. Coppens,[8] Joke de Meris,[9] Britta A. P. Laros-van Gorkom,[10] Eveline P. Mauser-Bunschoten,[11] Frank W. G. Leebeek,[1] Jeroen Eikenboom,[2] and the WiN study group

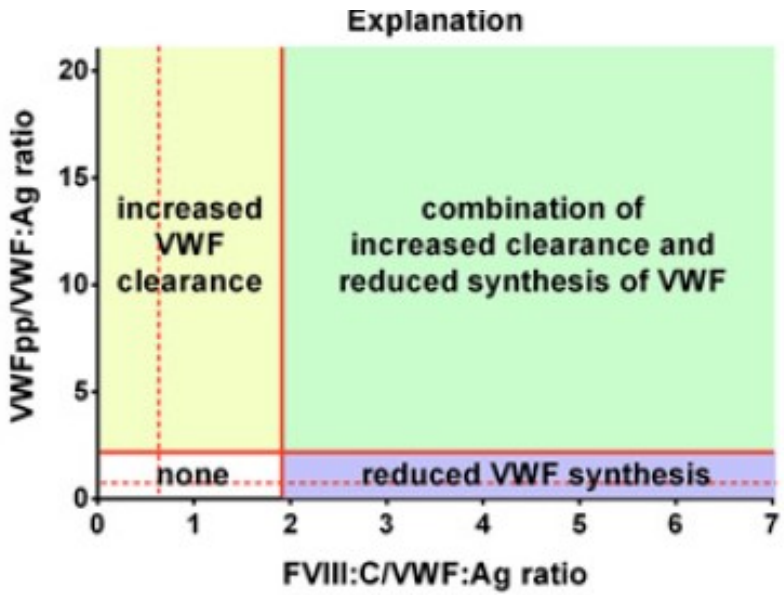


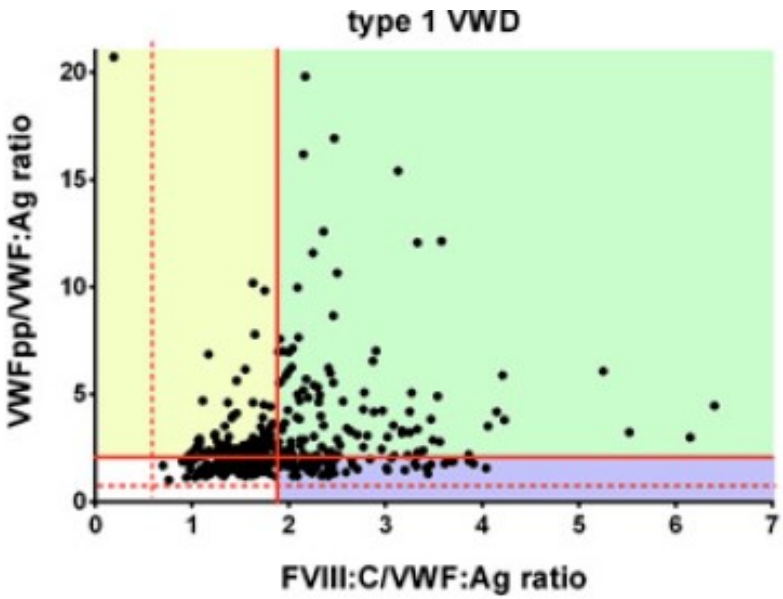




*Blood*. 2015;125(19):3006-3013

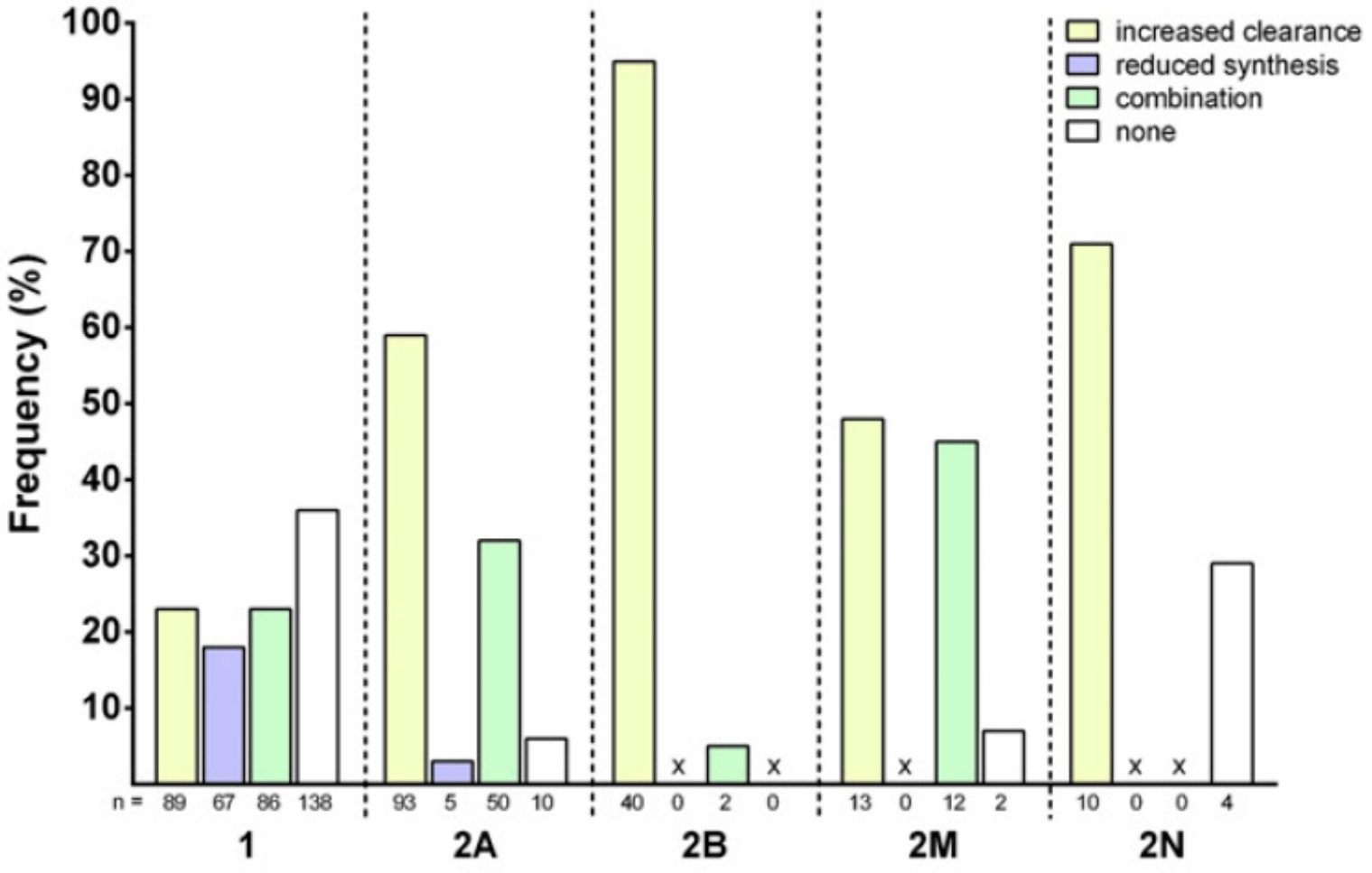
# VWF propeptide



# VWFpp measurement and VWF



# Discrimination between severe type 1 and type 3

| | Type 3 VWD patients | | | | |
|---|---|---|---|---|---|
| | Without VWFpp (n = 22) | | With VWFpp (n = 15) | | |
| Characteristic | No. | % | No. | % | *P* |
| **Age, y** | | | | | .636 |
| Median | 22 | | 35 | | |
| Range | 2-60 | | 4-65 | | |
| Male sex | 9 | 41 | 7 | 47 | .729 |
| Blood group O | 8 | 36 | 12 | 80 | .009 |
| **VWFpp, U/dL** | | | | | <.001 |
| Median | 0 | | 72 | | |
| 25%-75% IQR | 0-0 | | 37-94 | | |
| **VWF:Ag, IU/dL** | | | | | <.001 |
| Median | 0 | | 4 | | |
| 25%-75% IQR | 0-1 | | 2-4 | | |
| **VWF:Act, U/dL** | | | | | .036 |
| Median | 0 | | 1 | | |
| 25%-75% IQR | 0-0 | | 0-3 | | |
| **FVIII:C, IU/dL** | | | | | <.001 |
| Median | 2 | | 9 | | |
| 25%-75% IQR | 1-3 | | 8-13 | | |
| **Multimers** | | | | | |
| Absent | 19 | 86 | 3 | 20 | <.001 |
| Abnormal | 3 | 14 | 12 | 80 | |
| **BS** | | | | | .025 |
| Median | 19.5 | | 14.0 | | |
| 25%-75% IQR | 11.3-23.8* | | 7.0-17.0 | | |

# Additional VWF assays

- RIPA assay
  - Low dose (<0.5 mg/ml)  ristocetin induced platelet aggregation assay (type 2B or platelet type VWD)

- VWF: FVIII binding assay
  - To measure the ability of VWF to bind FVIII (type 2N)

- VWF multimer analysis

# Multimer analysis

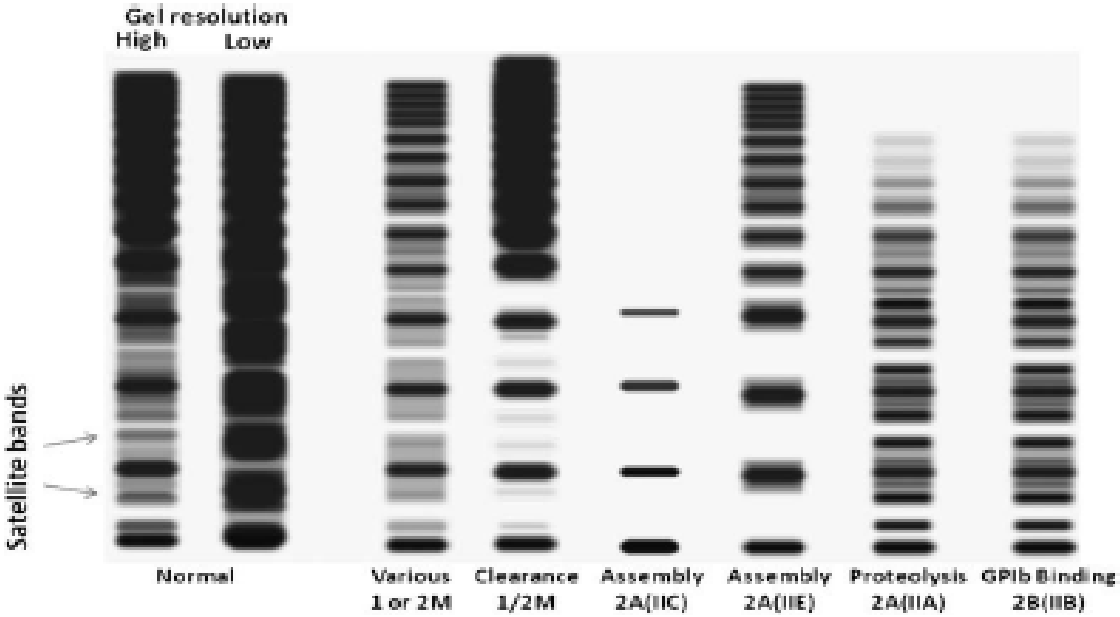
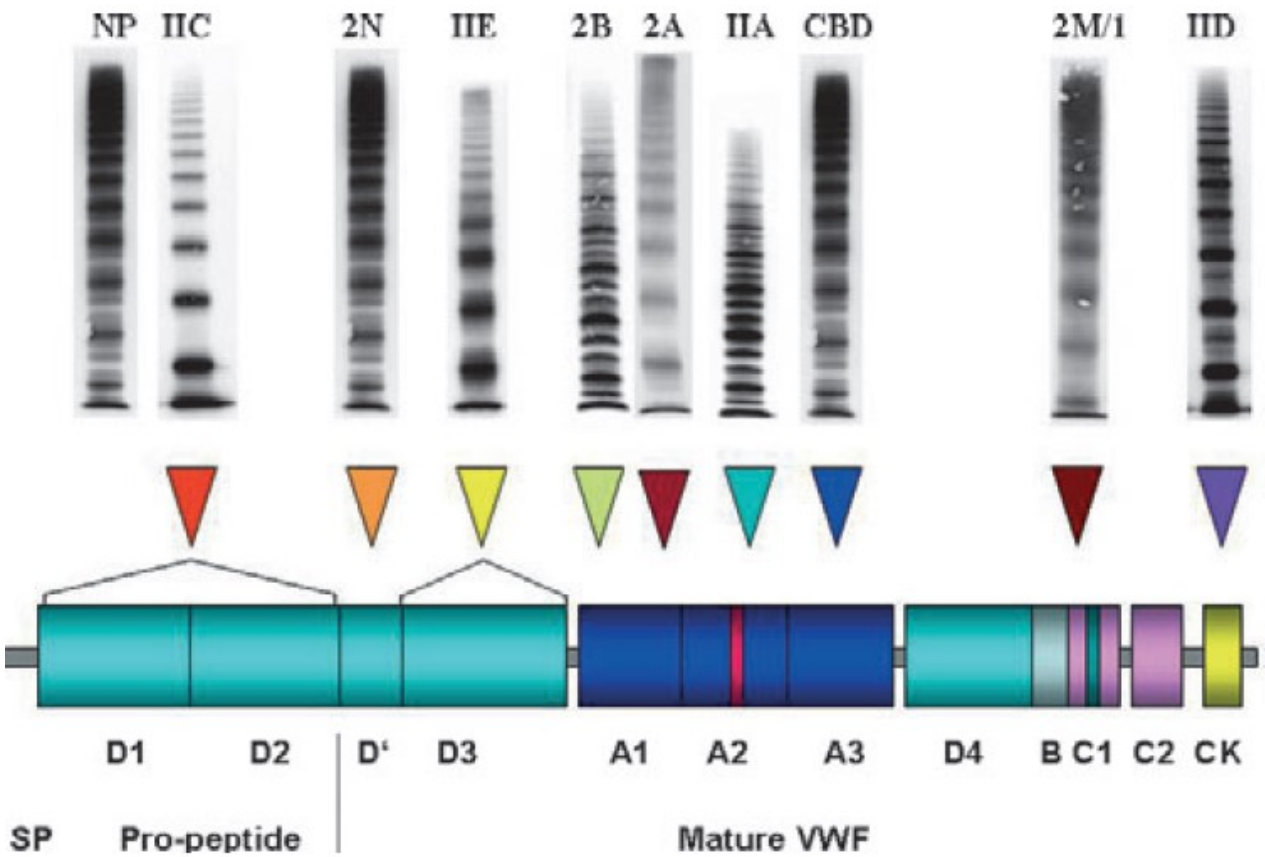



Fig. 3. Schematic representation of representative vWF multimer gels. Low-resolution gels show a distribution of multimers and are able to resolve broad patterns of small, intermediate, and large multimers. Higher resolution gels are needed to visualize satellite bands representing degradation products and flank main multimers. Various patterns are characterized predominantly by the main features of total intensity, distribution of sizes, and abnormalities of the satellite bands corresponding to different molecular mechanisms as discussed in the text.

Torres 2009

# Multimer analysis




Budde U, Haemophilia 2008;14s:27-38

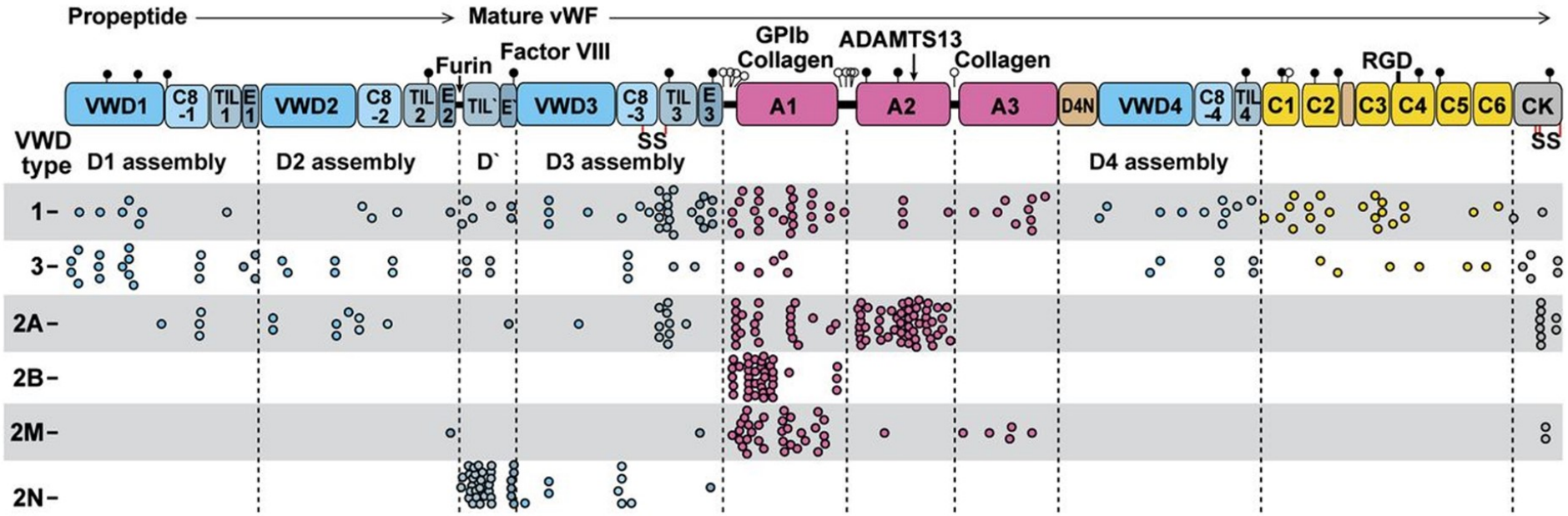
# Genetics of von Willebrand disease

*VWF* gene: chromosome 12p13.31, 178 kb, 52 exons

pseudogene on chromosome 22

highly polymorphous

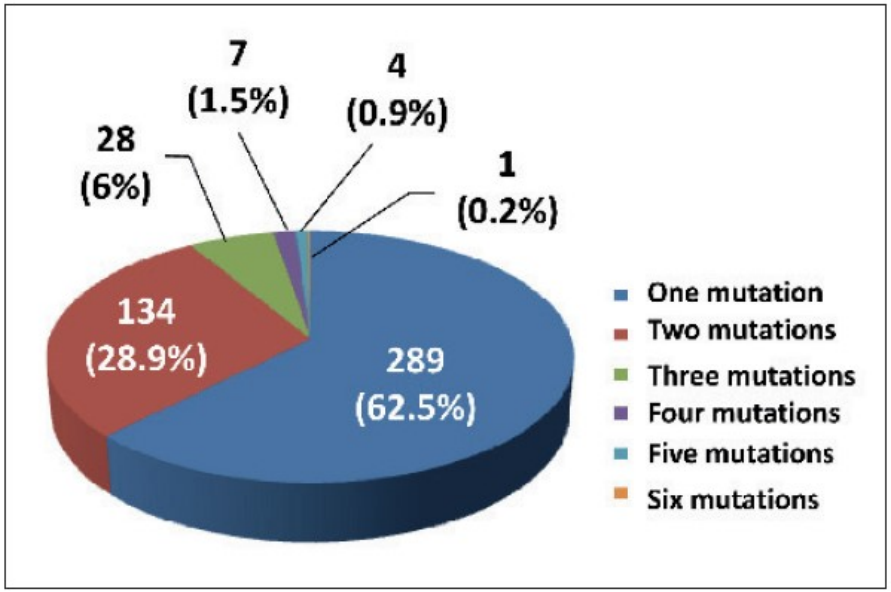
Over 500 mutations identified




Springer, Blood 2014

# Molecular and clinical profile of von Willebrand disease in Spain (PCM–EVW–ES): Proposal for a new diagnostic paradigm

| | PCM–EVW–ES |
|---|---|
| **VWF mutation detection rate** | |
| Unique mutations** | 238 |
| Type 1 | 89.2 % |
| Type 2 (A, B, M, N) | 100 % |
| Type 3 | 100 % |
| **VWD** | **n** |
| Type 1 | 138 |
| Type 2A | 109 |
| Type 2B | 35 |
| Type 2M | 38 |
| Type 2A/2M | 34 |
| Type 2N | 9 |
| Type 3 | 42 |
| **Polymorphisms** | 100 (34 in exon) |




Thromb Haemost 2016; 115: 40–50

# Mutations found in type 1 patients

## D3 mutations

p.R924Q
**p.S979N**
p.C1060Y
p.C1149R
p.1162L

p.R1205H
**p.P1240L**

## D4 mutations

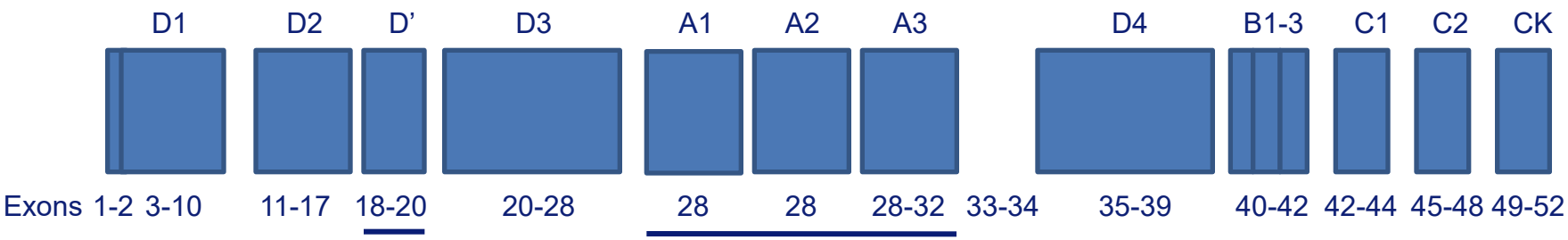
p.S2179F
**p.C2184Y**
p.R2185Q
**p.C2248Y**

## Propeptide mutations

p.D75_G178del
p.Y629C

## C mutations

p.R2535X
p.C2693Y




## D' mutations

p.P812RfsX31
p.H817Q
p.R854Q

## A1-A3 mutations

p.P1266Q
p.P1266L
p.V1279I
p.V1360A
p.R1374C
p.D1472H
p.V1499E

p.Y1584C
p.R1597W
**p.F1654I**
p.K1794E
p.V1360A
p.F1369I
p.S1378F

**Novel mutation**

# Conclusions

- VWD is a heterogeneous disorder with varying bleeding phenotype
- Laboratory diagnosis is based on several tests
- Use of algorithm is necessary for correct diagnosis
- New test are in development, but need to be validated
- Increasing role of genetic testing of *VWF*