Pro- and anticoagulant properties of Factor V in pathogenesis of thrombosis and bleeding disorders

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Holds patent for APC resistance testing and receives royalty
Factor Va a cofactor to factor Xa in activation of prothrombin on negatively charged phospholipids
Factor V a procofactor
Pro- and anticoagulant properties

PROTEOLYSIS MODULATES ACTIVITY
Activation of factor V by thrombin or factor Xa

R709  R1018  R1545

FVα
Activation and Propagation of Extrinsic Pathway of Coagulation
Inhibition of coagulation by the protein C system
Activation of protein C

TM (thrombomodulin)

PC

APC

EPCR

T

T*
Degradation of FVa by APC
protein S a cofactor
Degradation of FVIIIa by APC
FV and protein S synergistic cofactors
ANTICOAGULANT

FACTOR V

COFACTOR TO ACTIVATED PROTEIN C

IN DEGRADATION OF FVIIIa
FV and protein S
synergistic APC cofactors in degradation of factor VIII

Factor VIIIa activity (%)

Factor V (ug/ml)

Factor VIIIa activity (%) vs. Factor V (ug/ml) for FV, APC+FV, and APC+PS+FV.
Procoagulant system

Anticoagulant system

XII → XIIa

XI → XIa

IX → IXa

Ca²⁺

VIIa, TF

VIIIa

VIII, TF

Ca²⁺

X → Xa

Ca²⁺

VIIIi

C4b-binding protein

Protein S

Protein C

Activated protein C

Thrombomodulin

EPCR

Thrombin

Prothrombin

V
Procoagulant

Anticoagulant
Thrombophilia

Life-long increased risk of thrombosis
Family with Thrombophilia

Dahlbäck B et al. Proc Natl Acad Sci U S A. 90:1004-8, 1993
Activated protein C (ug/ml)

Clotting time (seconds)

Dahlbäck B et al. Proc Natl Acad Sci U S A. 90:1004-8, 1993
APC RESISTANCE

Arg506Gln mutation in FV (FV Leiden) results in loss of one cleavage site
Protein S and FV synergistic cofactors to APC in degradation of FVIIIa

Cleavage at Arg506 stimulates activity -> FVLeiden poor cofactor
APC RESISTANCE
(FVLeiden)

* Prevalence in population

Heterozygotes 0-15%
Homozygotes 0-0.5%
World Distribution of the FV:Q506 allele

Europe and North America 1-15%

- Americas 0%
- Brazil ≈ 2%
- Africa 0%
- Japan 0%
- China 0%
- Australia ≈ 5%
- Aboriginals 0%
Factor V in bleeding disorders

**Genetic**
Factor V deficiency - parahaemophilia
Combined Factor V and VIII deficiency
Factor V in East Texas Bleeding disorder

**Acquired**
Liver disease
Disseminated Intravascular Coagulation (DIC)
Inhibitory autoantibodies

**Deficiency in platelet factor V**
Quebec platelet disorder (high urokinase-type PA)
FV New York
Intriguing pathogenic mechanism of autosomal dominant East Texas bleeding disorder

Lisa M. Vincent, Tracy A. Bensend, Dianna M. Milewicz

Sinh Tran
Ruzica Livaja
Björn Dahlbäck
• Autosomal Dominant

• 22 affected family members

East Texas Bleeding

- Autosomal dominant
- Moderately severe bleeding phenotype
- Mildly or moderately prolonged PT and/or aPTT
- Normal levels of II, V, VII, VIII, IX, X, XI, XII
- Linked to *FV*-*gene* on chromosome 1q23-25
- Sequencing: 6 known and 1 new SNP(A2440G:Ser$^{756}$Gly)

Location of A2440G in FV Gene
Activates alternative splicing

FV-Short Δ702

Modified from Vincent et al, J Clin Invest, 2013
FV-Short in affected family members

FV A2440G mutation (+/-)

Modified from Vincent et al, J Clin Invest, 2013
Inhibition of thrombin generation in affected family members

Modified from Vincent et al, J Clin Invest, 2013
Inhibitory activity in affected plasma removed by FV-immunodepletion

Inhibitor associated with FV, however, recombinant FV-Short does not reproduce inhibitory phenotype

Conclusion
Inhibitor associated with FV, however, recombinant FV-Short does not reproduce inhibitory phenotype

Modified from Vincent et al, J Clin Invest, 2013
Possible explanations:

- Inhibitory activity an intrinsic property of FV-Short
- Inhibitor associated with FV-Short
Tissue factor pathway inhibitor, TFPI

TFPlα reported to interact with both protein S and FV in plasma

Broze and Girard, Frontiers in Bioscience, 2012
TFPI an Efficient Inhibitor of Blood Coagulation
TFPI\(\alpha\) in family members

FV A2440G mutation (+/-)

Modified from Vincent et al, J Clin Invest, 2013
Normalization of TGA by TPFI antibodies

Inhibition of TGA by rTPFIα

Modified from Vincent et al, J Clin Invest, 2013
TFPlα ASSOCIATED WITH FV-SHORiNT IN AFFECTED PLASMA

IMMUNE PRECIPITATION WITH ANTI-TFPlα

Modified from Vincent et al, J Clin Invest, 2013
**TFPIα COMPLEXED WITH FV-SHORT ALSO IN NORMAL PLASMA**

**BUT AT MUCH LOWER CONCENTRATION**

Normal plasma

FV-FL

FV-Short

TFPI

 Modified from Vincent et al, J Clin Invest, 2013

**IMMUNE PRECIPITATION WITH ANTI-TFPI WITH LARGER VOLUMES OF NORMAL PLASMA (5 ml vs 0.3 ML)**
TFPIα binds to FV-Short with higher affinity than to FV-FL.
East Texas Bleeding Disease

Conclusions

- Autosomal dominant
- Mutation in exon 13 of FV gene
- Alternative splicing – deletion of 702 amino acids of B domain
- TFPI\(\alpha\) binds with high affinity to FV-Short
- TFPI\(\alpha\) retained in circulation → 10-fold increase in TFPI\(\alpha\)
- Inhibition of coagulation
- FV-Short:TFPI\(\alpha\) in normal plasma at low concentrations
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