11th HEMOSTASIS SEMINAR

VWF and ADAMTS 13: Physiopathology

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ULSS 6 Vicenza

Werfen

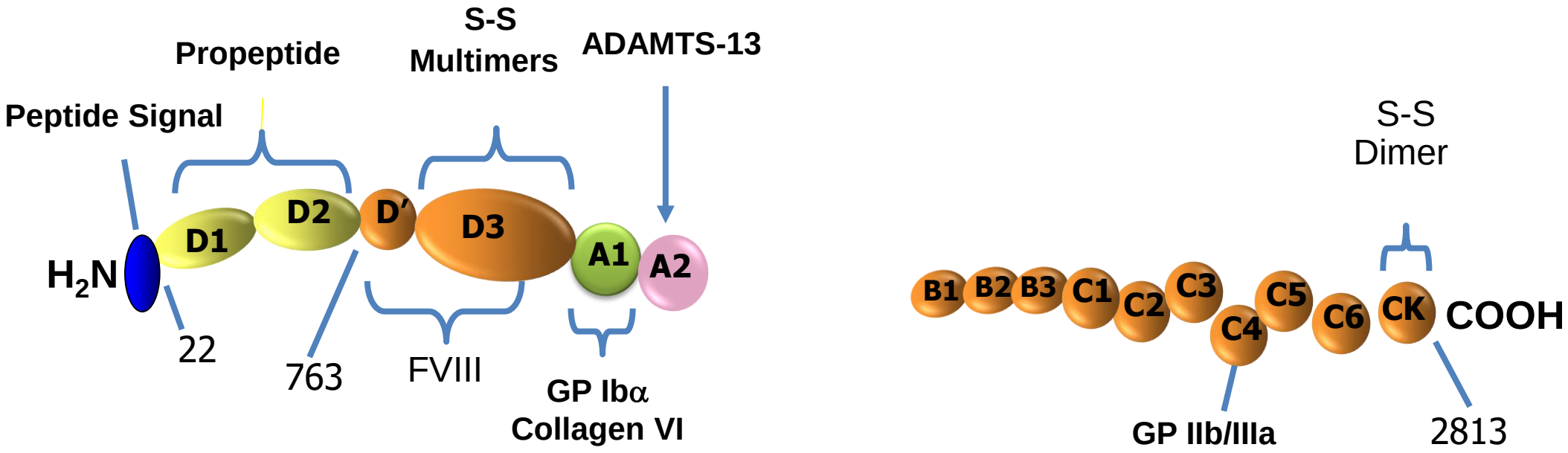
The Atlantic hagfish has circulating VWF

…but its closest Urochordata, the sea squirt, doesn’t

VWF evolved in the ancestral vertebrate following the divergence of the urochordates some 500 million years ago

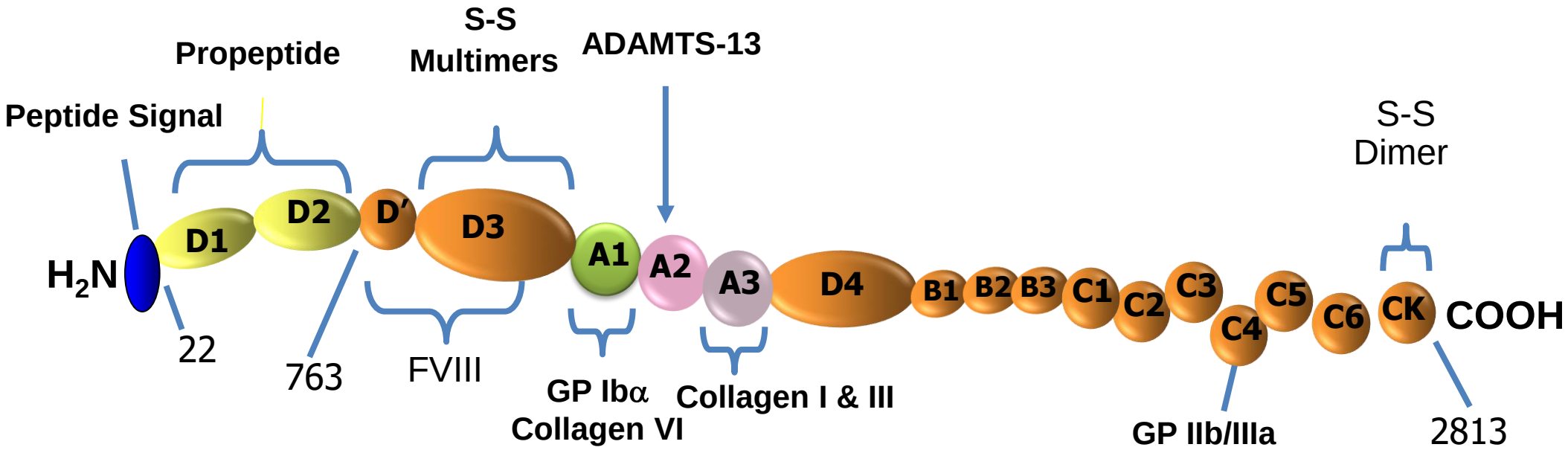
Grant et al. Blood, 2017.

The hagfish Von Willebrand Factor (VWF)



Grant et al. Blood, 2017.

The human Von Willebrand Factor (VWF)



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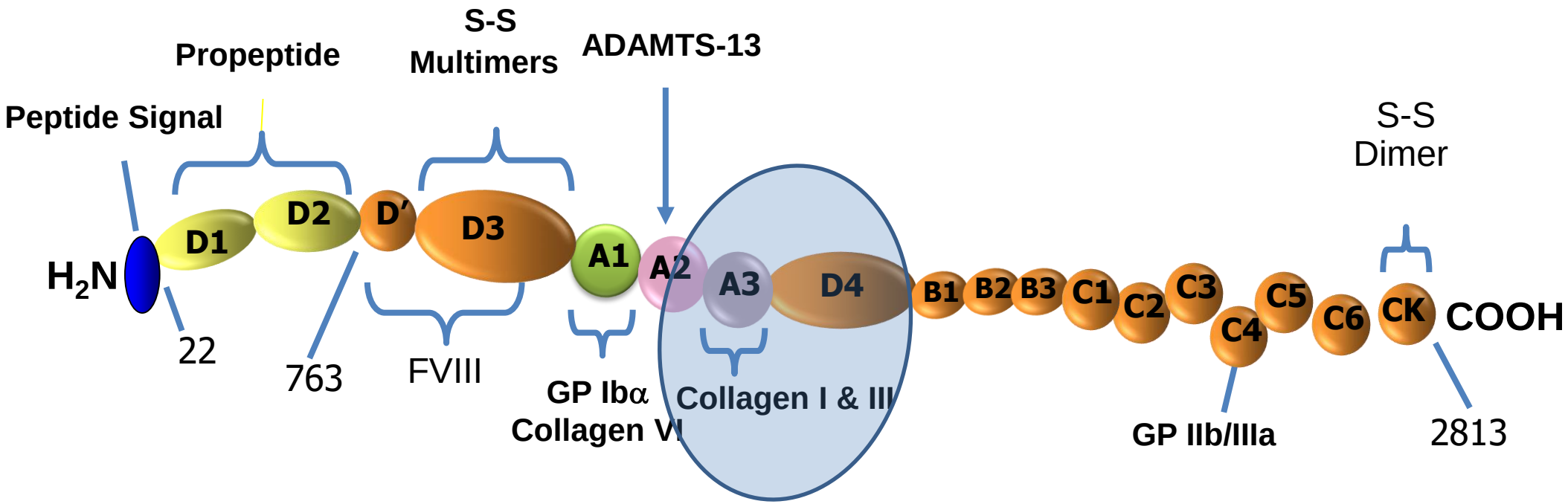
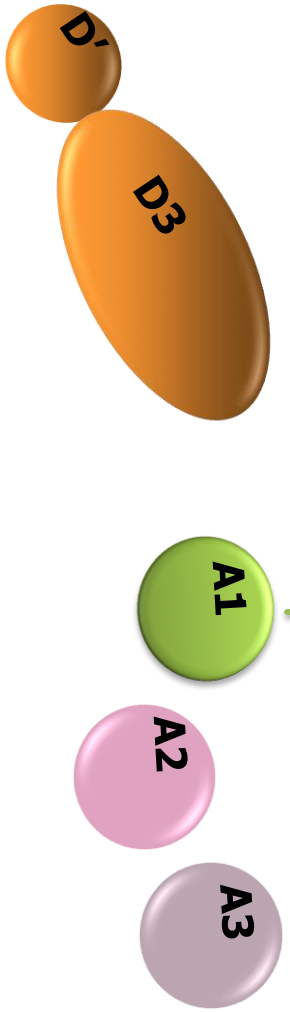


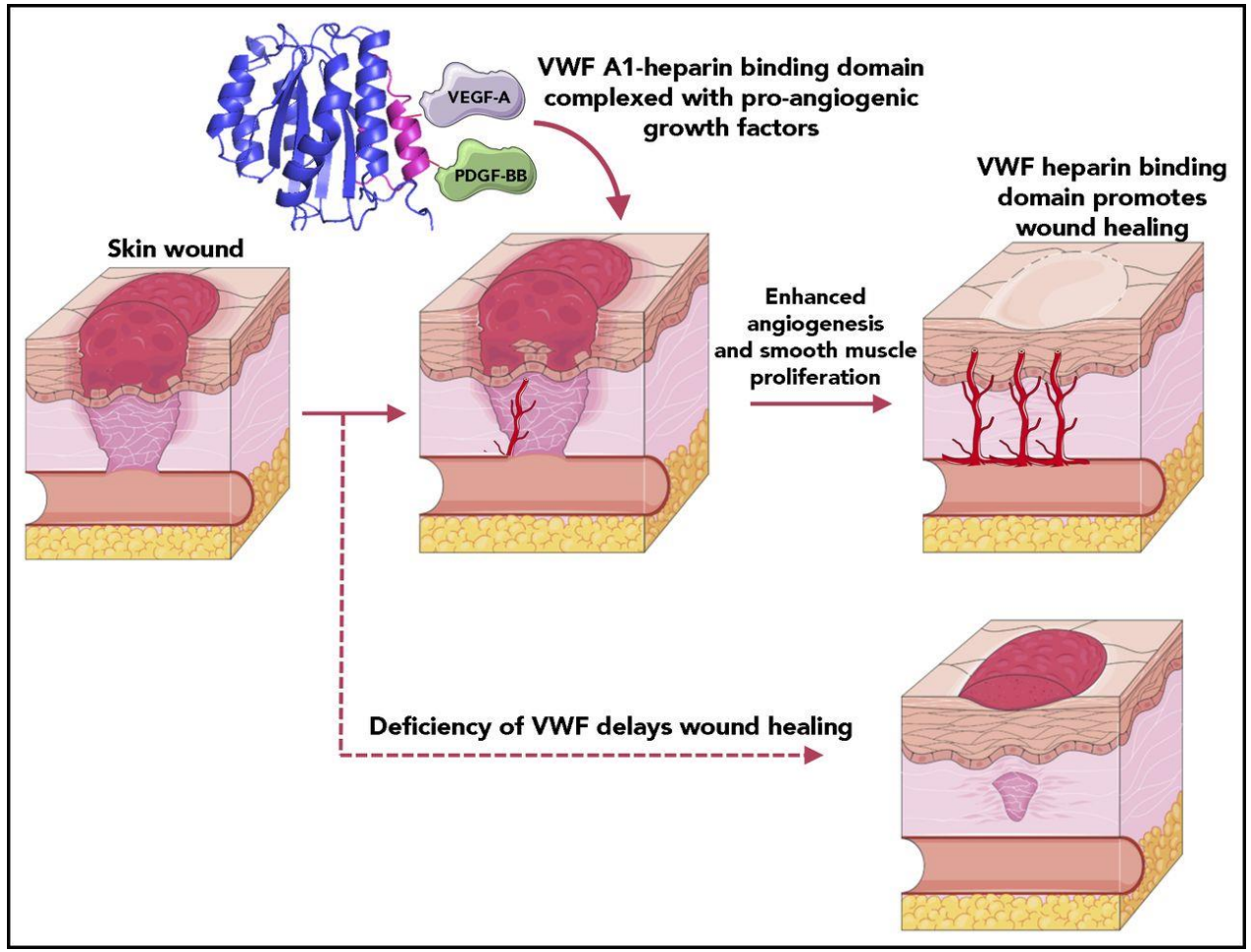
High-shear stress component

The revised VWF-related activities nomenclature



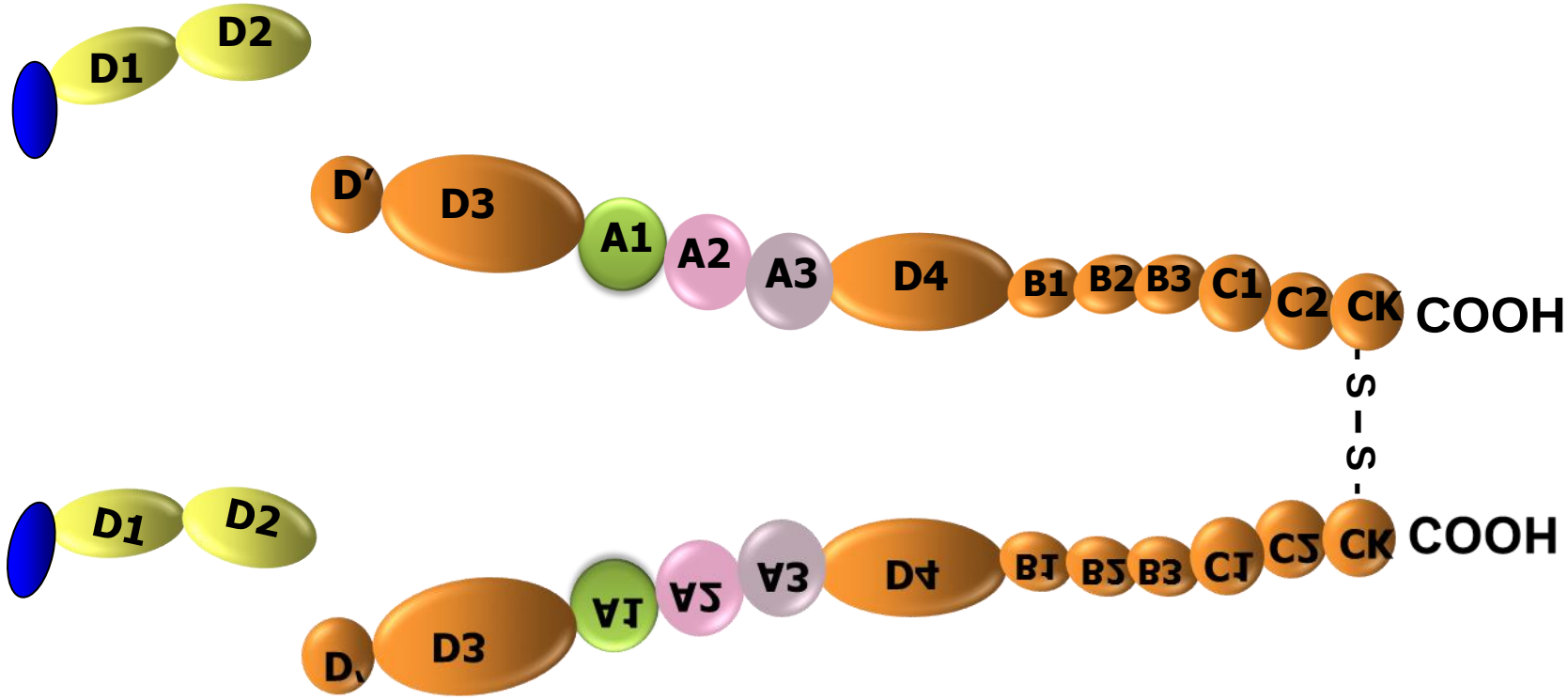
Test	Explored function
VWF:Ag	VWF antigen as measured by a polyclonal Ab
VWF:FVIIIB	FVIII Binding: all assays measuring D' domain binding to FVIII
VWF:Rco	Ristocetin Cofactor activity: all assays that use platelets and Ristocetin (A1 domain)
VWF:GPIbR	All assays that are based on the Ristocetin induced 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 (mAb) to a VWF A1 domain epitope
VWF:CBA	All assays measuring A3 domain binding to collagen

VWF promotes growth factor recruitment and wound healing

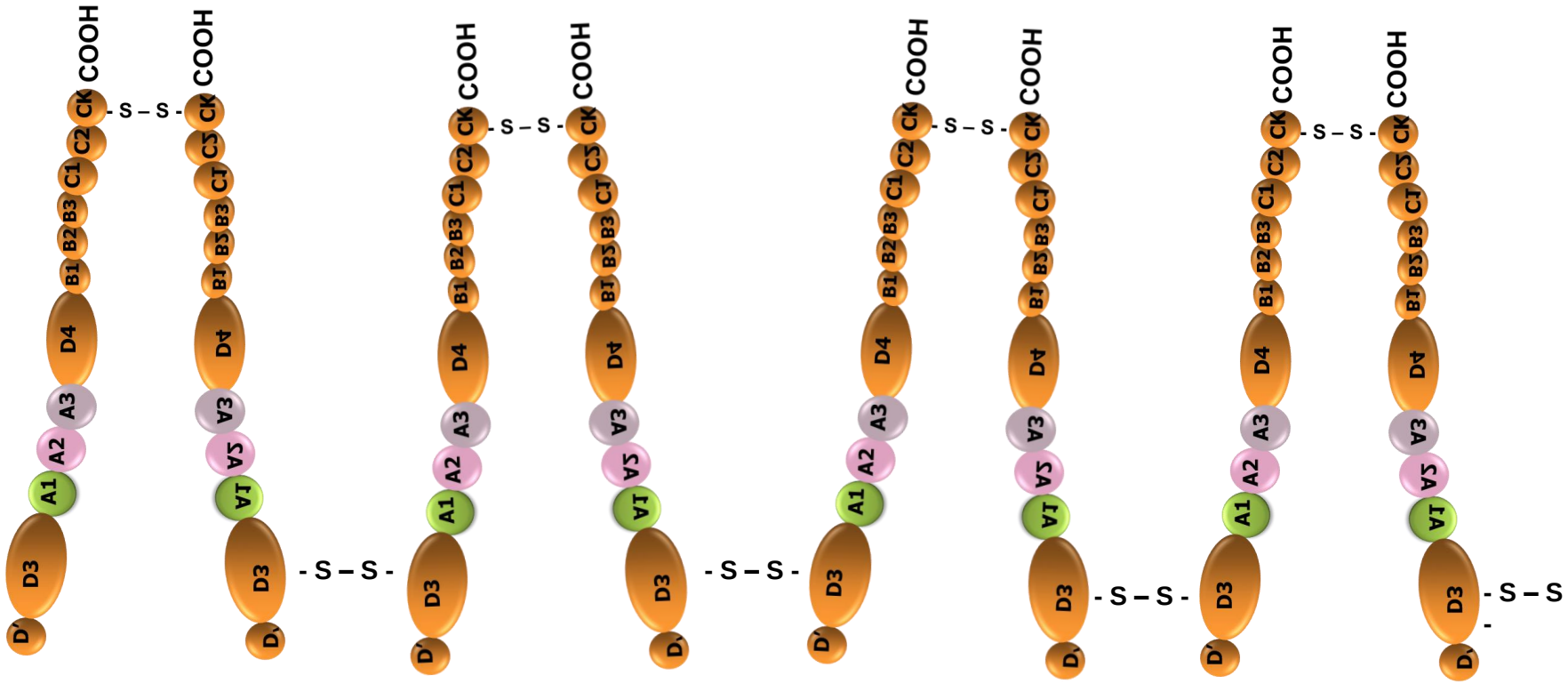


Ishihara et al, Blood, 2019

In the ER, propeptide is released and VWF dimerized

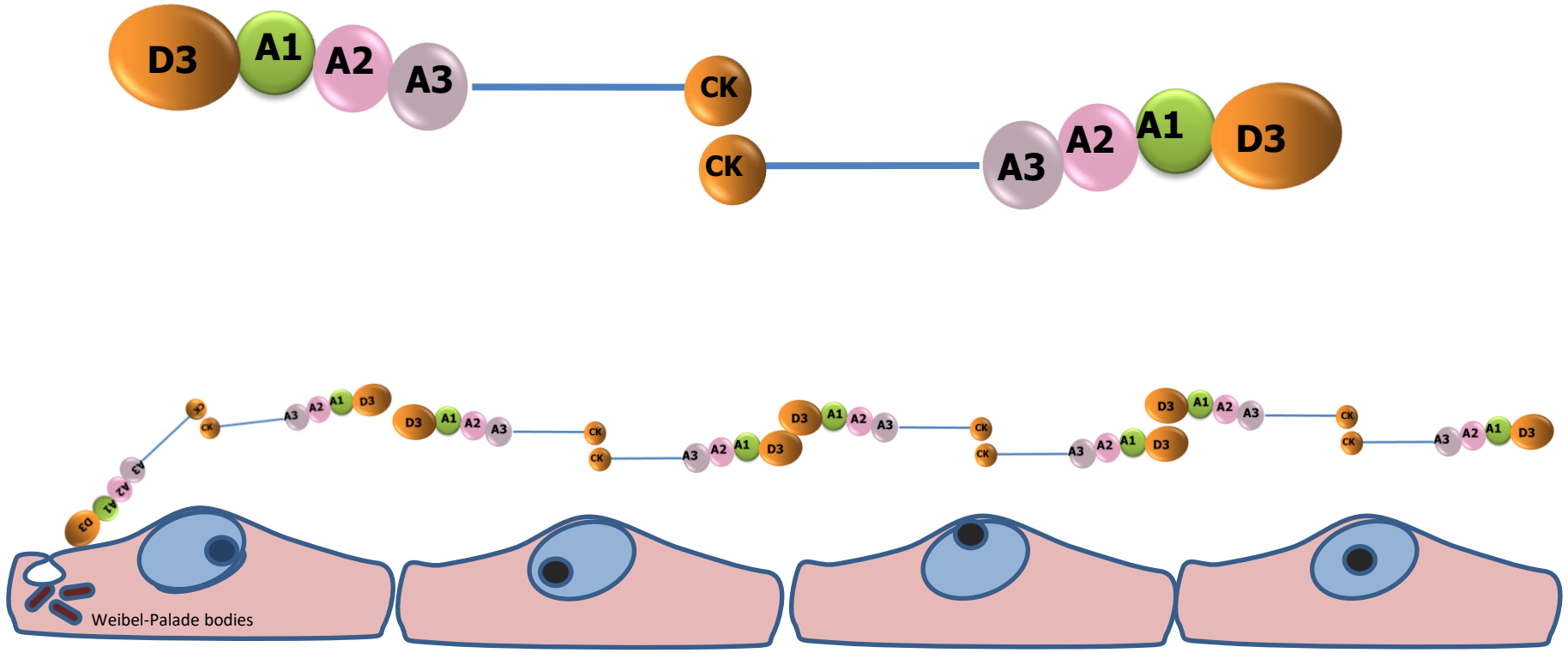


VWF dimers are stored in Weibel-Palade bodies as ultra-large multimers

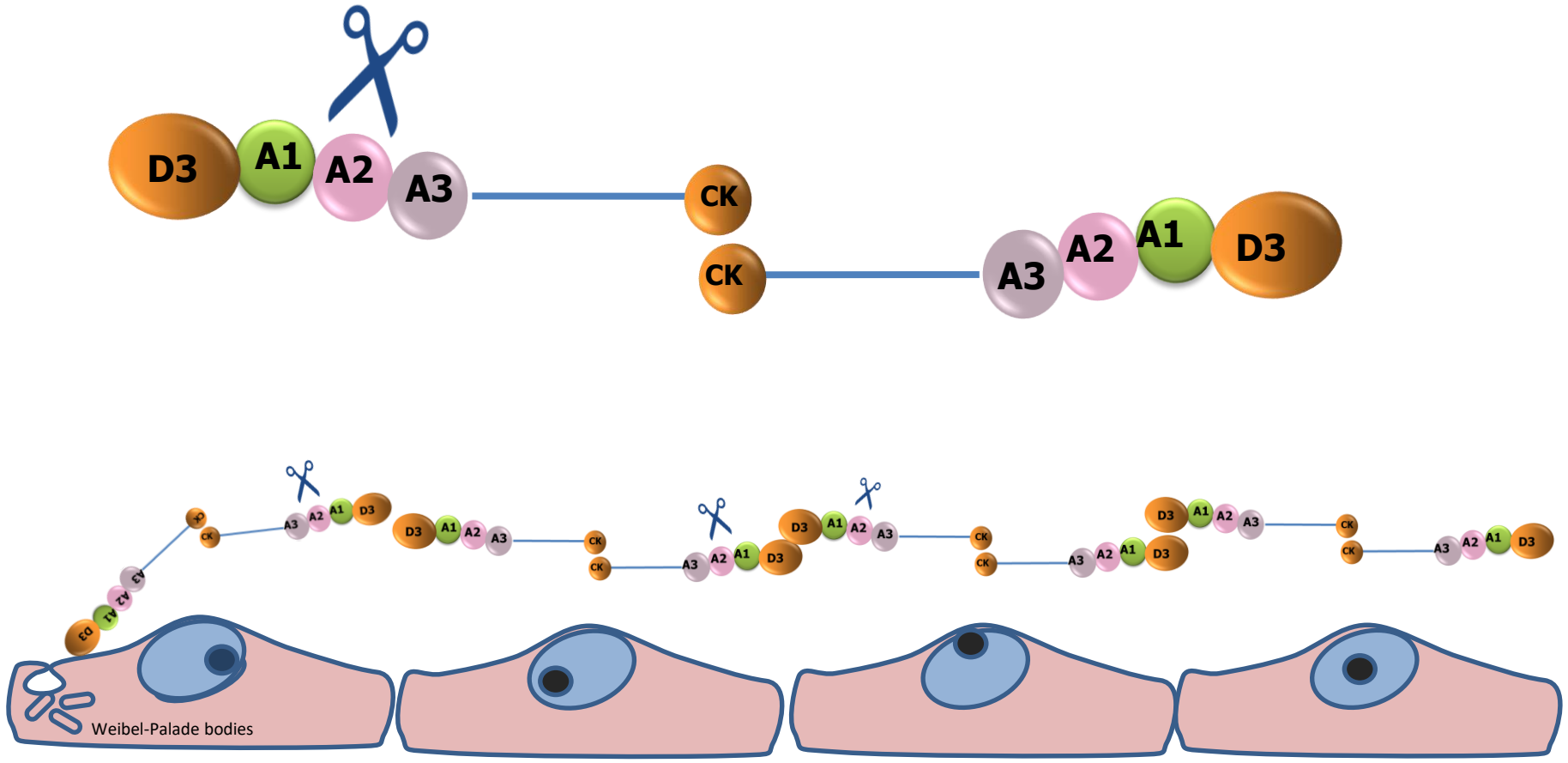


Mutations in CK and D3 domains are associated with defective (di)multimerization

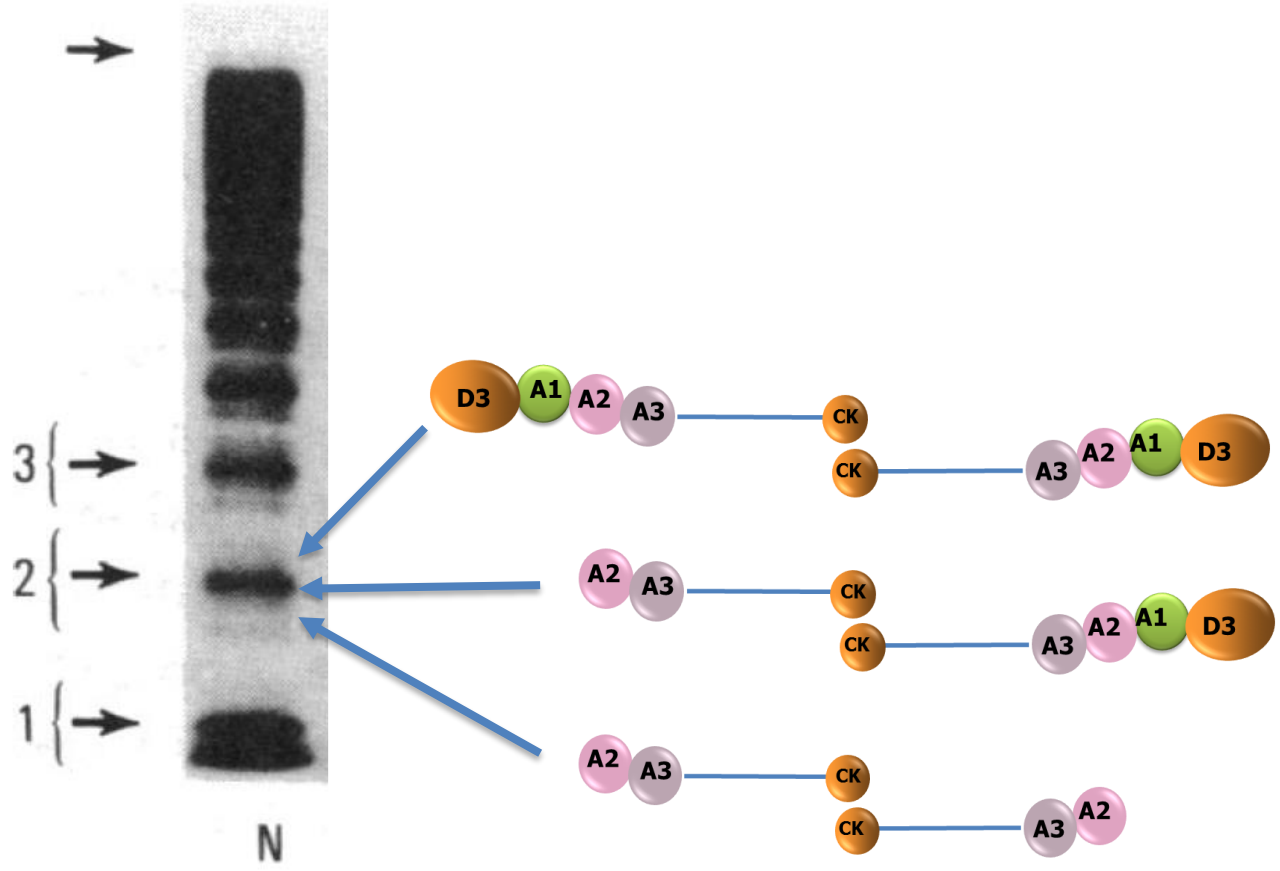
VWF is secreted from the endothelial cells as long VWF “strings”



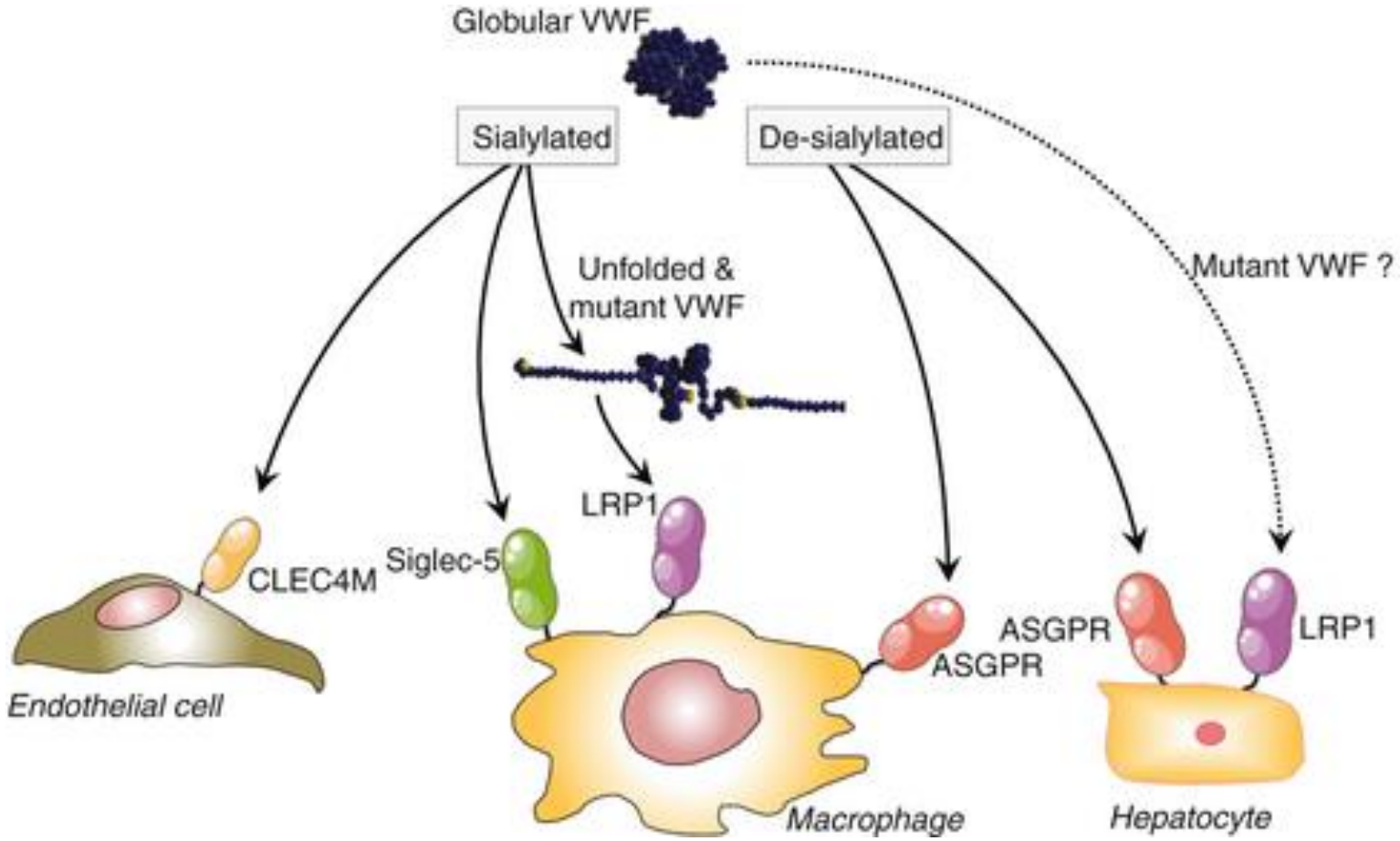
VWF “strings” are cut by ADAMTS at the A2 domain



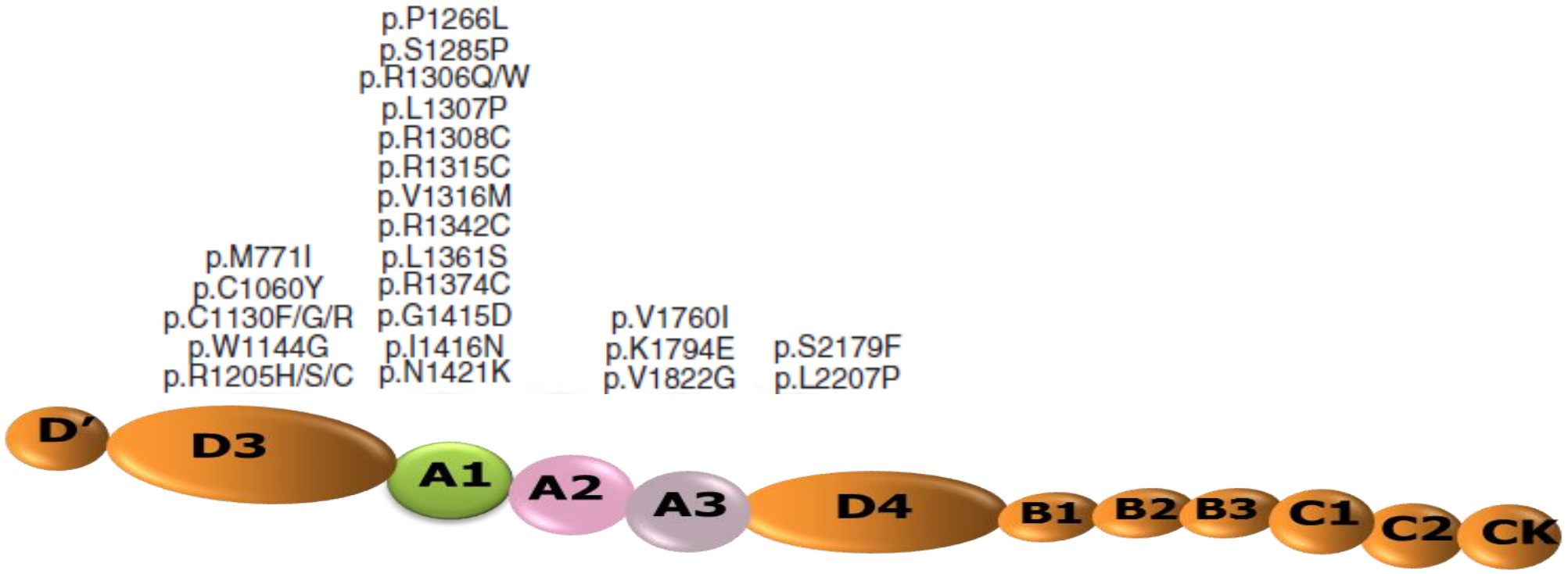
ADAMTS proteolysis is responsible for “triplets” in high-resolution gels



VWF clearance



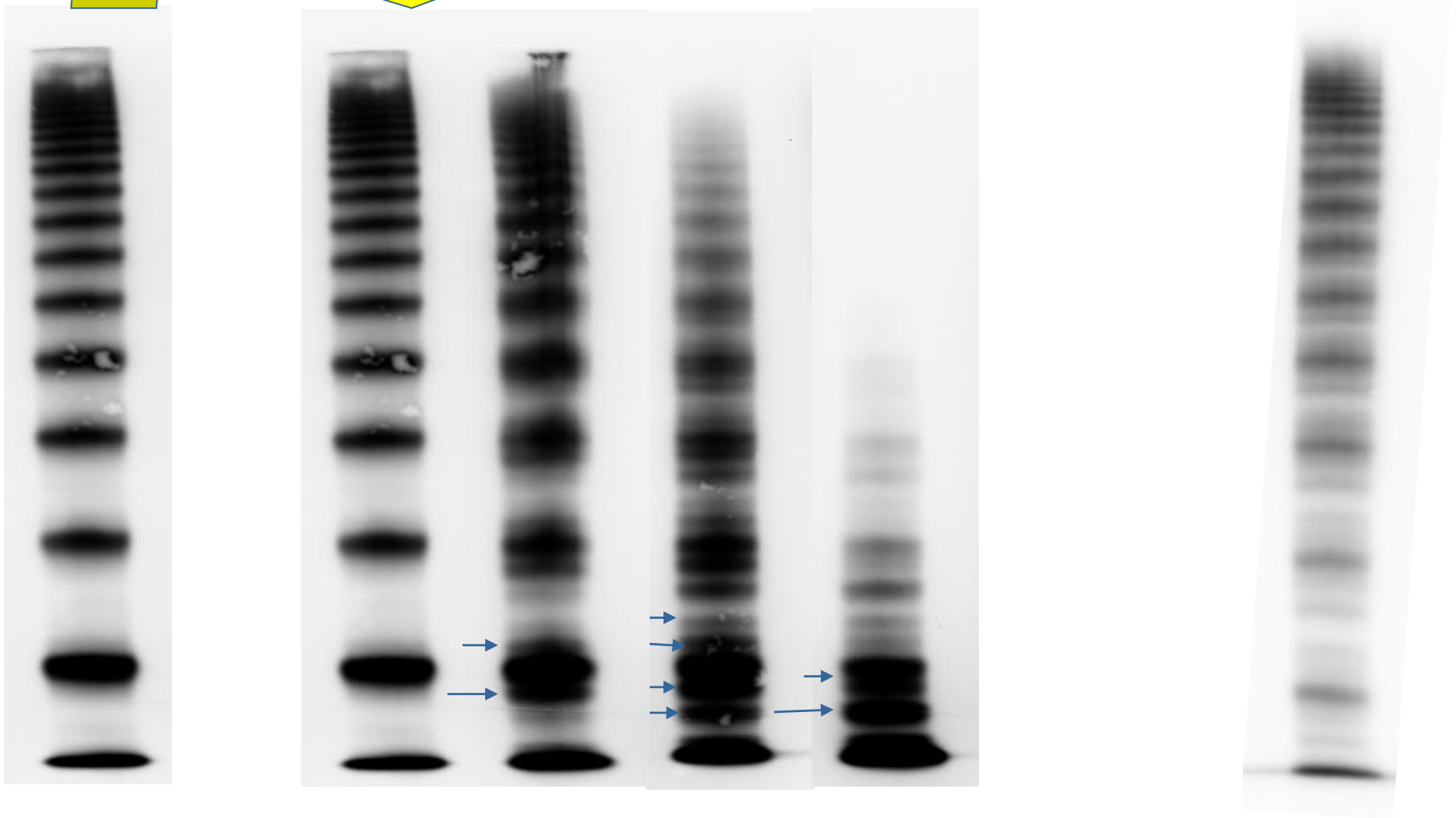
VWF clearance: the role of the D3-A1 domain



Sinthesis

Proteolisis
(ADAMTS-13)

Steady state
(Normal Plasma)

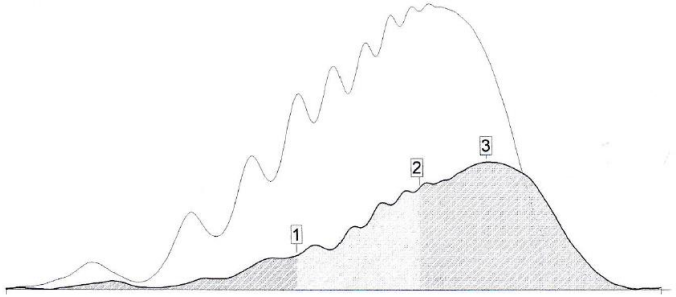


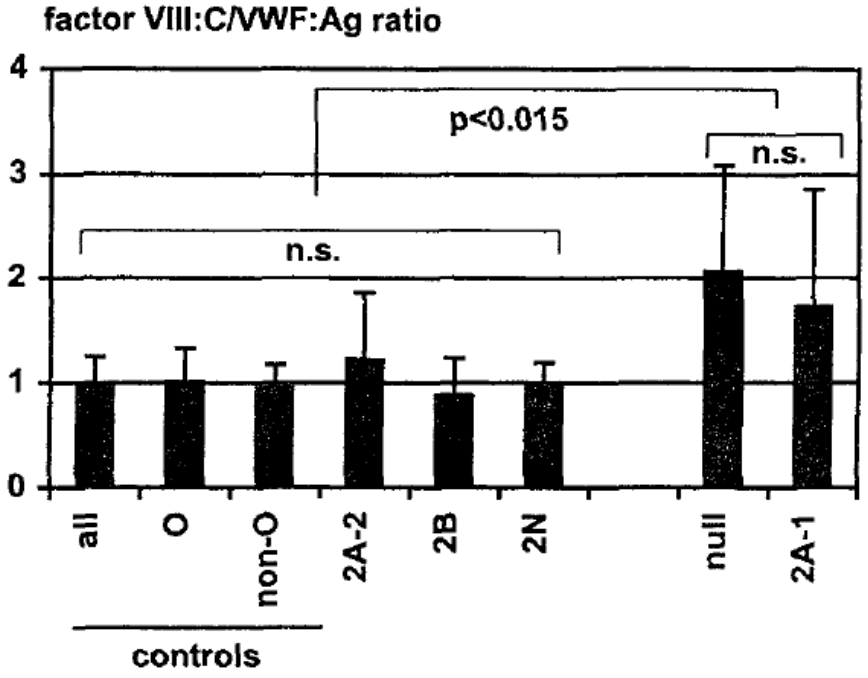
Mechanisms in VWD

- Decreased biosynthesis
  - Type 1 VWD

Mechanisms in VWD: FVIII/VWF:Ag ratio

- Patient SS, a 34 yo male
- Mild mucocutaneous bleeding diathesis
- FVIII:C: 19 IU/dL
- VWF:Ag: 8 IU/dL
- VWF:RCo 9 IU/dL
- FVIII:C/Ag ratio = 2.4





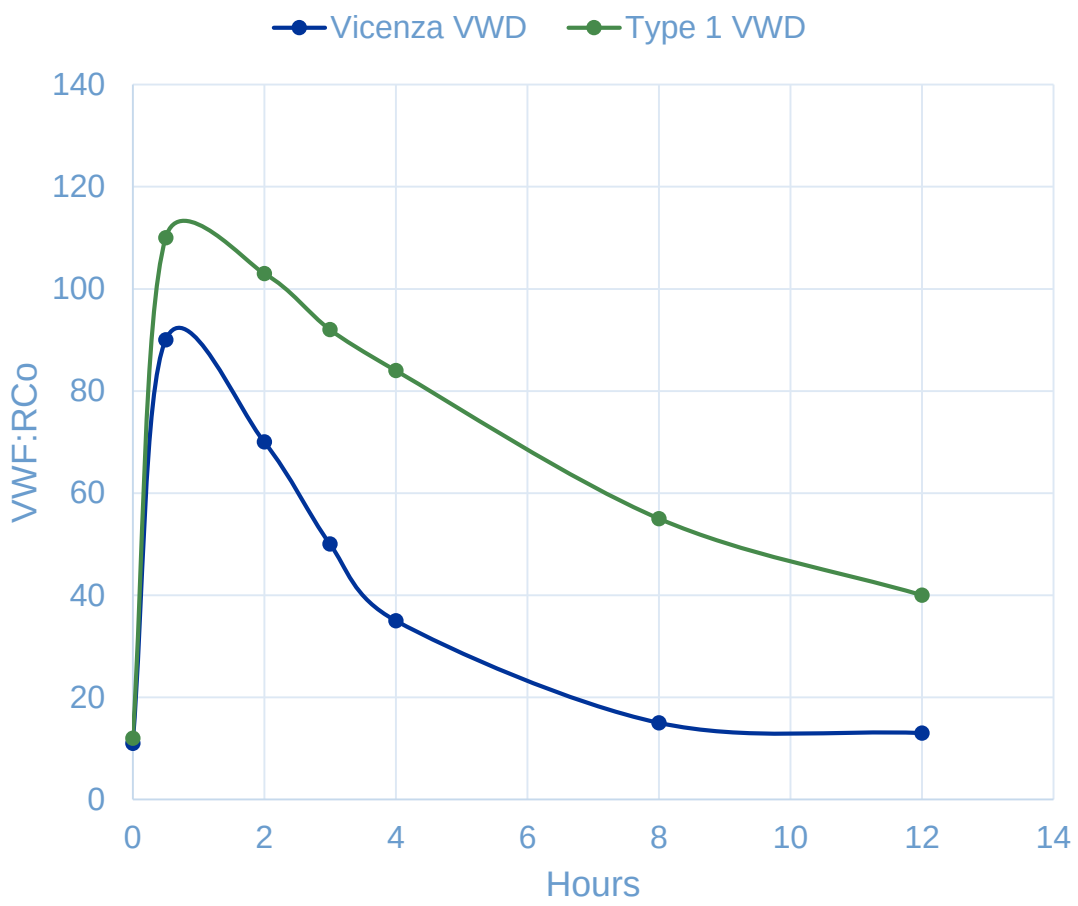
Increased FVIII/VWF ratio is suggestive for presence of reduced VWF biosynthesis

Eikenboom, et al. Thromb Haemost, 2002

Mechanisms in VWD

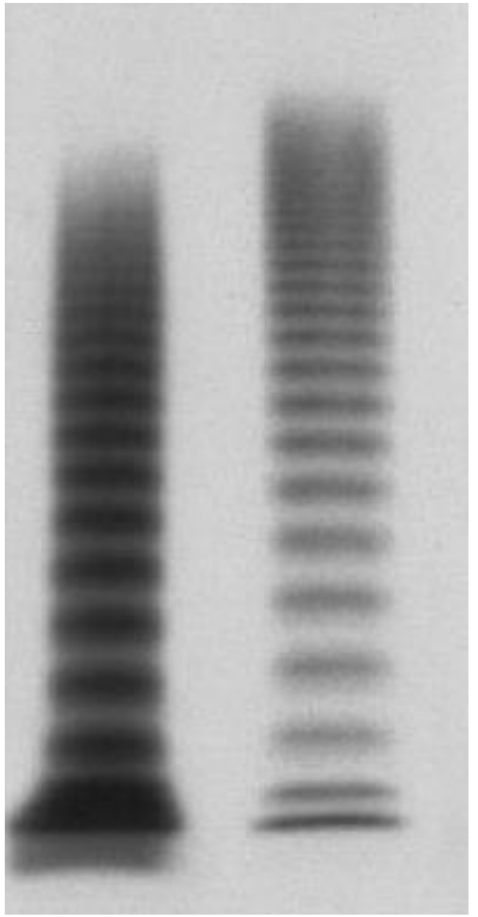
- Decreased biosynthesis
  - Type 1 VWD
- Increased clearance
  - Type 1 VWD (“Vicenza”)
  - Type 2B (increased affinity for GpIb)

Increased clearance is present in many type 1 VWD patients (type 1C)

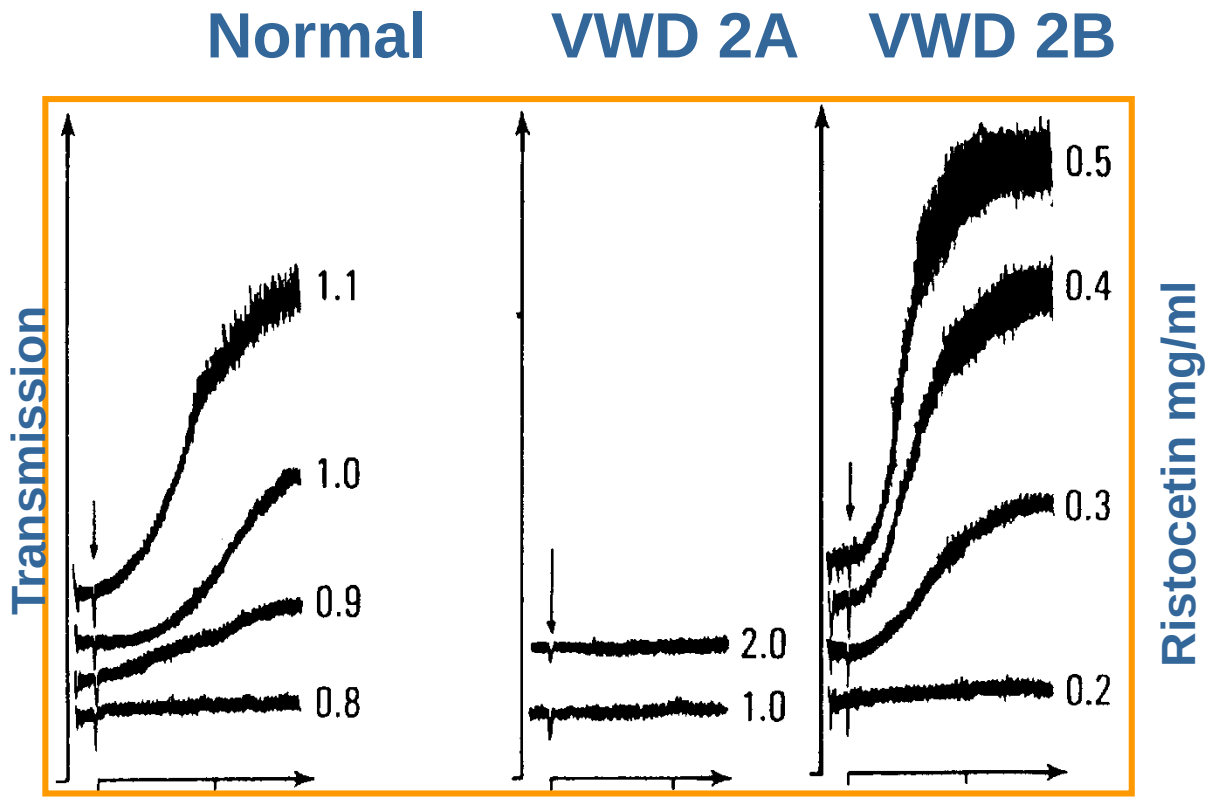


Increased clearance:

- Reduced response to DDAVP
- Multimeric pattern more closely resembling storage pools
- Increased propeptide/Ag ratios



VON WILLEBRAND FACTOR: RIPA
Ristocetin induced platelet agglutination

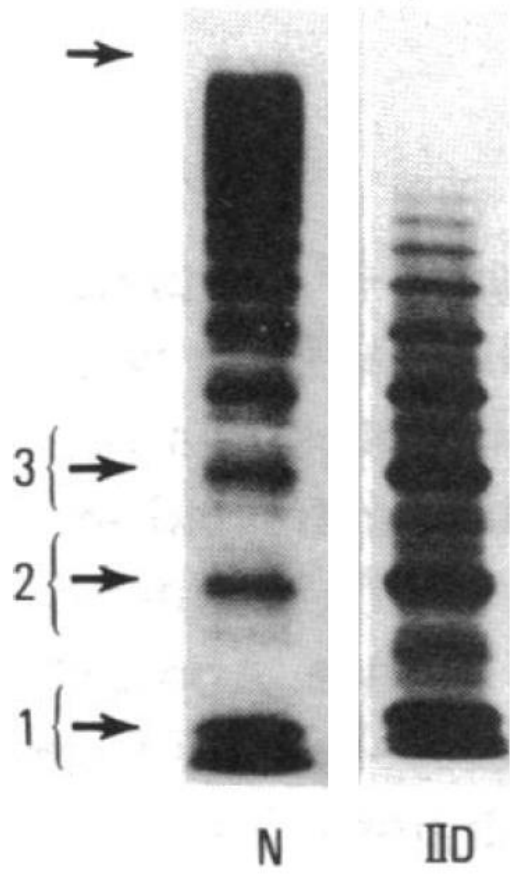


Platelet Rich Plasma from Patients + RISTOCETIN [0.2-2.0 mg/ml]

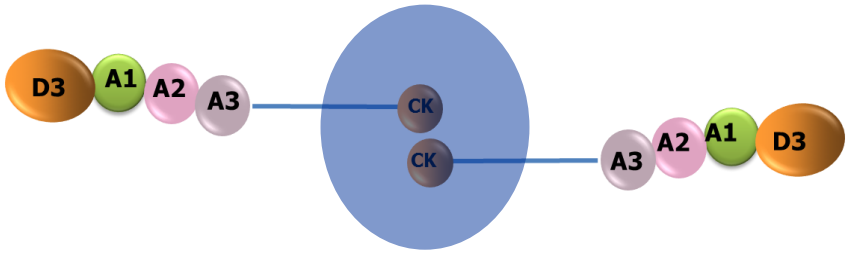
Mechanisms in VWD

- Decreased biosynthesis
  - Type 1 VWD
- Increased clearance
  - Type 1 VWD (“Vicenza”)
  - Type 2B (increased affinity for GpIb)
- Defective multimerization (biosynthesis and clearance abn.)
  - Type 2

“Variants” of type 2 VWD: defective multimerization and/or ADAMTS interaction



- Odd dimerization (IID): CK defects

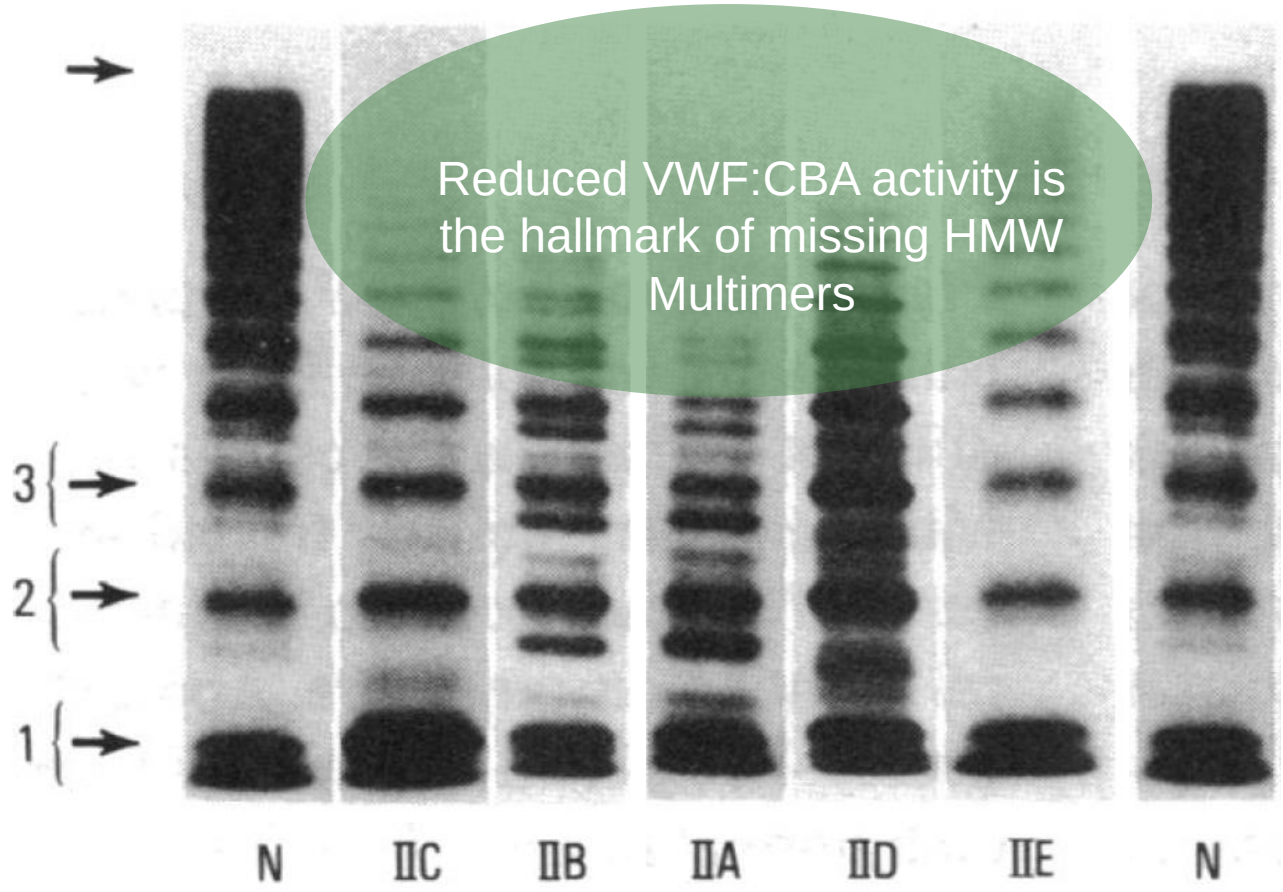


Zimmerman et al. J Clin Invest, 1986.

“Variants” of type 2 VWD

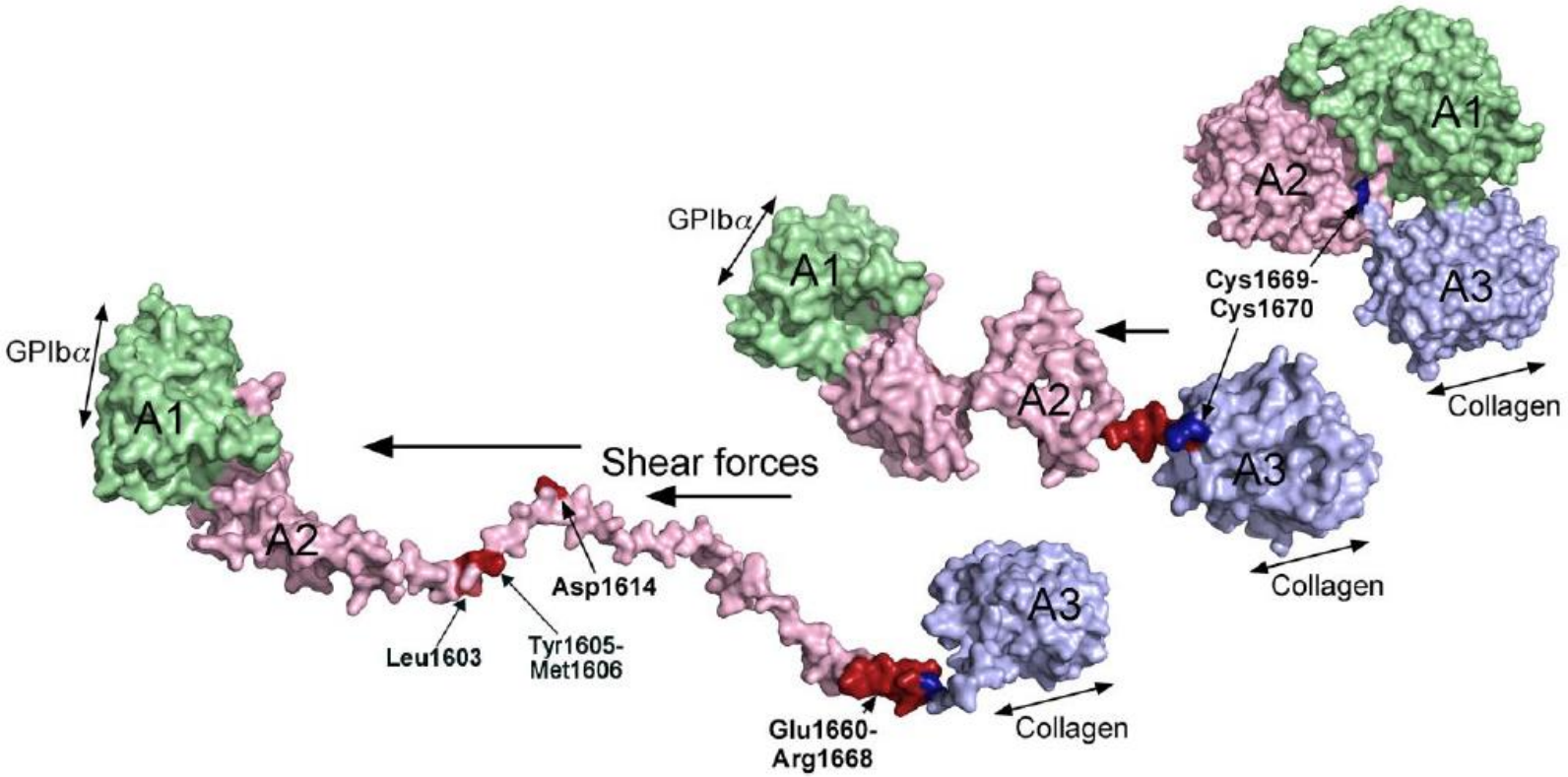
CBA

RCo



Reduced VWF:CBA activity is the hallmark of missing HMW Multimers

A1, A2, A3 domains are available only when VWF is elongated by shear stress

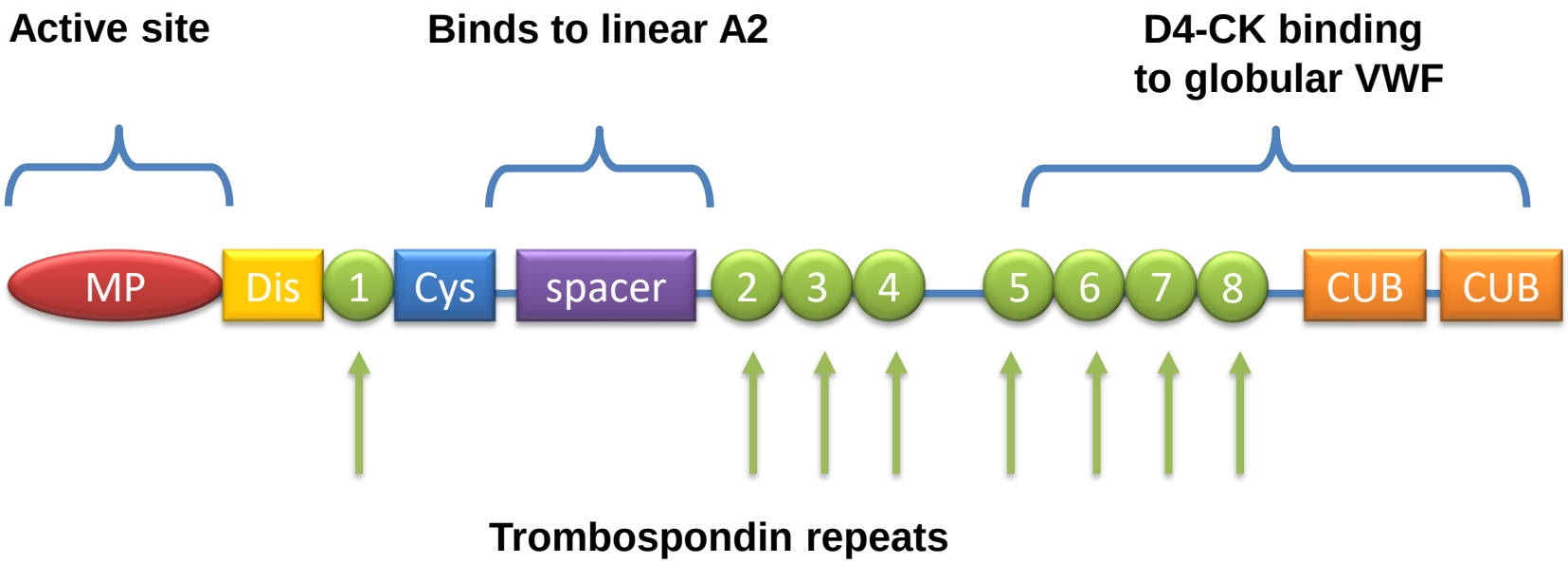


Crawley et al. Blood, 2011.

ADAMTS13 (*A D*isintegrin *A*nd *M*etalloprotease with *T*hrombo*S*pondin 1 repeats)

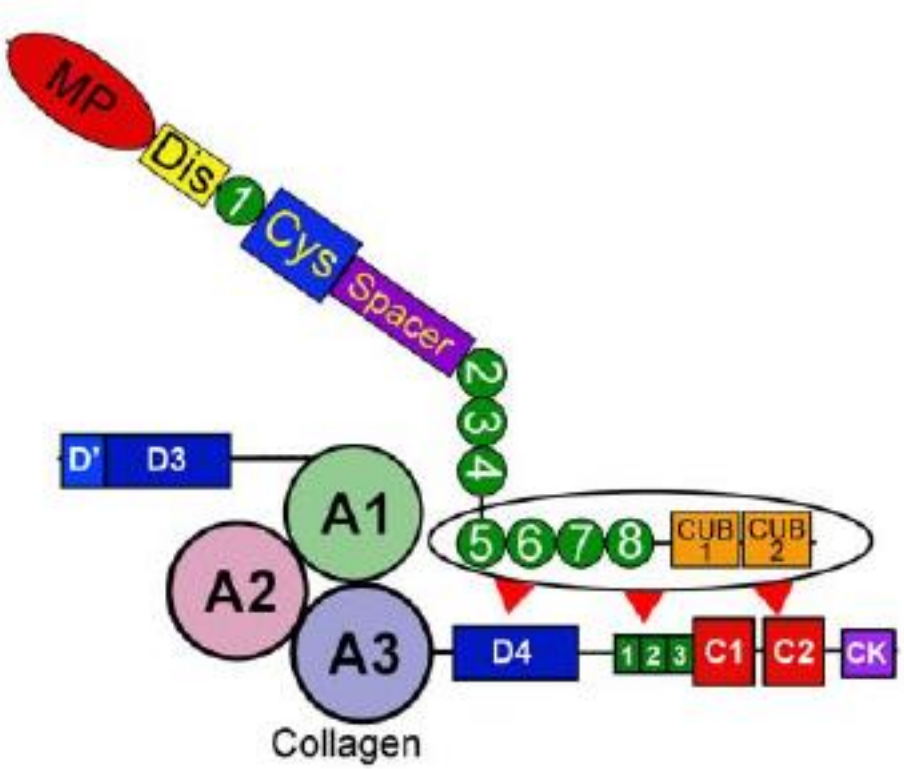
- Synthetized in the liver, endothelium and platelets
- Up-regulated upon activation by inflammatory cytokines
- Secreted as a constitutively active protease, no inhibitor identified to date.

ADAMTS13 structure



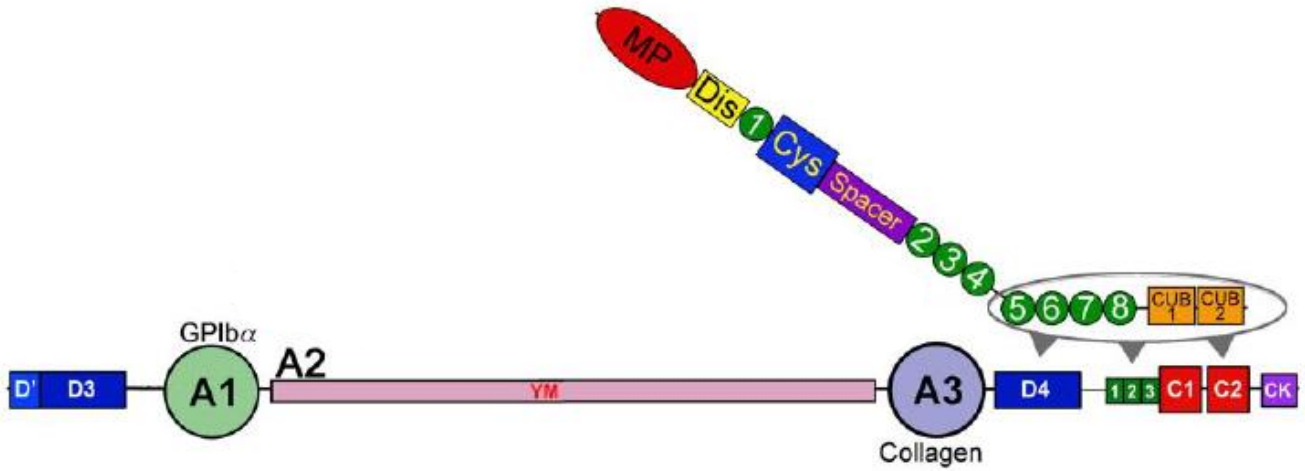
Zheng JTH, 2013.

ADAMTS13 TSP repeats bind to globular VWF



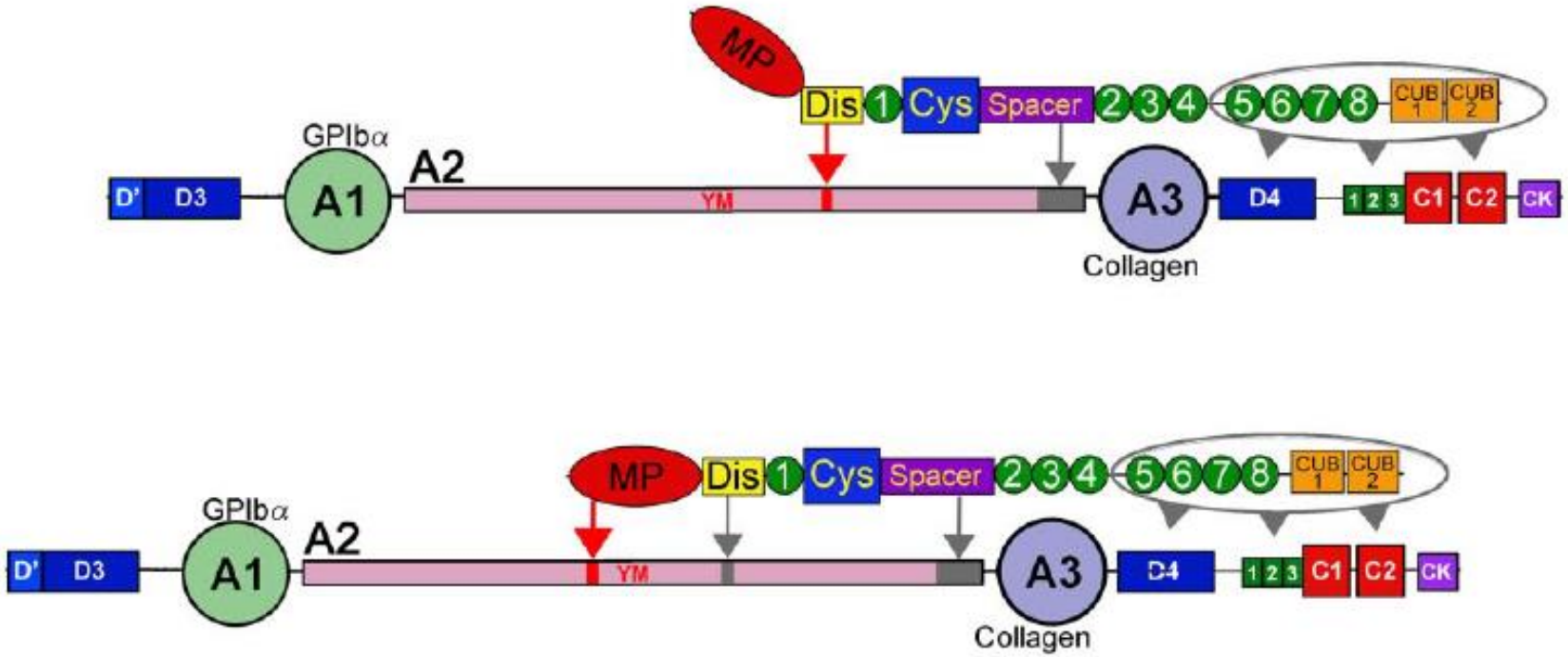
Crawley et al. Blood, 2011.
Zheng JTH, 2013.

Shear-stress elongated A2 permits binding and cleavage



Crawley et al. Blood, 2011.
Zheng JTH, 2013.

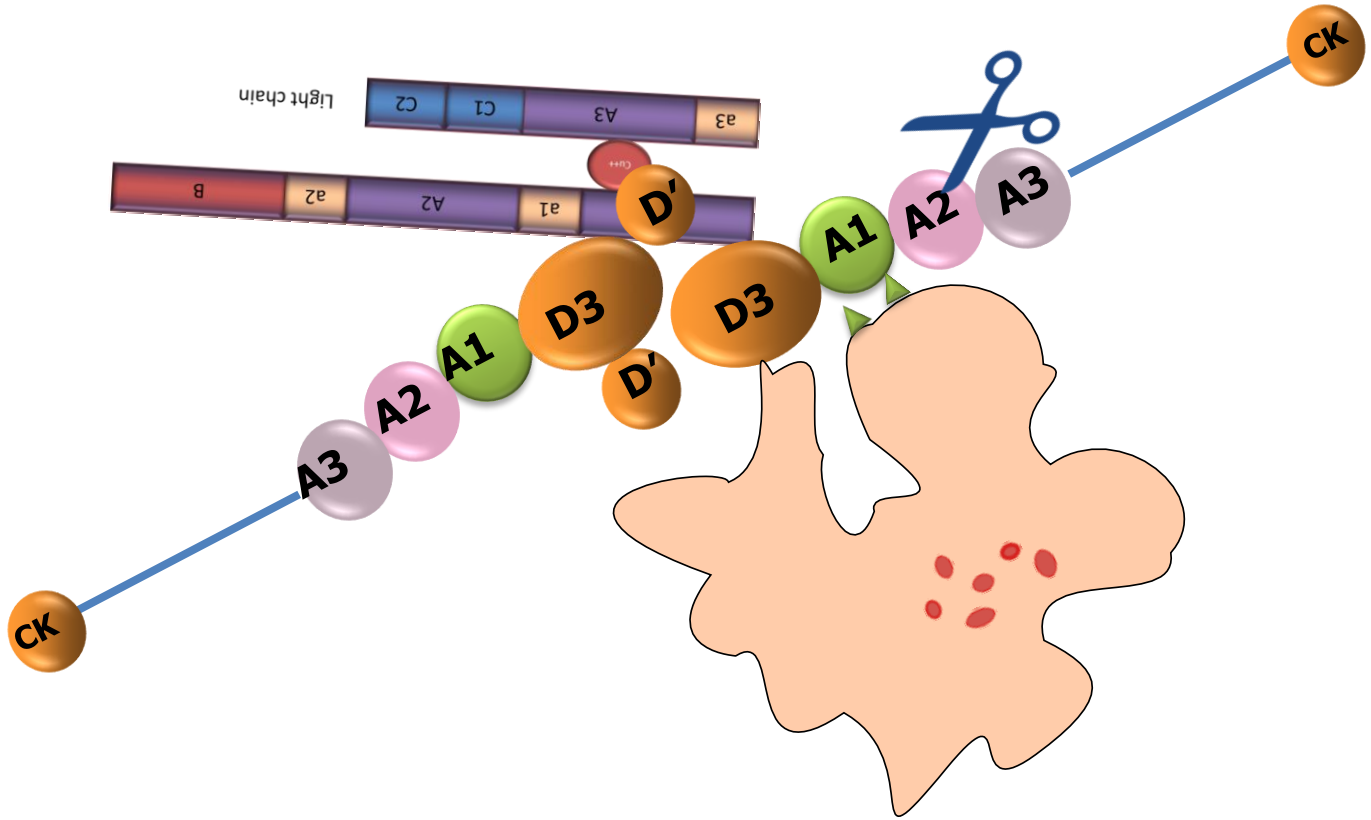
Shear-stress elongated A2 permits binding and cleavage



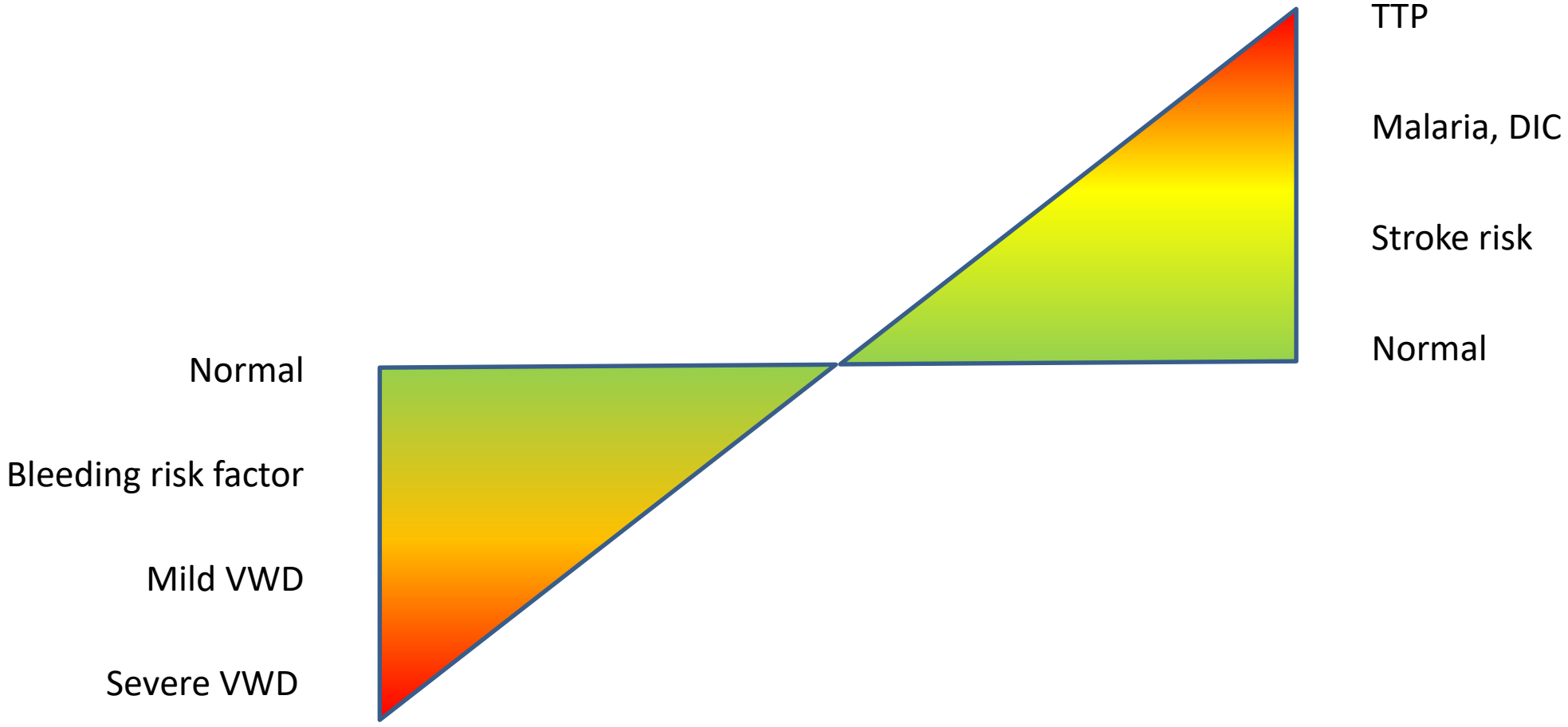
Crawley et al. Blood, 2011.
Zheng JTH, 2013.

ADAMTS13 cleavage is promoted by FVIII:C and GpIb binding

Binding of FVIII or GpIb discloses cleavage site in A2



Disorders of the VWF/ADAMTS system



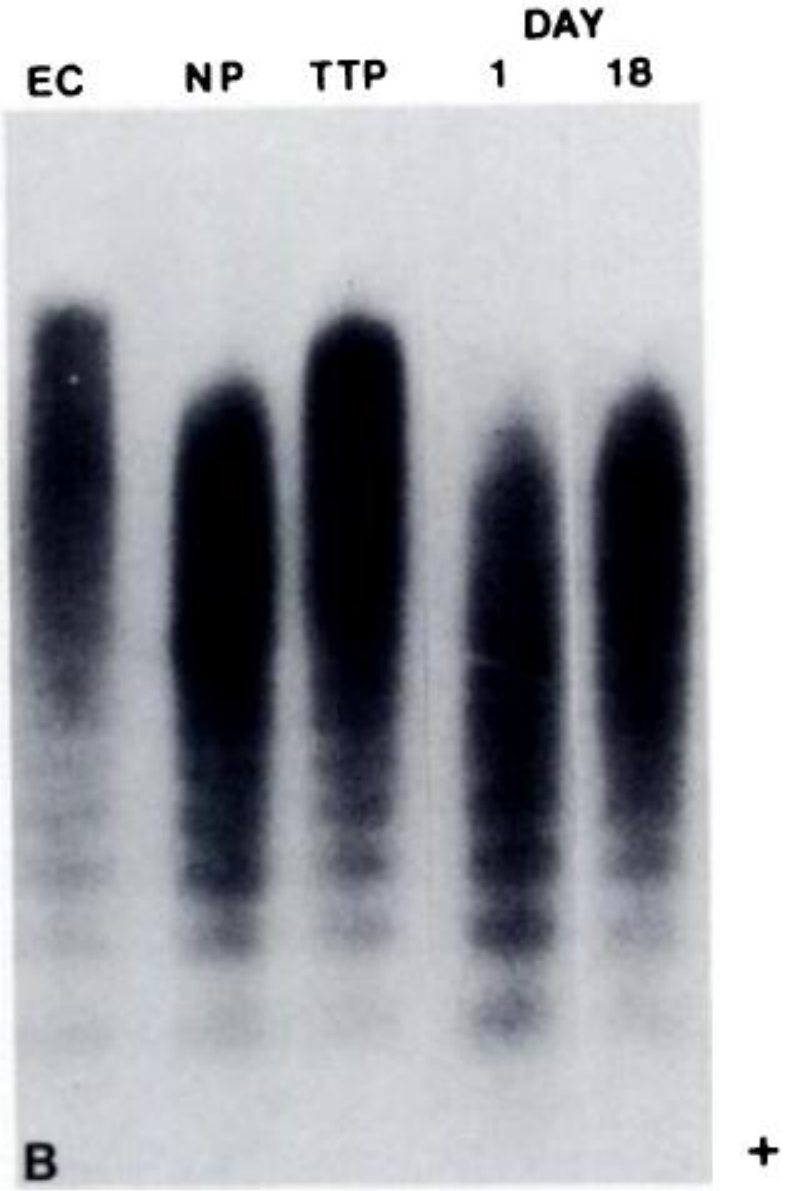
Multimeric pattern in TTP

EC: endothelial cell lysate

NP: normal plasma

TTP plasma during remission

Day 1, 18: after one or 18 days of plasma exchange



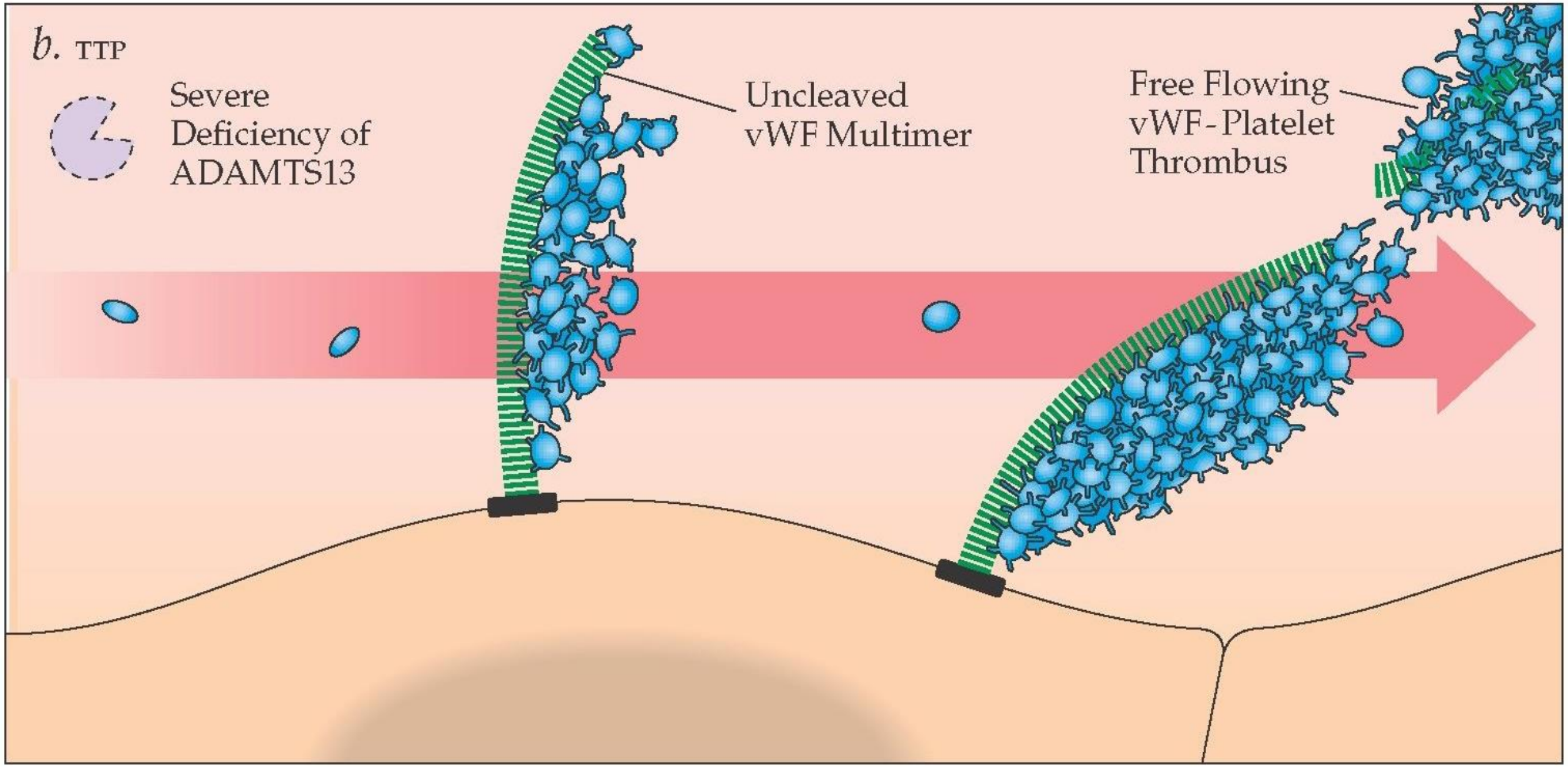
Moake et al. Blood, 1984.

In TTP, antibodies directed against Cys and spacer domains clear ADAMTS13 from circulation

- Associated with SLE, APA, pregnancy, drugs (cyclosporine, quinine, clopidogrel, ticlopidine), HIV infection, cancer
- 50% of cases are idiopathic

Zheng JTH, 2013
Joly et al Blood 2017.

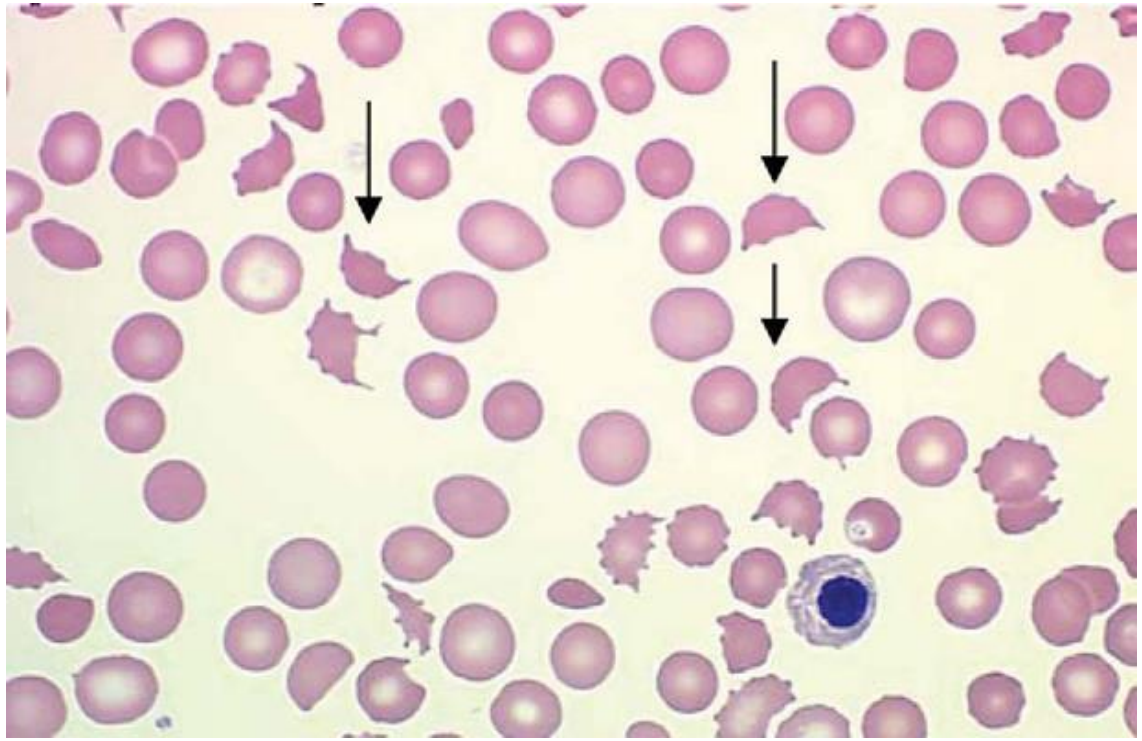
TTP: pathophysiology



TTP: a thrombotic microangiopathy

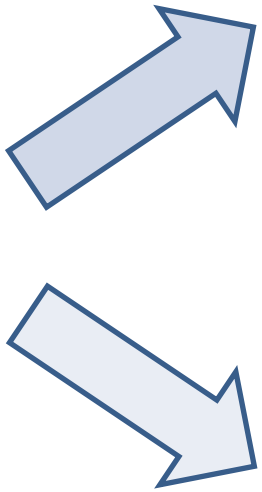
- Incidence: 1 new case/10^6

- Neurologic manifestations
- Hemolytic anemia
- Thrombocytopenia (but few bleeding)
- Marginal renal involvement
- Fever
- Mortality: 10-20%



Disorders of the VWF/ADAMTS system

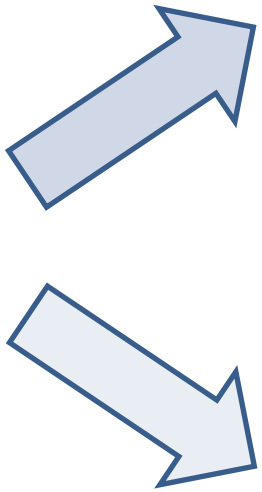
VWF multimers



Mulltimers are:
Increased: relative ADAMTS13 deficiency • TTP • Upshaw-Shulman • DIC, malaria, sepsis
Decreased: von Willebrand disease • Congenital • Acquired (antibody, ECMO, MPD)

Disorders of the VWF/ADAMTS system

VWF multimers



Mulltimers are:	Measured with:
Increased: relative ADAMTS13 deficiency • TTP • Upshaw-Shulman • DIC, malaria, sepsis	Multimer analysis ADAMTS13
Decreased: von Willebrand disease • Congenital • Acquired (antibody, ECMO, MPD)	VWF:Ag VWF:RCo VWF:CBA

Potential mechanisms of ADAMTS13 deficiency

	Mediator	Mode of action
Adamts13 deficiency		
Inhibition	IL6	Delays the rate of UL-VWF cleavage under flow
	Free haemoglobin/TSP	Competitive binding to VWF A2/A3 domain
	Leukocyte elastes, bacteria, thrombin	Proteolysis of ADAMTS13
	FVIII deficiency	FVIII accelerates VWF cleavage
Exhaustion		ULVWF multimers consuming ADAMTS13 molecules
Increased clearance		Non-neutralising antibodies

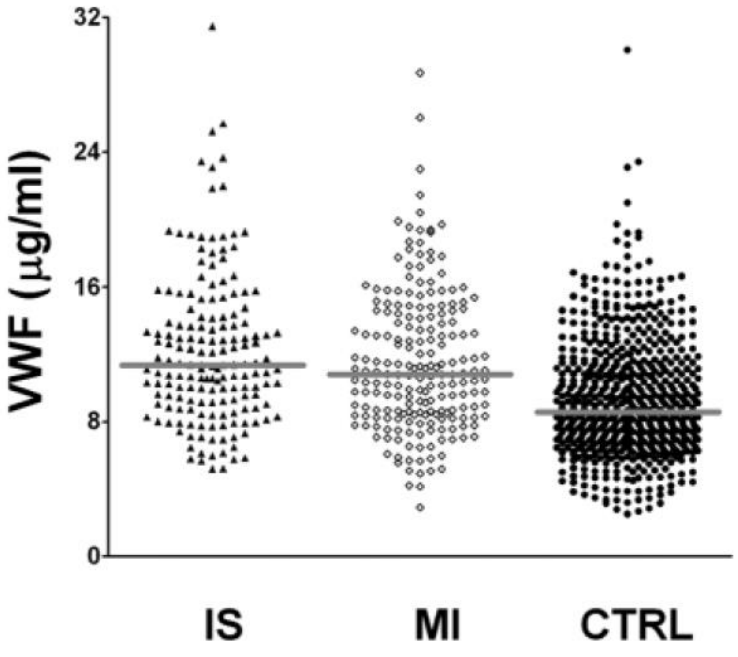
Adapted from Schwameis et al. Thrombosis and haemostasis, 2015.

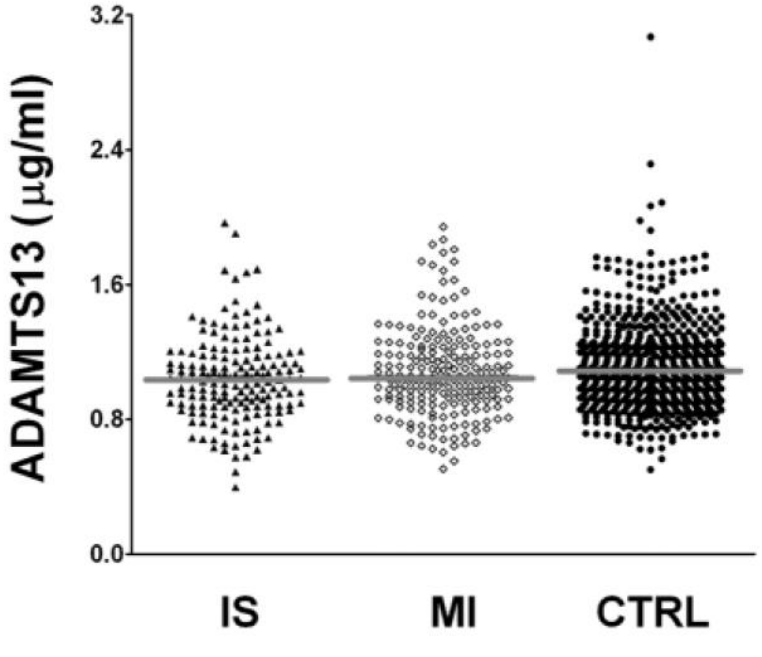
Potential mechanisms of VWF iper-release

	Mediator	Mode of action
VWF release		
	Cytokines	TNF, interleukin-8
	Endotoxin	LPS
	Biogenic amine	Histamine, Epinephrine
	Clotting factor	FVIIa

Adapted from Schwameis et al. Thrombosis and haemostasis, 2015.

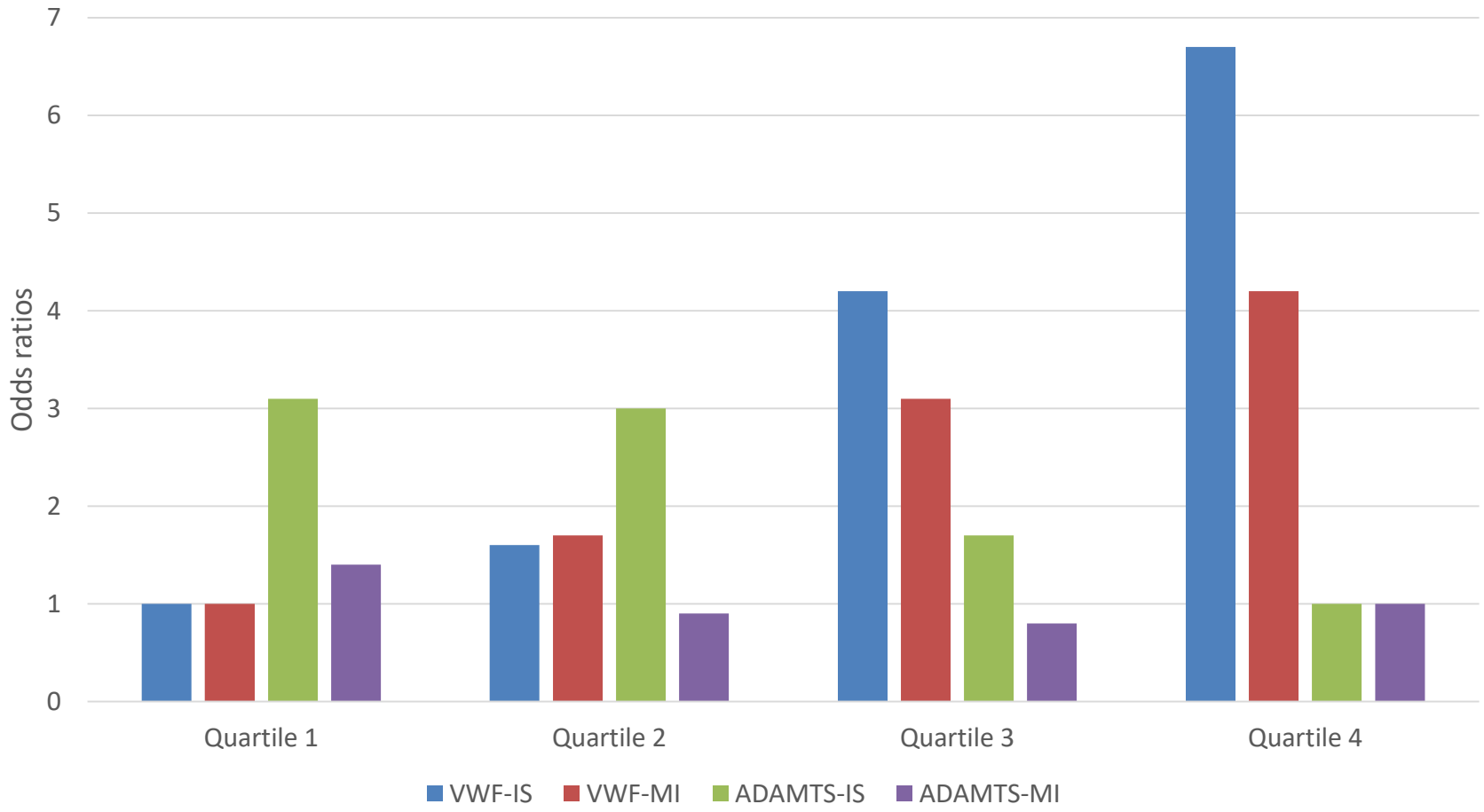
VWF and ADAMTS13 in non-acute conditions





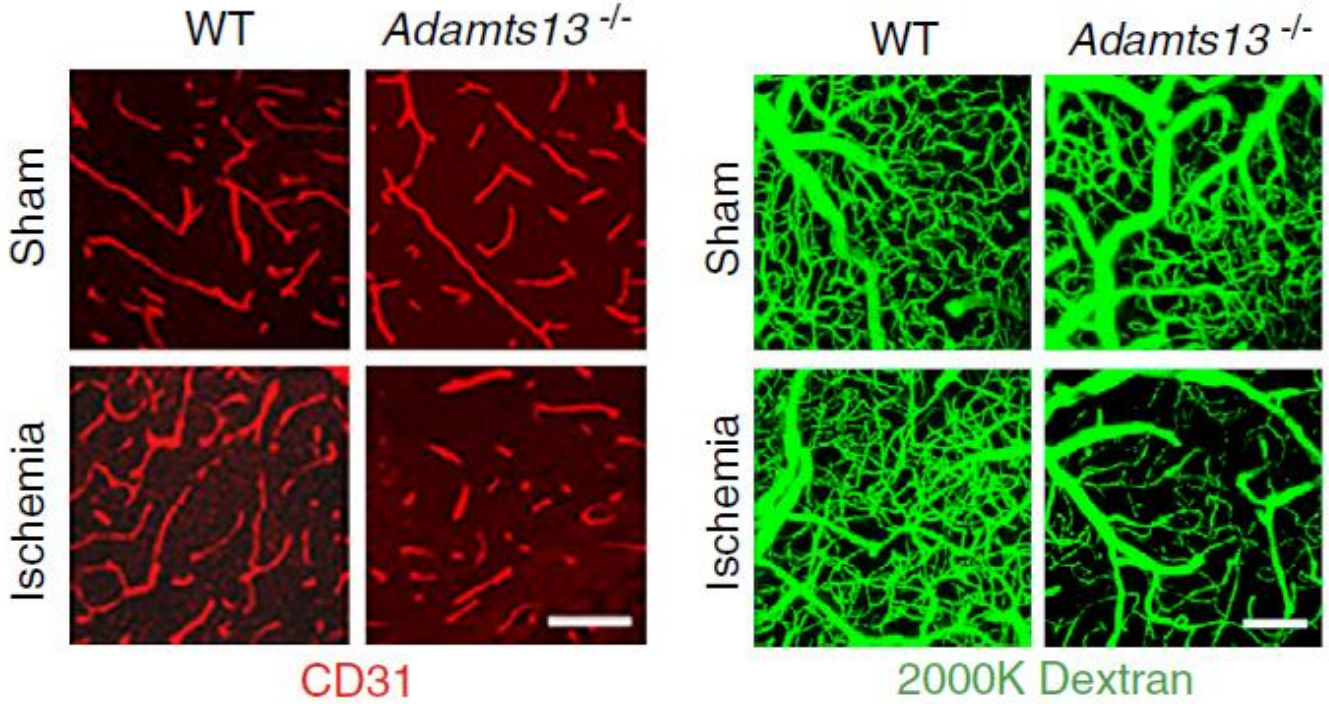
Andersson et al. Blood, 2012.

Relative Risk of Stroke or IMA



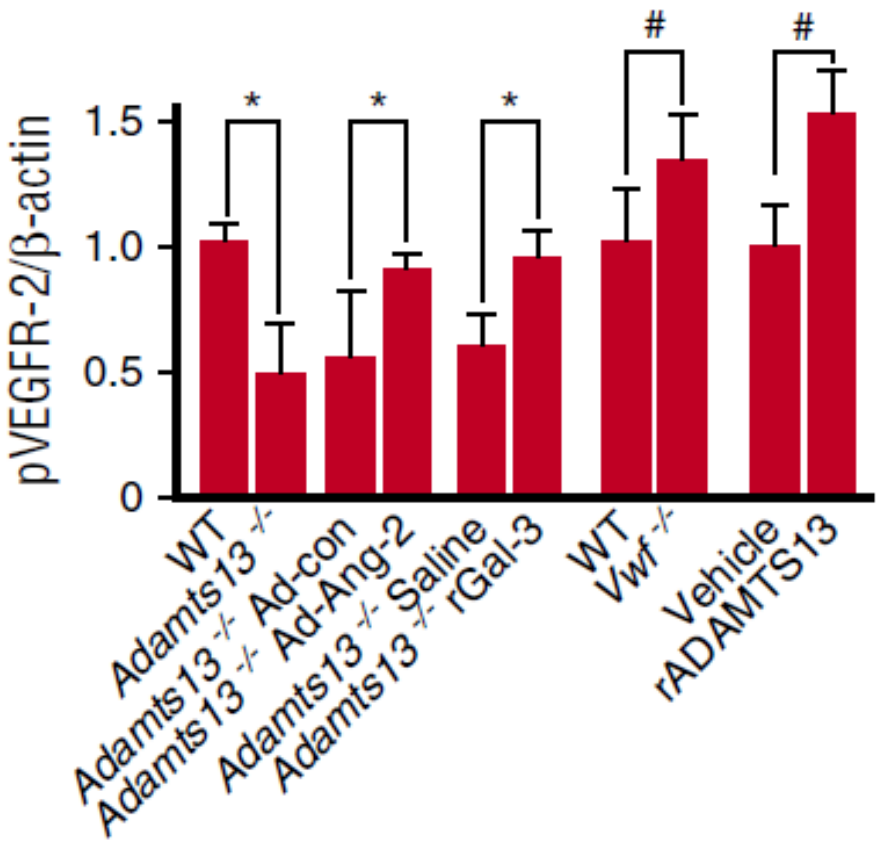
Andersson et al. Blood, 2012.

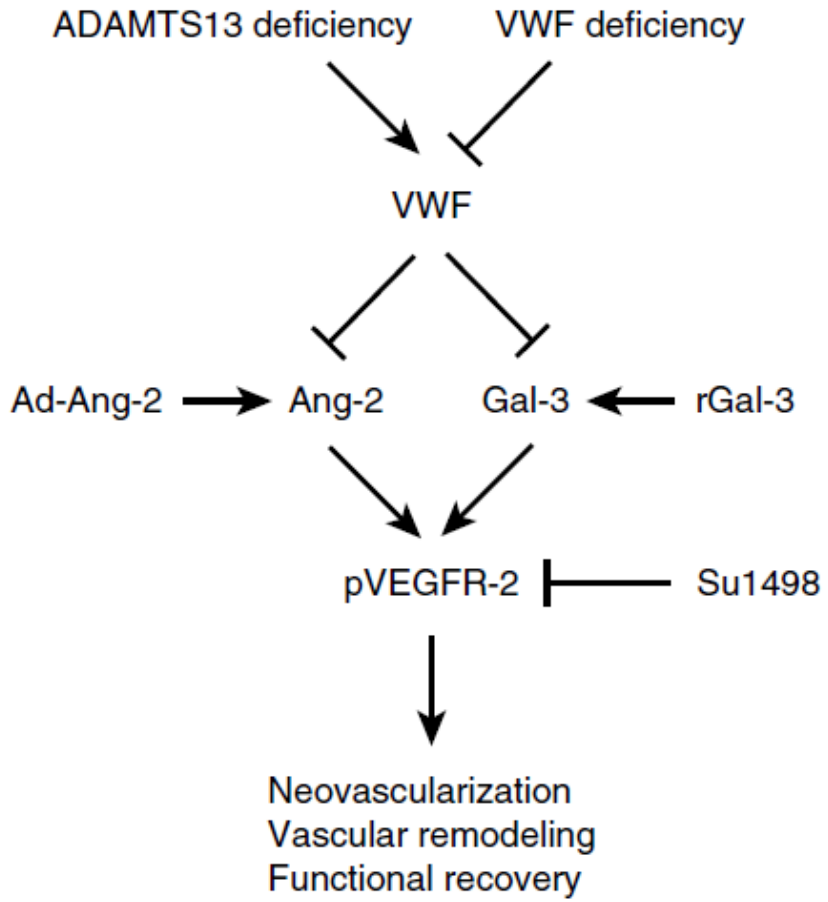
ADAMTS13 knockout mice has impaired neovascularization after stroke



Xu et al. Blood, 2017.

Infusion of rADAMTS-13 is capable of restoring VEGFR expression





Xu et al. Blood, 2017.

Conclusions

- HMW VWF multimers play an essential role in bleeding and thrombotic disorders
- Increase of HMW multimers is strongly associated with subsequent development of TMA
- Measurement of ADAMTS13 levels is still unfrequent in clinical labs, but required for hospitals in which a plasma exchange facility is available

Questions?

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