Presenter Disclosure Information

J. Evan Sadler, M.D., Ph.D.
von Willebrand Factor, ADAMTS13, and Thrombotic Microangiopathy

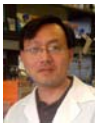
FINANCIAL DISCLOSURE:
No relevant financial relationship exists

UNLABELED/UNAPPROVED USES DISCLOSURE:
Rituximab for the treatment of TTP

von Willebrand Factor, ADAMTS13, and Thrombotic Microangiopathy


X. Long Zheng

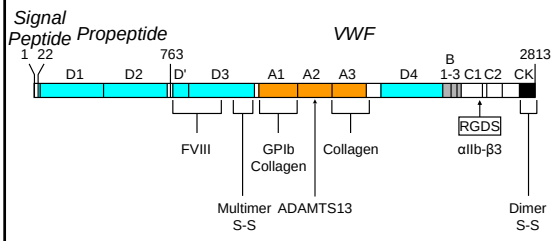

Elaine Majerus


Weiqiang Gao

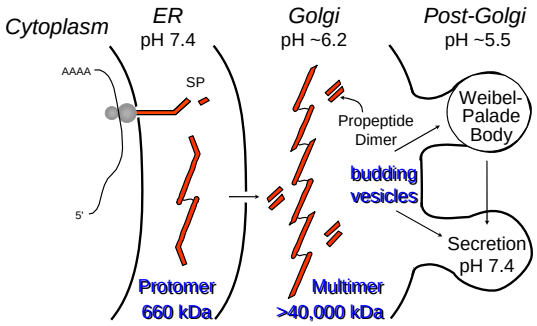

Hendrik Feys

- Pathophysiology of thrombotic thrombocytopenic purpura (TTP)
- Proteolysis of von Willebrand factor (VWF) by ADAMTS13
- Clinical uses of ADAMTS13 data

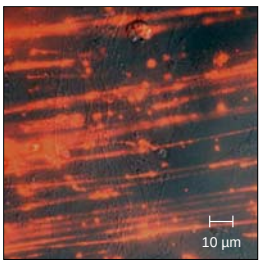
The von Willebrand Factor Precursor



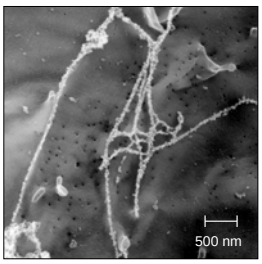
Biosynthesis of VWF



VWF Secreted by Endothelial Cells



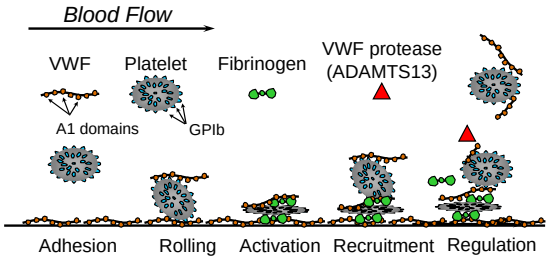
Histamine 100 µM, 10 min
AlexaFluor594 anti-VWF



Quick-freeze deep-etch
15 nm gold anti-VWF

Jing Huang et al, *Blood* 2009; **113**: 1589-97

VWF and Platelet Adhesion

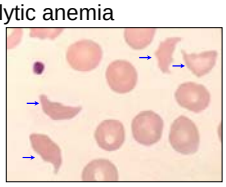


Thrombotic Thrombocytopenic Purpura

A Disorder of VWF Proteolysis

A classic pentad of signs:

- Microangiopathic hemolytic anemia
- Thrombocytopenia
- Neurologic dysfunction
- Renal disease
- Fever



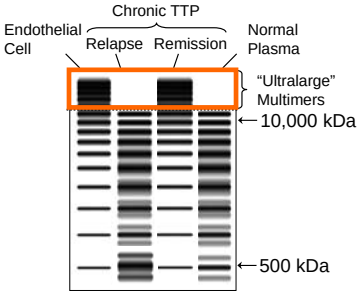
≈ 4 per million incidence

Strikes mainly young adult women

Untreated, mortality >90%

Treated with plasma exchange, mortality <20%

VWF Multimers in TTP



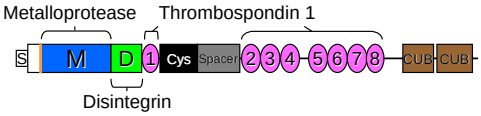
Failure to cleave ultralarge VWF causes TTP?

Moake et al, *N Engl J Med* 1982; **307:** 1432-1435

VWF Cleaving Protease in Plasma

- 1982: Predicted by Moake
- 1996: Discovered by Tsai and Furlan
- 1997: Absent in congenital TTP
- 1998: Absent in most idiopathic TTP (acquired IgG autoantibody inhibitor)
- 2001: Purified and sequenced by Fujikawa and Furlan

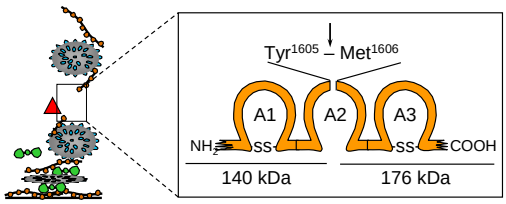
VWF Cleaving Protease (ADAMTS13)



*A D*isintegrin-like *A*nd *M*etalloprotease with *T*hrombo*S*pondin-1 repeats

Zheng et al, *J Biol Chem* 2001; **276**: 41059-63
Levy et al, *Nature* 2001; **413**: 488-94
Soejima et al, *J Biochem (Tokyo)* 2001; **130**: 475-80

Shear and VWF Proteolysis



Proteolysis increased by:

- Shear stress (aortic stenosis)
- VWD type 2A and 2B mutations

Why is ADAMTS13 Specific for VWF?

ADAMTS13 is constitutively active

"Stretched" or "sheared" VWF is the only known natural substrate

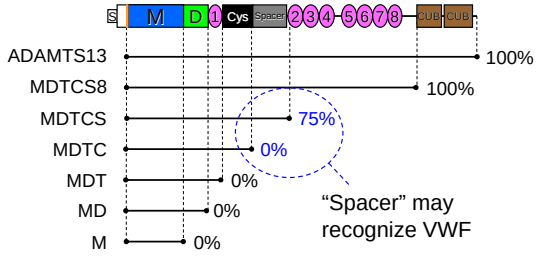
No ADAMTS13 inhibitors in vivo

Plasma is a tough environment:

- [ADAMTS13] = 1 µg/ml
- [VWF] = 10 µg/ml
- [Total Protein] = 80 mg/ml (8,000-fold higher)

Recombinant ADAMTS13 Activity

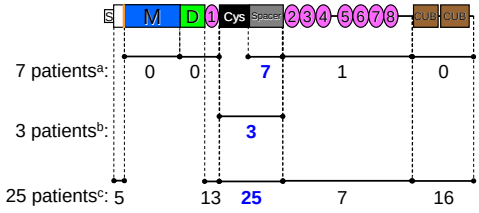
Against Plasma VWF



Zheng et al, *J Biol Chem* 2003; **278**: 30136-41

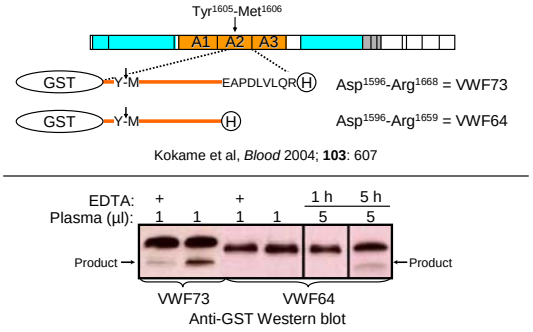
ADAMTS13 Inhibitors in TTP

Epitopes Map to Cys-Rich + Spacer



[a] Luken et al, *Thromb Haemost* 2005; **93:** 267-274
[b] Soejima et al, *Blood* 2003; **102:** 3232-3237
[c] Klaus et al, *Blood* 2004; **103:** 4514-4519

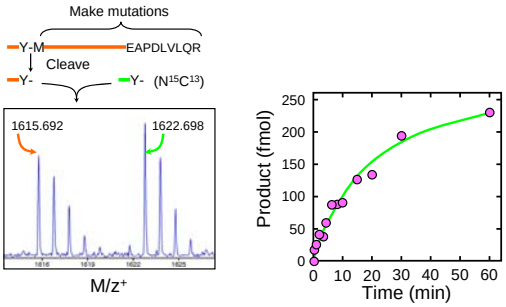
What Does ADAMTS13 Recognize?



Kokame et al, *Blood* 2004; **103**: 607

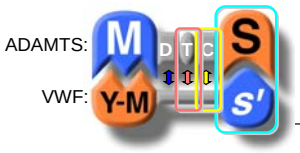
VWF-ADAMTS13 Interactions

Kinetic Analysis by MALDI-TOF MS



Gao et al, *Proc Natl Acad Sci USA* 2006; **103**: 19099-104

More Substrate-Exosite Interactions

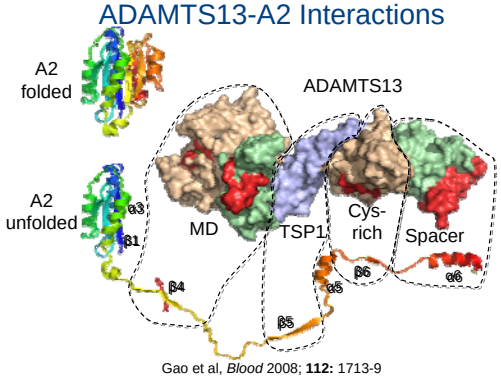


Catalytic Efficiency

	MD	MDT	MDTC	MDTCS
	k_{cat}/K_m (μM^{-1} s^{-1})			
VWF73 Y-M EAPDLVLQR^{1668}			0.1	2.0
VWF64 Y-M R^{1659}	0.01	0.01	0.1	0.1
VWF46 Y-M R^{1641}	0.0003	0.004	0.01	
VWF35 Y-M V^{1630}	0.0009	0.005	0.01	

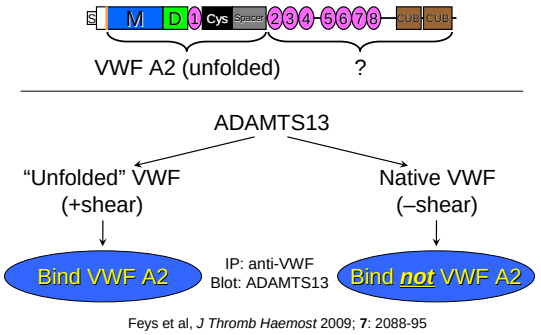
Gao et al, *Blood* 2008; **112**: 1713-9

ADAMTS13-A2 Interactions



Gao et al, *Blood* 2008; **112:** 1713-9
Akiyama et al, *Proc Natl Acad Sci USA* 2009; **106:** 19274-9

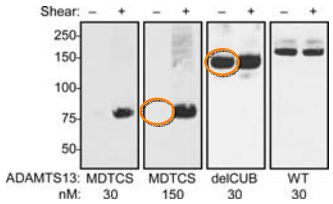
ADAMTS13 Binding to VWF



Feys et al, *J Thromb Haemost* 2009; **7**: 2088-95

ADAMTS13 Binding to VWF

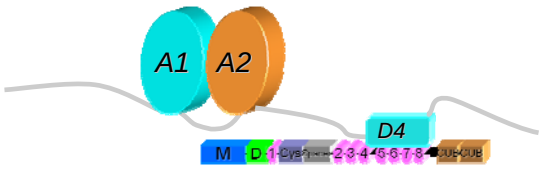
IP: anti-VWF
Blot: anti- ADAMTS13



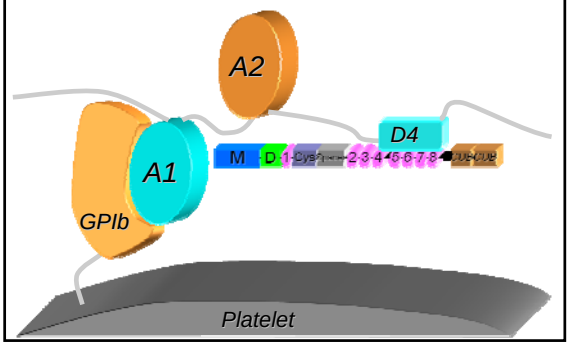
Distal TSP domains bind native VWF
MDTCS is sufficient to bind sheared VWF

Feys et al, *J Thromb Haemost* 2009; **7**: 2088-95

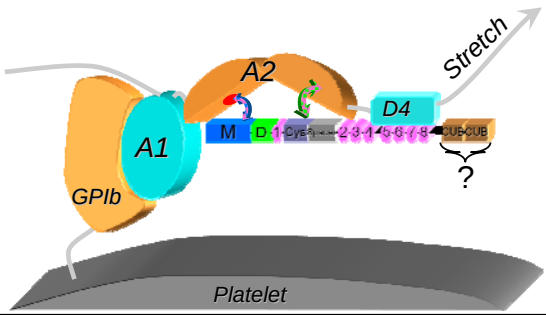
VWF Cleaving Complex



VWF Cleaving Complex

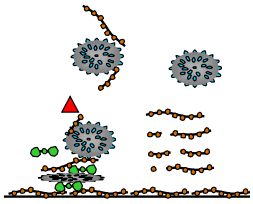


VWF Cleaving Complex



VWF, ADAMTS13 and TTP

With ADAMTS13



Normal VWF Multimers
Normal Hemostasis

Without ADAMTS13

Ultralarge VWF Multimers
Microvascular Thrombosis
(TTP)

23 yo AA Male (2007)

HPI: 1 wk of malaise, fevers, abdominal pain, nausea and vomiting, somnolence

PE: Temp 37.6 ˚C, BP 184/133, Pale, disoriented

CBC:
- Hgb 6.5
- Platelets 10K
- 20 schistocytes/HPF

Other Labs:
- Cr 2.9 mg/dL
- LDH >1800 IU/L
- Troponin I 15 ng/mL

Diagnosis: Thrombotic thrombocytopenic purpura

Treatment: Prednisone, plasma exchange…

TTP – Clinical Course

80% respond to plasma exchange in average of 16 days (range, 3-36)

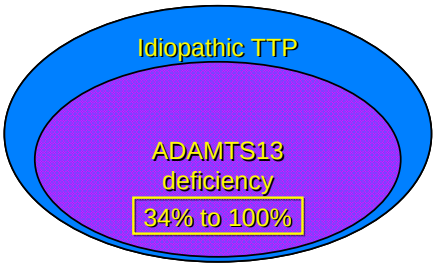
20% dead within with 5 weeks

40% of responders have exacerbations within one week

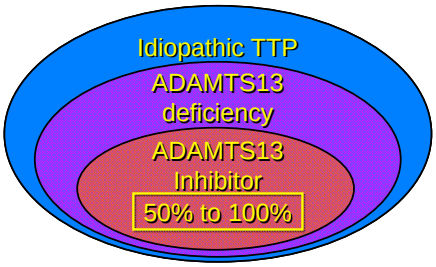
30% of responders relapse within 2 years

Rock et al, *New Eng J Med* 1991; **325:** 393-397

Autoimmune Idiopathic TTP
Acquired ADAMTS13 Deficiency



Autoimmune Idiopathic TTP
Acquired ADAMTS13 Deficiency



ADAMTS13 – Clinical Correlations

ADAMTS13 deficiency (<5-10%) predicts:
- "Idiopathic" (primary) TTP
- Response to plasma exchange
- Survival (long term)
- Relapse

ADAMTS13 inhibitor predicts:
- Prolonged time to complete response
- Death (early)
- Relapse

Reviewed in Coppo et al, *Br J Haematol* 2005; **132:** 66-74
(≈391 total patients)

ADAMTS13 – Clinical Correlations

Character	Odds Ratio
ADAMTS13 <10%:	
• Survival	(≈5)
• Primary TTP	≈8
• Female Gender	≈4
• African Ancestry	≈8
• Obesity	High
ADAMTS13 Inhibitor Present:	
• Relapse	≈13

Kremer Hovinga et al, *Blood* 2010; **115**: 1500-11
Zheng et al, *Blood* 2004; **103**: 4043-9
Vesely et al, *Blood* 2003; **102**: 60-8
Veyradier et al, *Blood* 2001; **98**: 1765-72

ADAMTS13 Deficiency and Relapse

At Presentation:

- ADAMTS13 < 10%: 30% relapse in 2 years, and risk continues
- For example, *at WUMS: 11/16 (70%) relapsed, 5/16 (30%) dead, by 6 years of follow up
- ADAMTS13 > 10%: 4% relapse in 2 years

Kremer Hovinga et al, *Blood* 2010; **115**: 1500-11
*(follow up of patients in) Zheng et al, *Blood* 2004; **103**: 4043-9

ADAMTS13 Deficiency and Relapse

In Remission:

- ADAMTS13 < 10%: 21/35 (60%) relapsed
- ADAMTS13 > 10%: 14/74 (19%) relapsed

At Relapse:

- ADAMTS13 < 5%: all patients

Peyvandi et al. *Haematologica* 2008; **93:** 232-9

25 yo AA Male (2009)

HPI: Found down with R hemiparesis. Discharged 21 days ago for relapsed TTP, treated with PE, rituximab x 1. Did not keep clinic appointments.

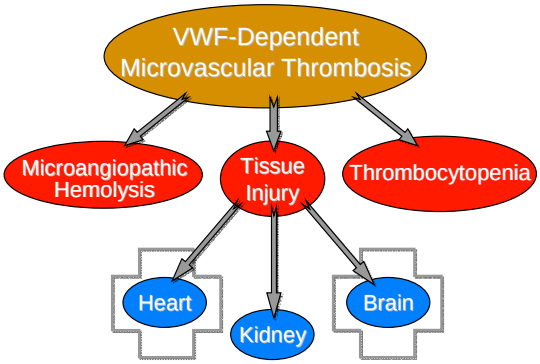
PE: Temp 36.5 ˚C, BP 114/63, Pale, R hemiparesis

CBC:
- Hgb 5.1
- Platelets 346K
- 0-1 schistocytes/HPF

Other Labs:
- Cr 1.31 mg/dL
- LDH 187 IU/L
- Haptoglobin 251 mg/dL

MRI: Acute to subacute infarcts medial L parietal lobe and deep in L cerebral white matter

Thrombosis Preceding TTP?



25 yo AA Male (continued)

Diagnosis: Exacerbation of TTP (without MAHA)

Course: PE daily with resolution of UE weakness by day 2, persistent LE weakness requiring a walker. Rituximab #2,3,4 given in hospital...

ADAMTS13:

Date	Activity	Inhibitor	Comment
3/2007	<5%	<0.4 U/ml	Diagnosis
4/2007	<5%	3.2 U/ml	Refractory to PE
8/2009	<5%	3.6 U/ml	Relapse
9/2009	<5%	<0.4 U/ml	Exacerbation

Rituximab for Refractory TTP

>37 reports, ≈100 patients failed PE, many failed steroids, vincristine, splenectomy

Rituximab 375 mg/m^2 weekly, up to 8 doses

- 95% durable complete responses in 1-3 weeks
- 10% late relapses (>1 yr), almost all achieved CR upon retreatment
- Serious reactions (one each): cardiogenic shock, gastrointestinal Strongyloides, CMV reactivation, HZV myelitis/encephalitis

Elliott MA et al, *Eur J Haematol* 2009; **83:** 365-42
Ling HT et al, *Am J Hematol* 2009; **84:** 418-21

TTP – Summary

Look for hemolysis and schistocytes in all patients with anemia and thrombocytopenia

Severe ADAMTS13 deficiency is:

- Uncommon in secondary TTP
- Common in primary TTP

ADAMTS13 inhibitors correlate with:

- Relapse risk
- Early death

Refractory TTP may respond to rituximab

TTP – Questions

Can ADAMT13 assays diagnose atypical presentations of TTP or predict relapses?

Should the goal of treatment be a normal ADAMTS13 level?

Does plasma exchange help patients with normal ADAMTS13?

von Willebrand Factor, ADAMTS13 and Thrombotic Microangiopathy

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