# Factor V Short and TFPI

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# Characterization of a novel autosomal dominant bleeding disorder in a large kindred from east Texas

Shao-Qing Kuang, Sumera Hasham, Martin D. Phillips, David Wolf, Ying Wan, Perumal Thiagarajan, and Dianna M. Milewicz

BLOOD, 15 MARCH 2001 • VOLUME 97, NUMBER 6 1549

- Proband
  - 35 year old male
  - Bleeding diathesis since childhood
    - Bruising, epistaxis, bleeding from gums, significant bleeding after trauma (requiring transfusion)
    - No haemarthrosis or spontaneous haematoma
- Siblings presented with similar bleeding symptoms, sisters +menorrhagia
- Classified as a moderately severe bleeding disorder

| Laboratory test | Proband data | |
|---|---|---|
| PT, seconds | 18.4 | Prolonged |
| aPTT, seconds | 48.7 | |
| Fibrinogen, (μmol/L) | 6.1 | Normal |
| (mg/dL) | (208) | |
| Factor II, % | 94 | |
| Factor V, % | 111 | |
| Factor VII, % | 70 | |
| Factor VIII, % | 59 | |
| Factor IX, % | 74 | |
| Factor X, % | 81 | |
| Factor XI, % | 76 | |
| Factor XII, % | 82 | |
| Thrombin time, seconds | 20.1 | |
| Antithrombin III function, % | 90 | |
| Antithrombin III antigen, mg/L | 260 | |
| (mg/dL) | (26) | |
| vWF, ristocetin cofactor | 54 | |
| Bleeding time, minutes | 6.0 | |
| Platelet number and aggregation | Normal | |
| RVVT, seconds | 26.6 | |
| Protein C, % | 68 | |
| Activated protein C resistance, ratio | 2.8 | |

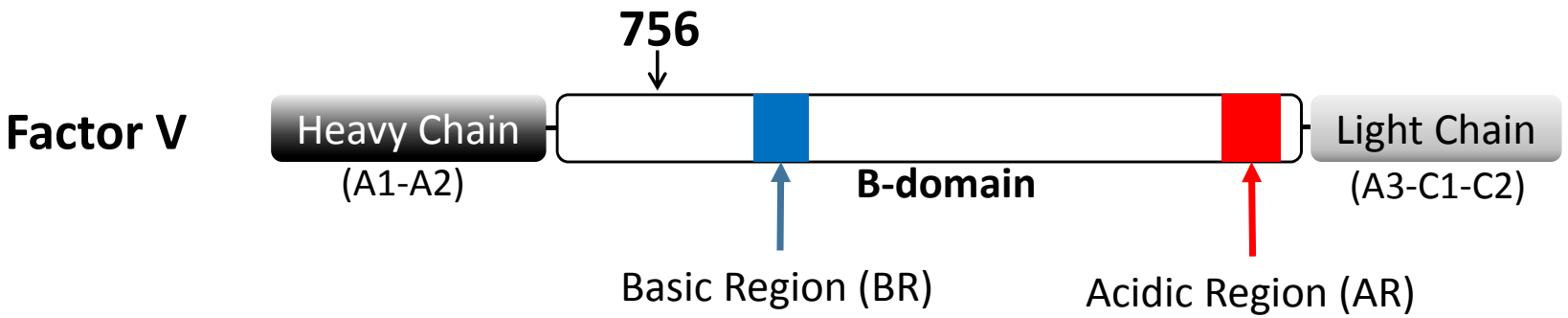
| Control plasma, % | Patient plasma, % | aPTT (immediate), seconds | aPTT, (1 h), seconds |
|---|---|---|---|
| 100 | 0 | 28.7 | 30.7 |
| 80 | 20 | 28.2 | 31.1 |
| 60 | 40 | 30.1 | 34.6 |
| 50 | 50 | 29.7 | 33.3 |
| 40 | 60 | 32.7 | 35.5 |
| 20 | 80 | 33.1 | 39.7 |
| 0 | 100 | 42.1 | 47.3 |

The presence of a novel slow-acting, soluble coagulation inhibitor was proposed

Genetic analysis revealed that all affected individuals had a novel *F5* mutation

- A→G at nucleotide 2440 (Exon 13)
- S→G at amino acid 756

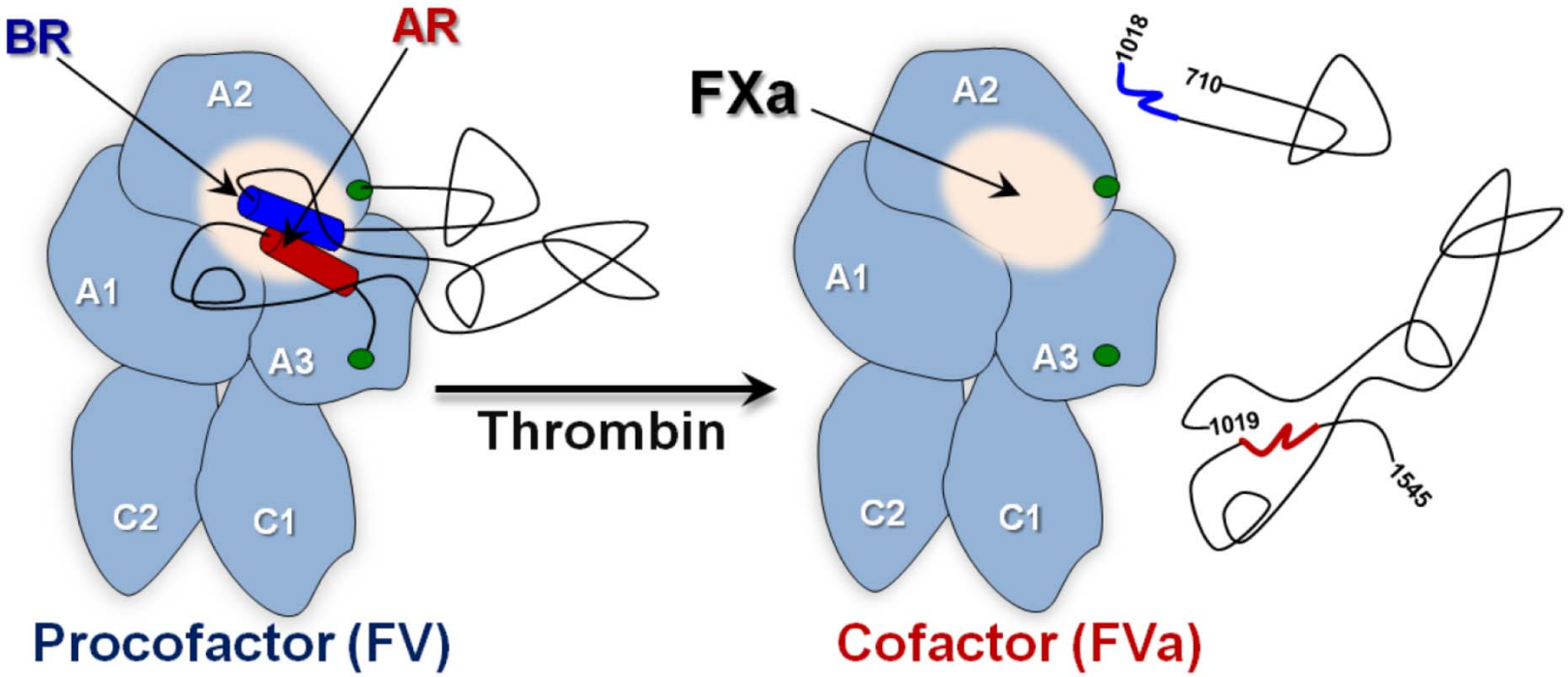
# Where is this mutation?




The basic and acidic regions bind to keep Factor V in an inactive state

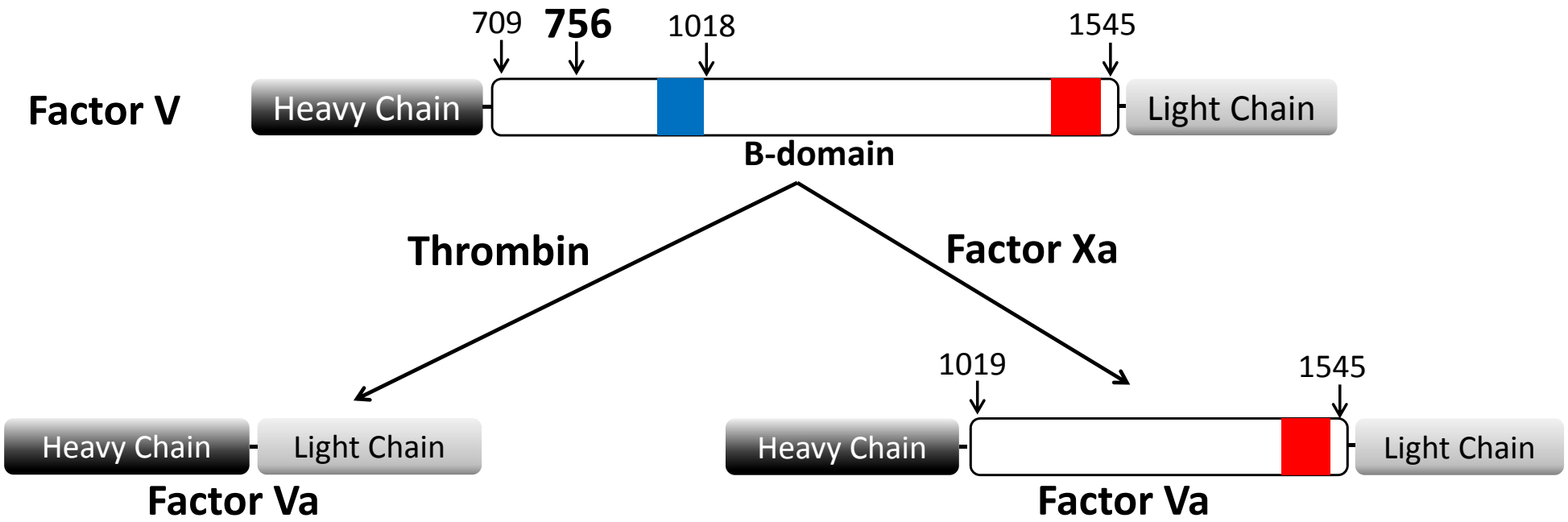
Zhu, Toso, and Camire. *J Biol Chem* 2007;282(20):15033-9*;* Camire and Bos. J Thromb Haemost 2009;7;1951; Bos and Camire. *J Biol Chem* 2012;287(31):26342

# Factor V activation




Bos and Camire. *J Biol Chem* 2012;287(31):26342

# Factor V activation *in vivo*




Both forms of fVa have equal co-factor activity

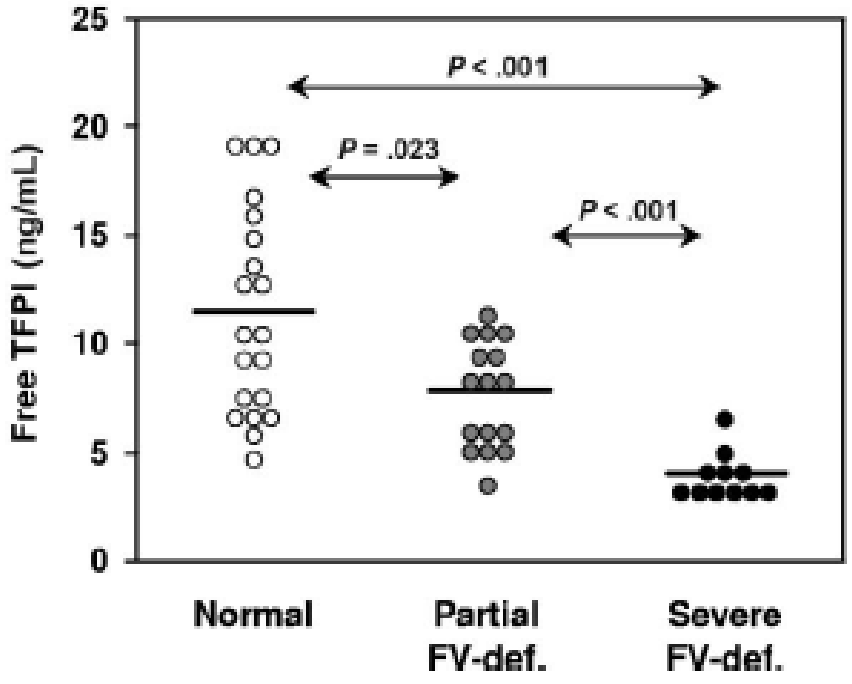
Jenny et al. *PNAS* 1987;84:4846; Monkovic and Tracy *Biochemistry* 1990;29:1118; Bos and Camire. *J Biol Chem* 2012;287(31):26342

# A link between Factor V and TFPI…….

## Low plasma levels of tissue factor pathway inhibitor in patients with congenital factor V deficiency

Connie Duckers,[1] Paolo Simioni,[2] Luca Spiezia,[2] Claudia Radu,[2] Sabrina Gavasso,[2] Jan Rosing,[1] and Elisabetta Castoldi[1]

BLOOD, 1 NOVEMBER 2008 • VOLUME 112, NUMBER 9 3615

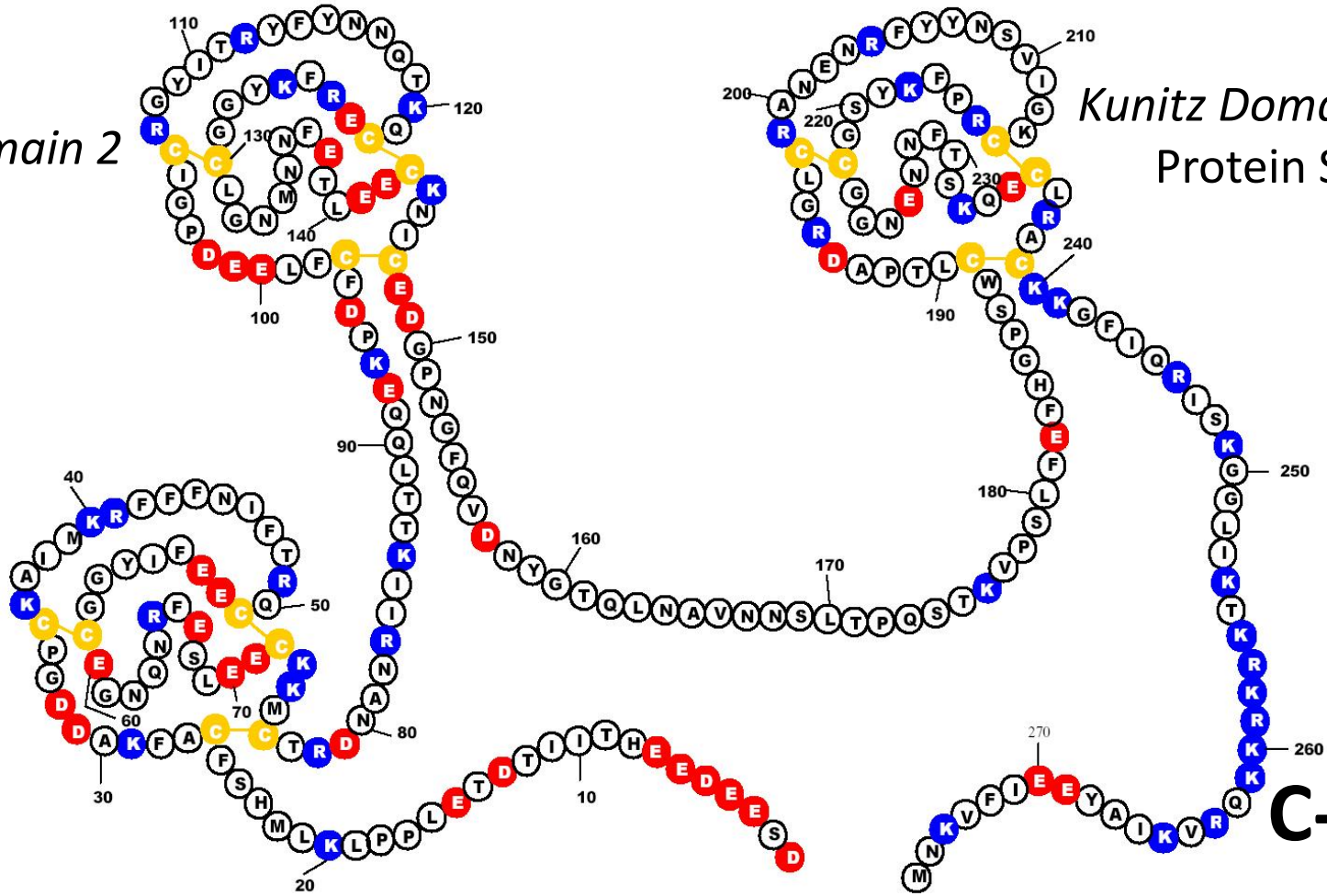


# Tissue Factor Pathway Inhibitor alpha (TFPIα)

*Kunitz Domain 2*
fXa

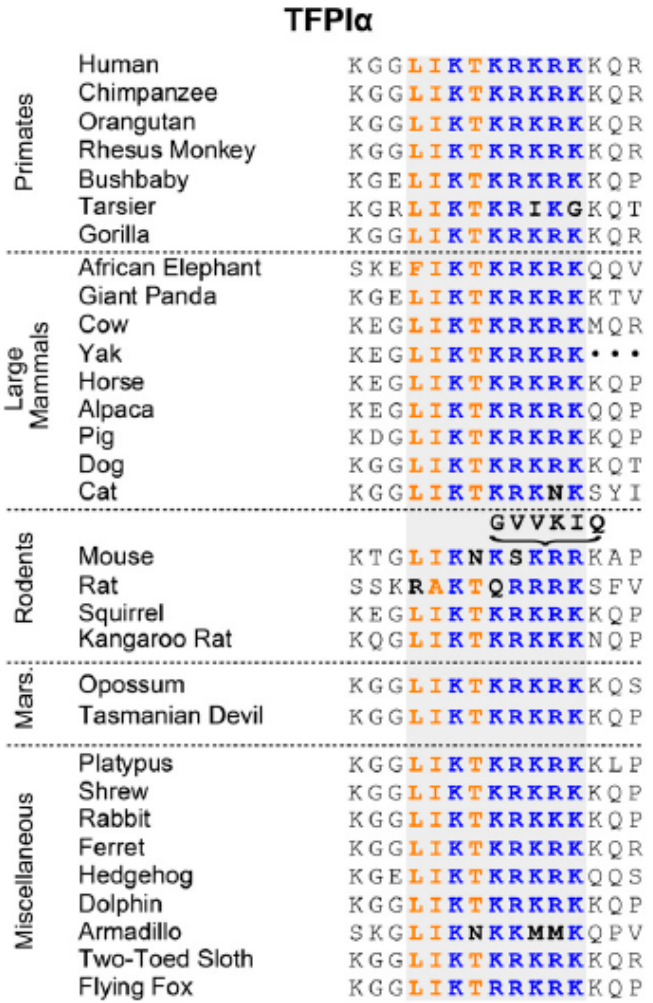
*Kunitz Domain 3*
Protein S

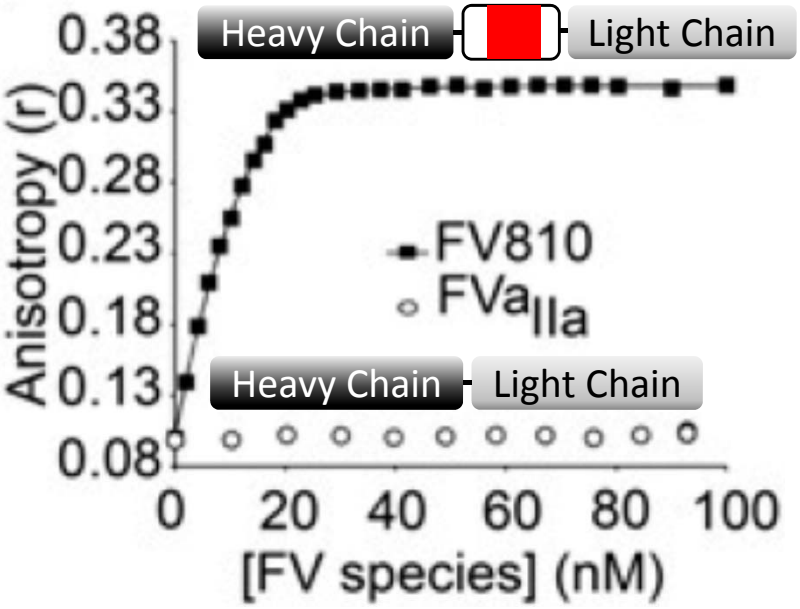
*Kunitz Domain 1*
fVIIa



**Basic C-terminus**

For reviews, see Ellery and Adams. *Semin Thromb Hemst.* 2014;40(8):881 and Wood et al. *Blood* 2014;123(19):2934


TFPIα
Primates
Human KGGLIKTKRKRKKQR
Chimpanzee KGGLIKTKRKRKKQR
Orangutan KGGLIKTKRKRKKQR
Rhesus Monkey KGGLIKTKRKRKKQR
Bushbaby KGELIKTKRKRKKQP
Tarsier KGRLIKTKRIKGKQT
Gorilla KGGLIKTKRKRKKQR
Large Mammals
African Elephant SKEFIKTKRKRKQQV
Giant Panda KGELIKTKRKRKKTV
Cow KEGLIKTKRKRKMQR
Yak KEGLIKTKRKRK•••
Horse KEGLIKTKRKRKKQP
Alpaca KEGLIKTKRKRKQQP
Pig KDGLIKTKRKRKKQP
Dog KGGLIKTKRKRKKQT
Cat KGGLIKTKRKNKSYI
GVVKIQ
Rodents
Mouse KTGLIKNKSKRRKAP
Rat SSKRAKTQRRRKSFV
Squirrel KEGLIKTKRKRKKQP
Kangaroo Rat KQGLIKTKRKKKNQP
Mars.
Opossum KGGLIKTKRKRKKQS
Tasmanian Devil KGGLIKTKRKRKKQP
Miscellaneous
Platypus KGGLIKTKRKRKKLP
Shrew KGGLIKTKRKRKKQP
Rabbit KGGLIKTKRKKKKQP
Ferret KGGLIKTKRKRKKQR
Hedgehog KGELIKTKRKRKQQS
Dolphin KGGLIKTKRKRKKQP
Armadillo SKGLIKNKKMMKQPV
Two-Toed Sloth KGGLIKTKRKRKKQR
Flying Fox KGGLIKTRRKRKKQP


Heavy Chain
Light Chain
Anisotropy (r)
0.38
0.33
0.28
0.23
0.18
0.13
0.08
FV810
FVa_IIa
Heavy Chain
Light Chain
0
20
40
60
80
100
[FV species] (nM)

# Back to the East Texas bleeding disorder

## Coagulation factor $V^{A2440G}$ causes east Texas bleeding disorder via TFPIα

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Dianna M. Milewicz,[1] and Björn Dahlbäck[2]

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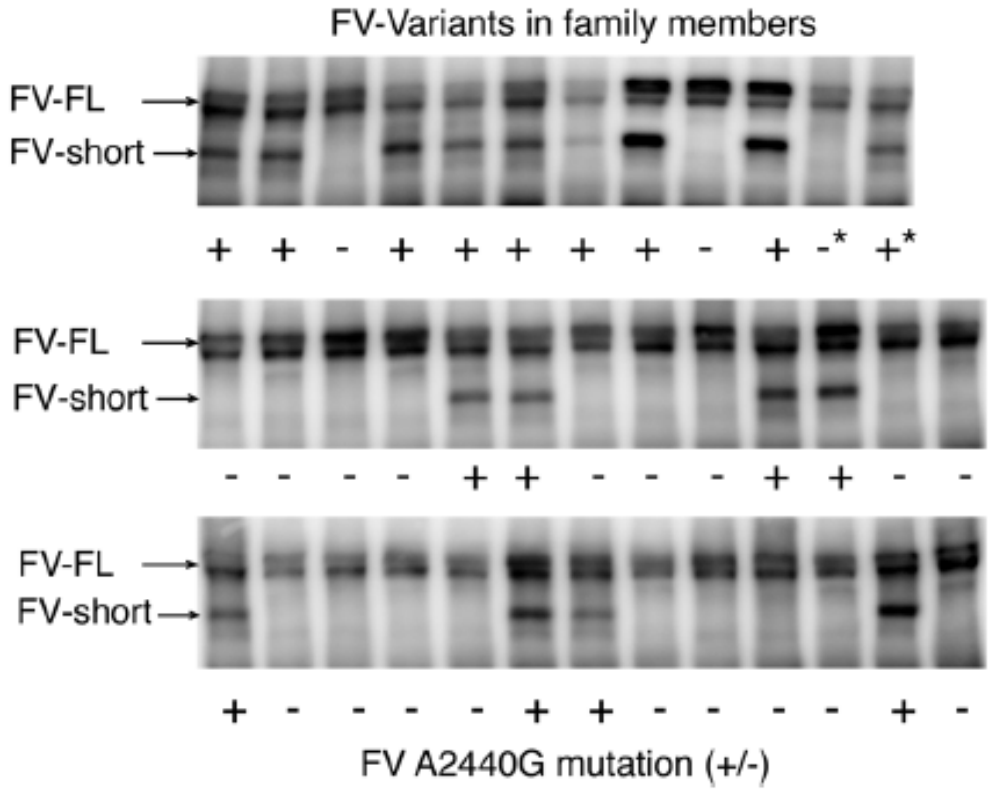
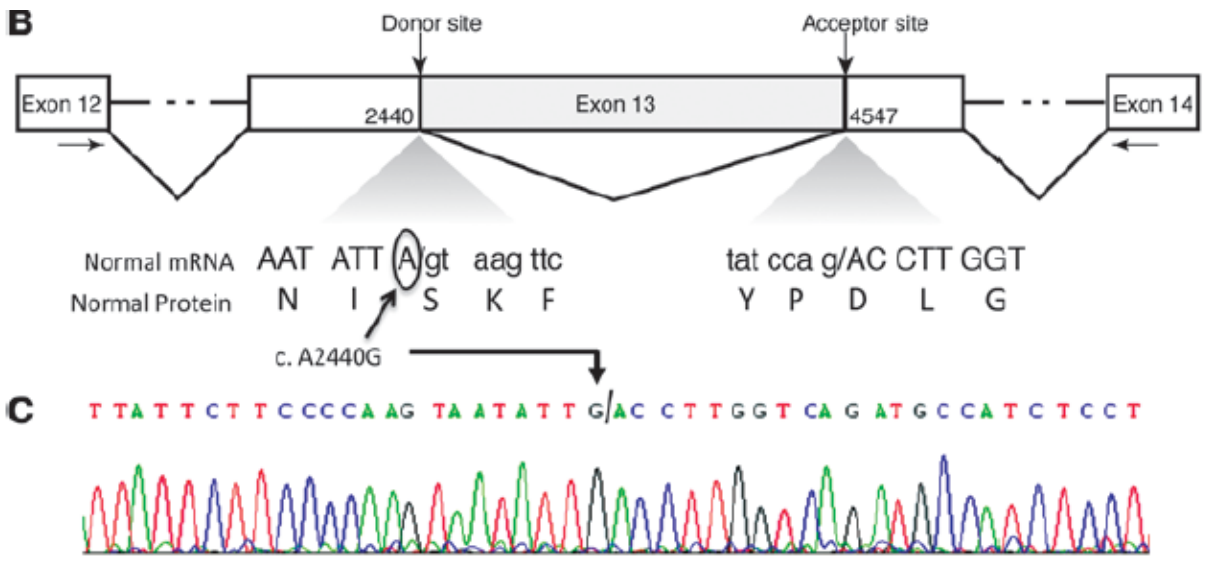
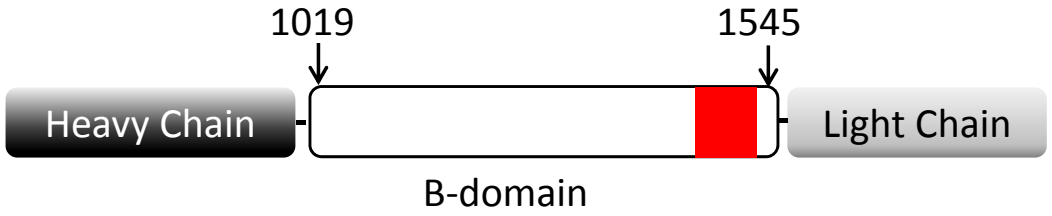
The Journal of Clinical Investigation http://www.jci.org Volume 123 Number 9 September 2013 3777

# Affected individuals produce FV-short

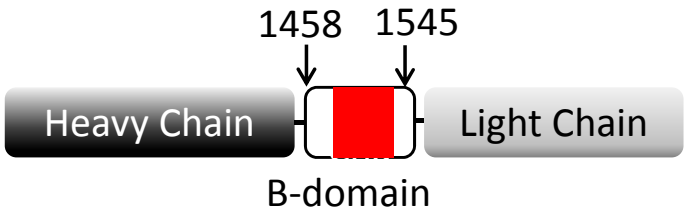


# The FV-Short protein resembles fXa-activated fV

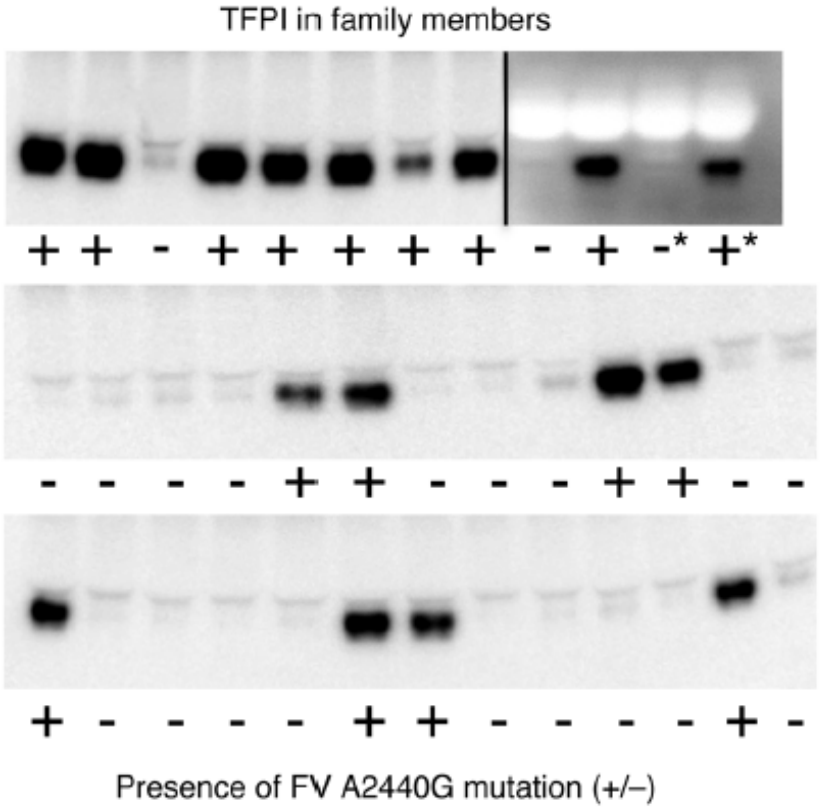


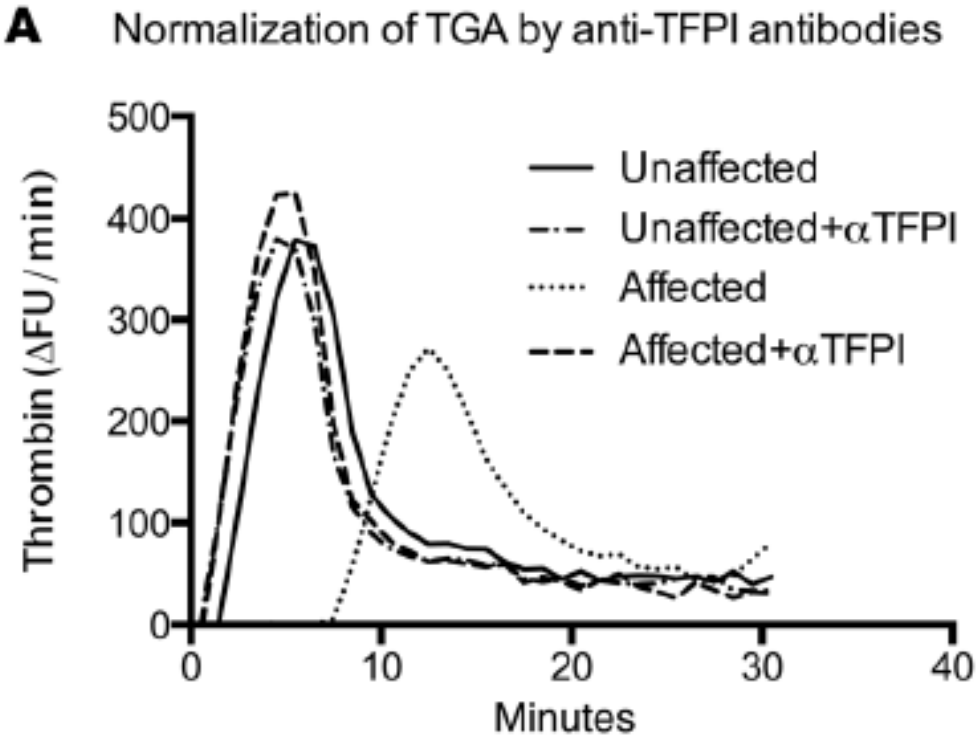

**Xa-activated Factor Va**




**fV-Short**

# Affected individuals have elevated plasma TFPIα



# Potential mechanisms of bleeding

- Binding of TFPIα → FV leads to ↑ circulating half-life
- Elevated plasma TFPIα directly inhibits TF-fVIIa
- TFPIα protects aa 1545 in FV-Short from proteolytic cleavage
- fVa-bound TFPIα is an excellent inhibitor of “early” Prothrombinase

# Factor V Amsterdam ≈ Factor V East Texas

**A novel mutation in the *F5* gene (factor V Amsterdam) associated with bleeding independent of factor V procoagulant function**

Marisa L. R. Cunha,[1,2] Kamran Bakhtiari,[1] Jorge Peter,[1] J. Arnoud Marquart,[1] Joost C. M. Meijers,[1,3] and Saskia Middeldorp[2]

1822 BLOOD, 12 MARCH 2015 • VOLUME 125, NUMBER 11

**Key Points**

- A novel gain-of-function mutation in factor V leading to increased levels of TFPI and bleeding was identified by whole exome sequencing.
- Factor V Amsterdam (*F5 C2588G*) resembles the mutation (*F5 A2350G*) leading to East Texas bleeding disorder.

# Summary

- Factor V East Texas and Factor V Amsterdam
  - Moderately severe bleeding disorders
  - Prolonged PT and/or aPTT
  - Mutation in exon 13 produces a novel donor splice site, resulting in truncated *F5* mRNA
    - In-frame deletion of a large portion of the region encoding the fV B-domain
  - Produce fV-Short, in which the BR of the B-domain is deleted but the AR is retained
  - Binds TFPIα with high affinity, resulting in markedly elevated plasma TFPIα
  - Indirect, gain-of-function mutations

- Investigations of the interactions between FV and TFPIα highlight a potential role of TFPI in thrombotic disease

# Acknowledgements


- THANZ Workshop Organising Committee
- Mast Lab, Blood Research Institute, Blood Center of Wisconsin