FACTOR V LEIDEN

Conference 12/20/2005

INTRODUCTION

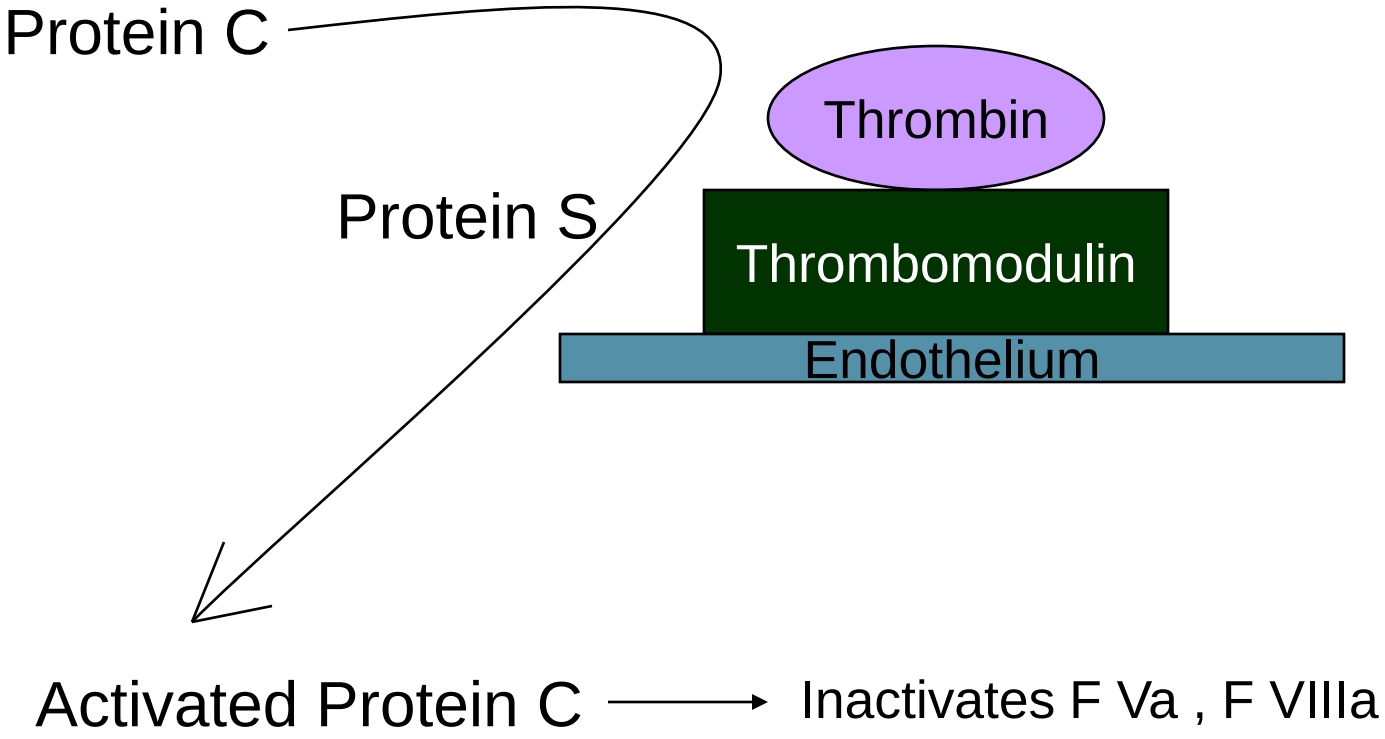
- Dahlback described an inherited (autosomal dominant) disorder associated with venous hypercoagulation (1993).
- This disorder is due to a mutation in Factor V gene on chromosome 1 (the mutated gene is called Factor V Leiden). Mutation at nucleotide 1691: Guanine-> Adenine, causing substitution at position 506: Arginine-> Glutamine

Note: FV HR2 haplotype (A4070G, His199Arg) has unknown risk

INTRODUCTION (cont'd)

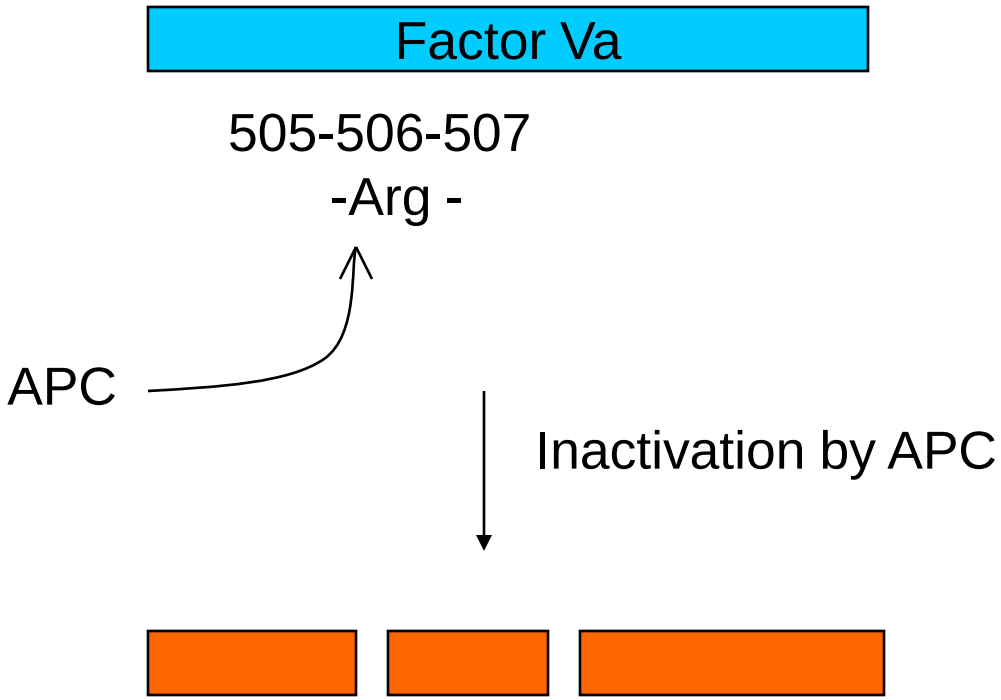
- Review of Protein C pathway: down-regulation of coagulation with activated protein C complex (APC)

Protein C Pathway



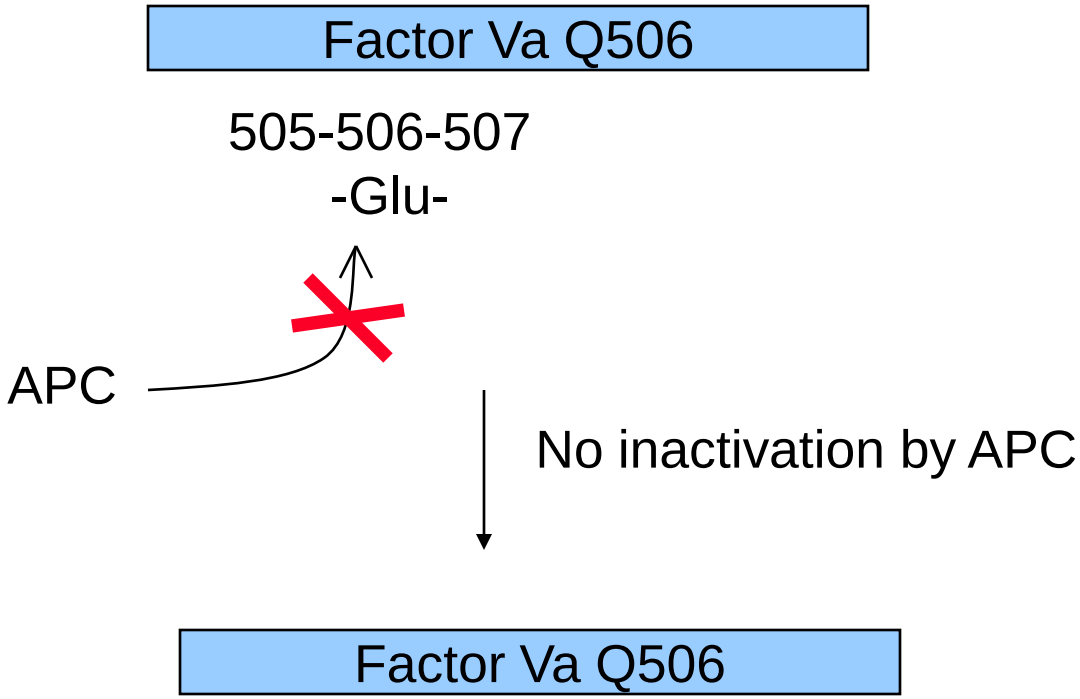
Cleavage Site on Factor V by APC:

Inactivation of Factor V in normal patient



Cleavage Site on Factor V by APC:

No inactivation of Factor V in patient with Factor V Leiden (95% of APC resistance cases)



Prevalence of Inherited Disorders in Hypercoagulation

- Factor V Leiden (12-40% of hypercoagulation cases), V
- Prothrombin gene mutation (6-18%), V
- Protein C deficiency (6-10%), V
- Protein S deficiency (5-10%), V
- Antithrombin III (AT III) deficiency (5-10%), V

- ***Legends: A (arterial thrombosis), V (venous thrombosis)
- Hypercoagulation incidence: 1/1,000/year

Prevalence of Inherited Disorders in Hypercoagulation (cont'd)

- Lupus anticoagulant (LA) (10-20%), A+V
- Anticardiolipin antibodies (ACA) (5-10%), A+V
- Heparin-induced thrombocytopenia, A+V
- Hyperhomocysteinemia (10-20%) secondary to Methylenetetrahydrofolate reductase mutation, A+V

Two Forms of Factor V Leiden

- Heterozygous: 3-7% of general population, 3-5 fold increase in risk of deep vein thrombosis, 20% have thrombosis by 33 y/o (mean age of first thrombotic episode)
- Homozygous: 0.06-0.25% of general population, 50-100 fold increase in risk of deep vein thrombosis, 40% have thrombosis by 33 y/o

Other Relevant Information on Factor V Leiden

- Some patients do not have thrombosis unless exposed to hemostatic challenge
- Increased risk for hypercoagulation in combination with other risk factors (such as Protein C or S deficiency)
- Factor V procoagulant activity is normal
- Treatment: heparin, coumadin

Testing for Factor V Leiden

- Clot-based testing (blue top tube)
- Polymerase chain reaction (PCR) testing (purple top tube)

Clot-based Testing

- Determines the resistance to APC, using platelet-poor plasma
- Principle of test: in patient with APC resistance, Factor V is not inactivated by APC, hence (PTT with APC) is not prolonged. This will shorten the APC Ratio (APCR)

Clot-based Testing (cont'd)

- APCR = (PTT with APC)/(PTT without APC)

APCR > 2 -> negative for APC resistance
APCR < 2 -> positive for APC resistance

- Considerable overlap between FV Leiden heterozygous and normal

Note: cut-off value is dependent on particular test kits

Clot-based Testing (cont'd)

- Inaccurate result with:intrinsic factor deficiency, lupus anticoagulant, anticoagulant (need to get pre-treatment sample)
- New generation test (COATEST by Chromogenix)
  1. Predilution of patient sample with FV deficient plasma before testing: alleviates coumadin interference
  2. Polybrene: alleviates heparin intereference

PCR Testing

- Amplifies the mutated gene fragment. Results: negative, heterozygous, homozygous.
- Results not effected by factor deficiency, lupus anticoagulant, anticoagulant
- PCR testing cannot detect APC resistance not due to FV Leiden

PCR Testing (cont'd)

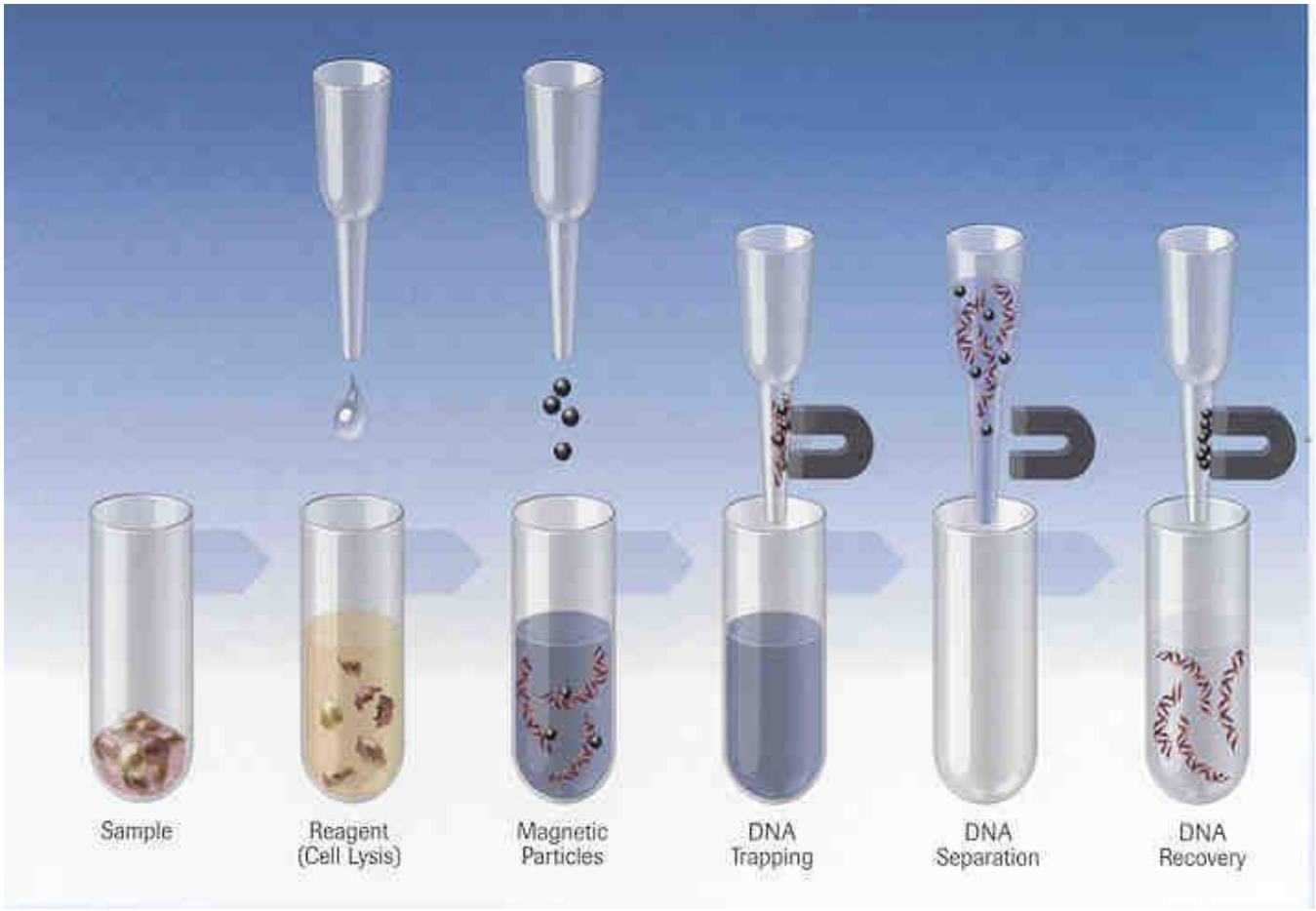
- Genomic DNA from lymphocytes
- DNA sequence flanking the mutation site is amplified by PCR, resultant product is analyzed by restriction enzyme digestion
- Normal (wild type): two normal FV alleles
  Heterozygous: one abnormal allelle
  Homozygous: two abnormal allelles

New PCR Testing: LightCycler (Roche)

- Melting curve analysis method
- “Real time” analysis
- 35 thermal cycles in 25 min, followed by melting curve analysis in 5 min -> results in 30 min
- Batch of 32 samples
- Designed for clinical lab setting
- Optional module for automated DNA extraction (60 min for 32 sample extraction)

MagNA Pure LC and LightCycler

Magnetic Bead Technology for DNA Extraction



LightCycler Schematics

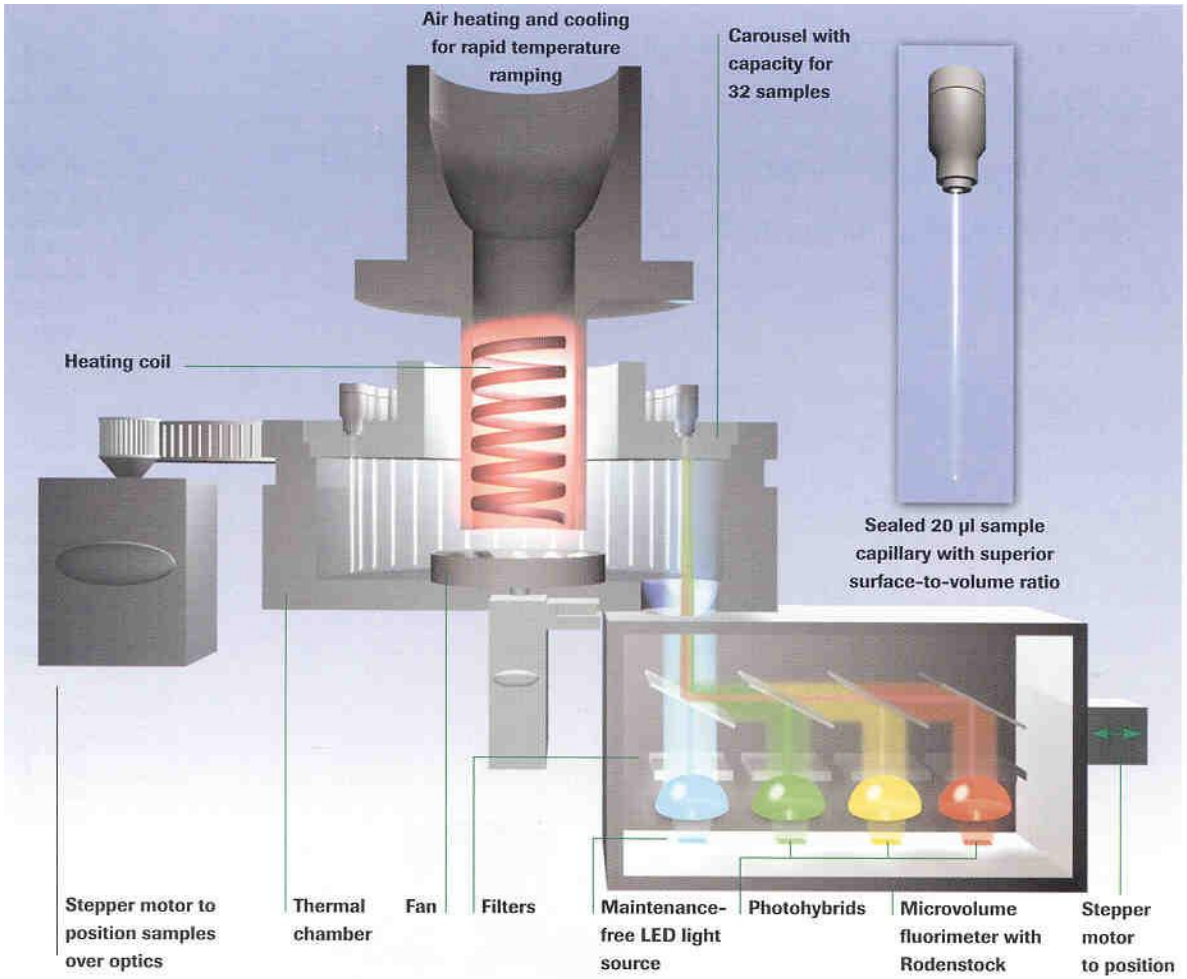
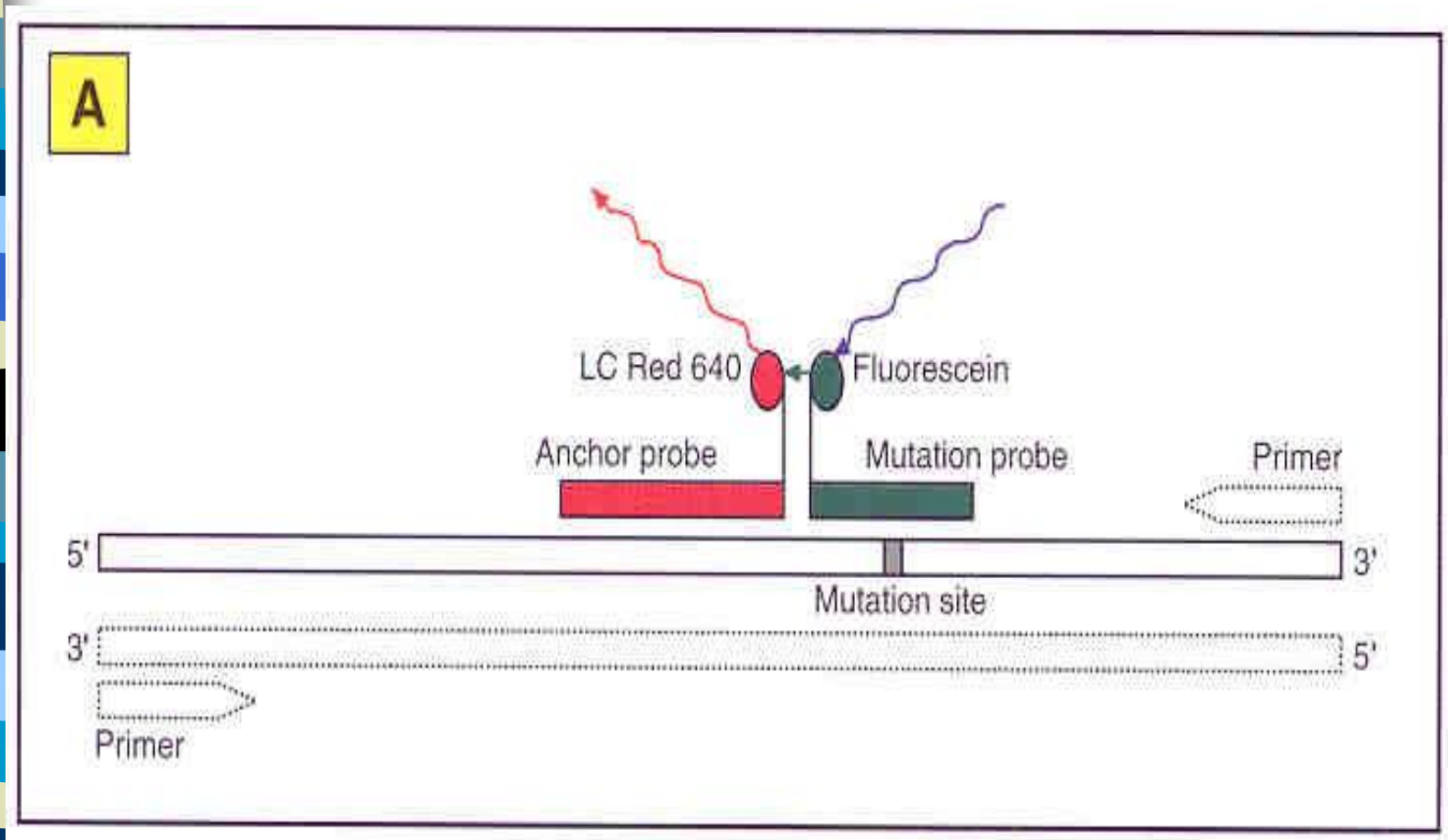


Figure 1. Schematic of the LightCycler System.

FV Leiden Mutation: Hybridization Probe with Fluoresence Resonance Energy Transfer (FRET)



FV Leiden Mutation: Melting Curve Analysis