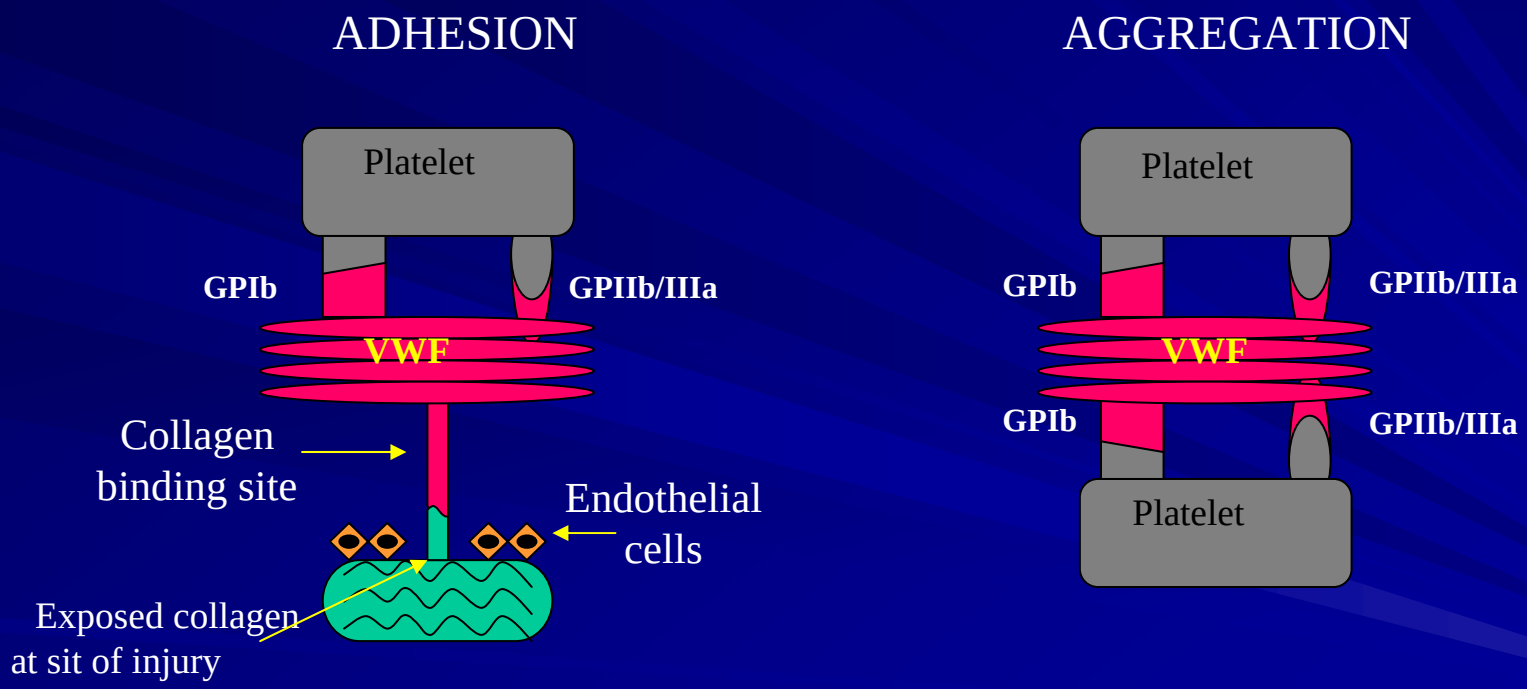
Thrombophilia

Dr. Adel M. Abuzenadah

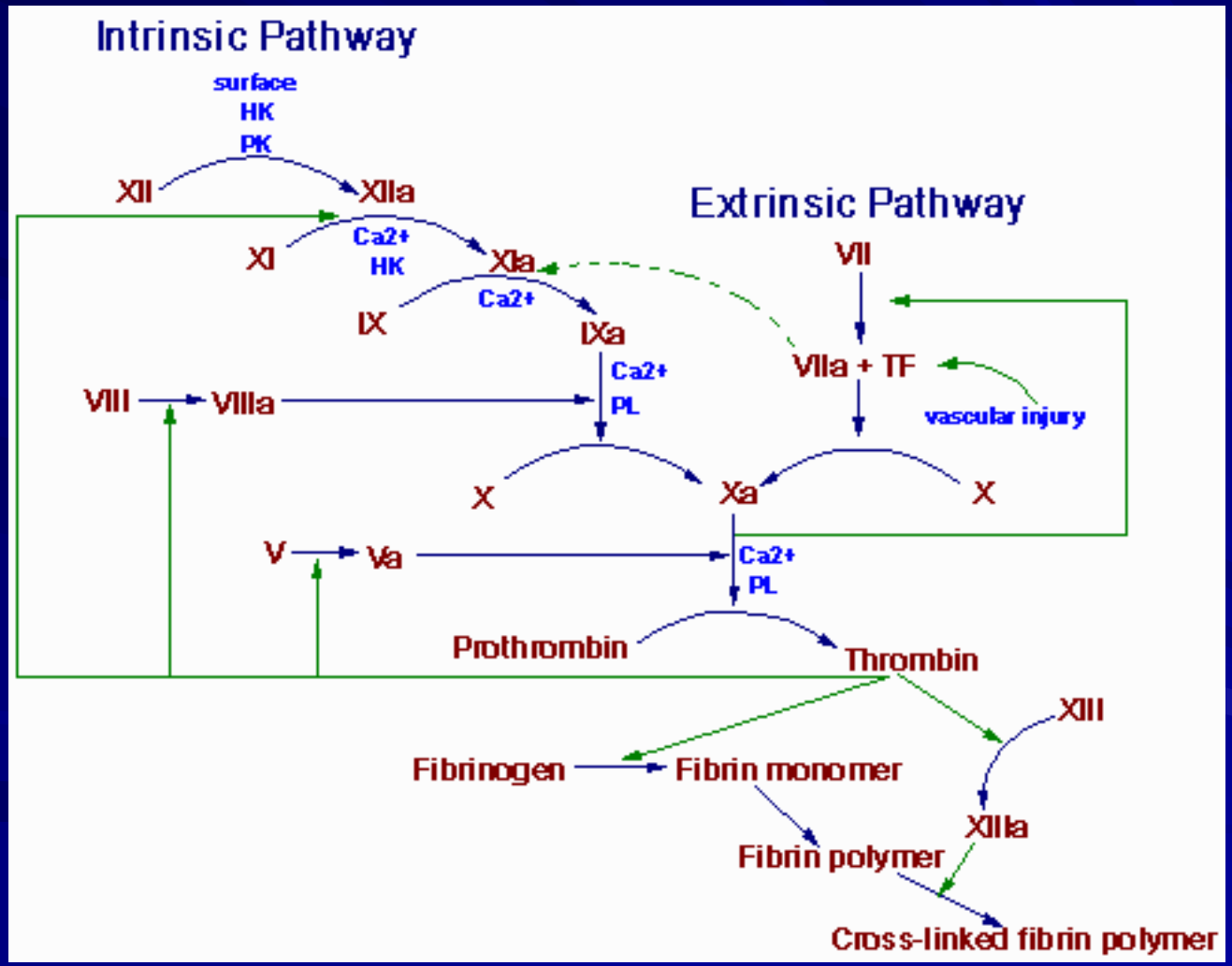
Function of Hemostasis

- Stop bleeding.
  - Primary and secondary hemostasis.
- Maintain blood in fluid state.
  - Control mechanisms of coagulation.
- Removal of clot after healing of injury.
  - Fibrinolytic system.

Primary Hemostasis



Coagulation Cascade



Control mechanism of coagulation

- Naturally occuring inhibitor (TFPI)
- Serien protease inhibitors
  - Antithrombin
  - Heparin and heparin cofactor
- Protein C system

Definition of Thrombophilia

British committee for standard in hematology 1999: Disorders of the hemostatic mechanisms which are likely to predispose to thrombosis.

In North America:
Clinicians use it to describe patients who have developed venous thrombosis either spontanously or due to recognized stimulus, patients who have recurrent venous thrombotic events and patients who develop venous thrombosis at early life.

Why Perform Thrombophilia Testing?

- To provide knowledge of the pathologic basis of the thrombosis and provide the opportunity to communicate etiiologic factors to patients.
- To influence duration and intensity of therapy during a thrombotic episode.
- To offer thrombosis prophylaxis for high risk patients during periods of potential increased thrombosis stimulus.
- To alert patient's kindred to the presence of inherited risk factors.
- To determine the need for alternative laboratory testing when condition affects primary testing mode.

Reasons to Order Laboratory Workup for Thrombophilia

- Venous thrombosis before 40-50 years age.
- Unprovoked thrombosis at any age.
- Recurrent thrombosis at any age.
- Thrombosis at unusual sites.
- Positive family history of thrombosis.
- Thrombosis secondary to pregnancy, oral contraceptives, or hormone replacement therapy.
- Unexplained abnormal laboratory test such as prolonged PTT.

Heritable Thrombophilia

Limited number of genetic variants are proven to be independent risk factors for venous thrombosis:

- Mutations in the genes encoding the natural anticoagulants:
  - Antithrombin
  - Protein C
  - Protein S
- Mutations in the genes encoding the clotting factors:
  - Fibrinogen
  - Prothrombin
  - Factor V
- Mutation in the MTHFR enzyme causing hyperhomocysteinemia

Antithrombin

- The most important physiologic inhibitor of thrombin.
- Its action is accelerated at least 2000-fold in the presence of heparin.
- Deficiency is associated with 8-fold increased risk of thrombosis.
- 1 in 2-5000 in normal population
- 0.5-1% of thrombotic patients
- Gene on long arm of chromosome 1.
- Several mutations have been reported some of them in independent reports in different families.
- Functional assay to determine antithrombin activity
- Ag assay when activity is consistently low
- Mutation detection is not applicable for routine testing.

Proteins C and S

Protein C

↓ Thrombin-thrombomodulin complex

APC

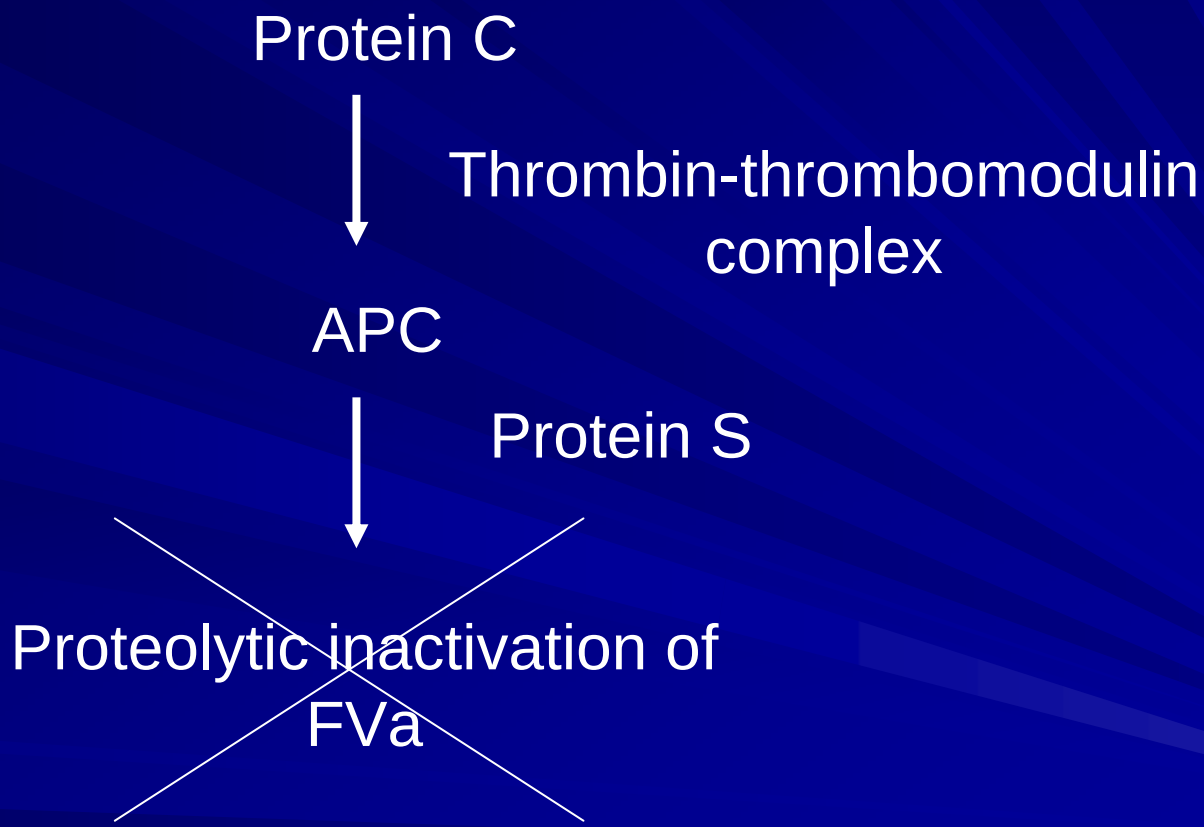
↓ Protein S

Proteolytically inactivates
Factors Va and VIIIa

Proteins C and S

- Proteins C and S deficiencies associated with 8-fold increased risk of thrombosis.
- Genes are located on chromosome 2 and 3.
- Wide variety of mutations were reported in both genes causing deficiencies of the proteins.
- Activity of both proteins are evaluated by functional assays.
- Protein C Ag and free and total protien S Ag should be measured if activity is consistently low.
- Mutation detection is not applicable for routine testing.

Activated Protein C Resistance (APCR)



APCR

- The most frequent laboratory abnormality in patients with history of thrombosis.
- FV Leiden mutation (R506Q)
  - Heterozygous 2-8 fold increased risk of thrombosis
  - Homozygous 80-100 fold increased risk of thrombosis
  - Oral contraceptive and heterozygosity increase thrombotic risk in female 30-50 fold
  - Heterozygosity in pregnancy also increase risk of thrombosis
  - Present in 5% normal Caucasians
  - Explains 20-40% thrombotic Caucasians
- Other mutations in FV (e.g. FV Cambridge and FV Hong Kong in codon 306.

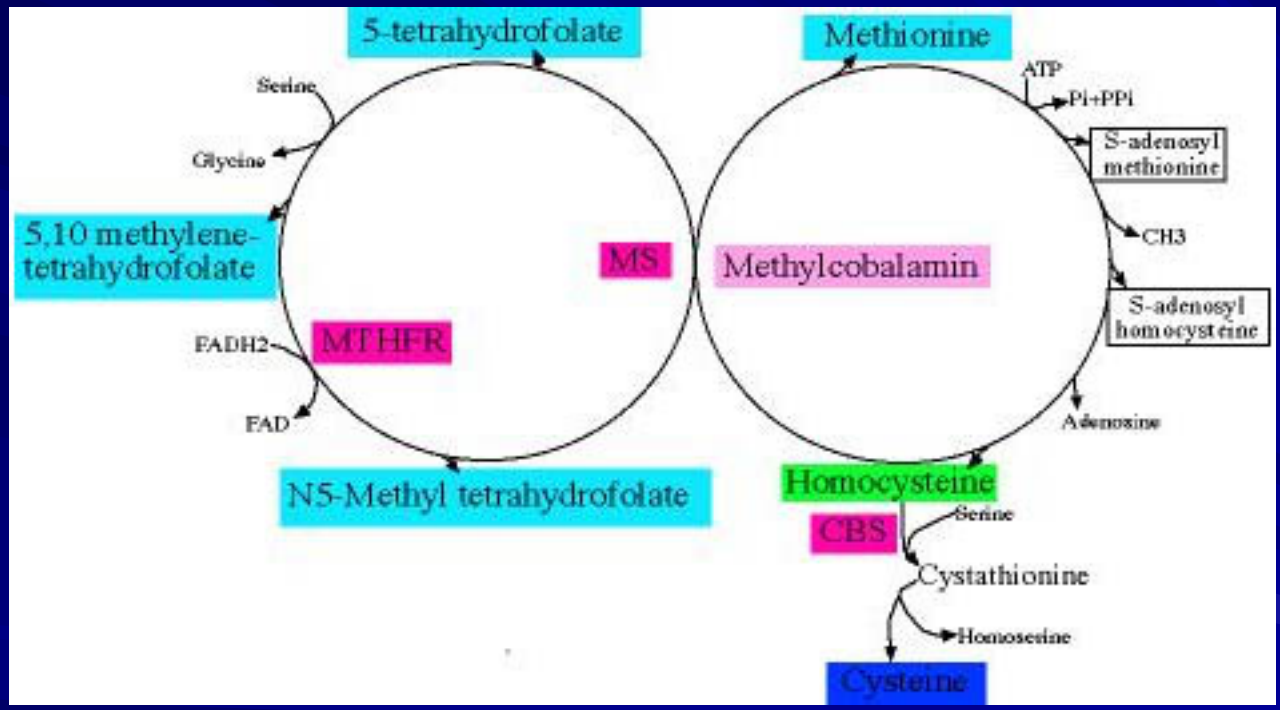
Prothrombin Mutation G20210A

- Reported by Bertina group 1996
- Heterozygous 2-4 fold increase risk of thrombosis
- 2% in normal population
- 6-7% unselected patients with DVT
- 18% in selected patients with history of venous thrombosis
- Coinheritance occur with FV Leiden in 1% of thrombophilia patients. This result in thrombotic event at very young age (20-25 years of age).
- No functional assay to detect the phenotypic varient
- DNA-based procedure is required.

Hyperhomocysteinemia

- Increased blood-level of homocystein was reported as risk factor for arterial and venous thrombosis.
- 2-fold increase risk of thrombosis.
- 4-fold increase risk of thrombosis in conjunction with oral contraseptive.
- Mutations in enzymes responsible of homocystein metabolic pathway (MTHFR).
- C677T in the MTHFR gene.
- Heterozygous in 40% and homozygous in 10% normal Caucasian population.

Metabolism of homocystein



Summary of Heritable Thrombophilia

Factor	General Population	People With Thrombosis
APCR	3-8% Caucasians	20-40%
Prothrombin G20210A	2-3% Caucasians	4-8%
Antithrombin def.	1 in 2-5000	1%
Protein C def.	1 in 300	2-10%
Protein S def.	1%	3%
Hyperhomocyst.	11%	-

Recommended Molecular Testing in Thrombophilia Investigation

- FV Leiden Mutation
- Prothrombin G20210A mutation
- MTHFR C677T mutation ???

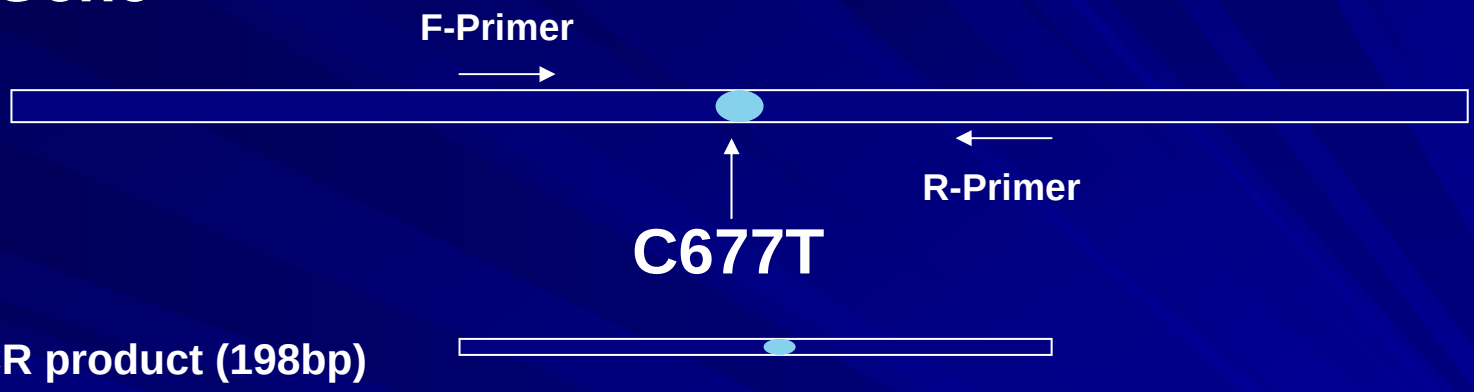
Are these applicable in our population?

PCR Amplification for MTHFR

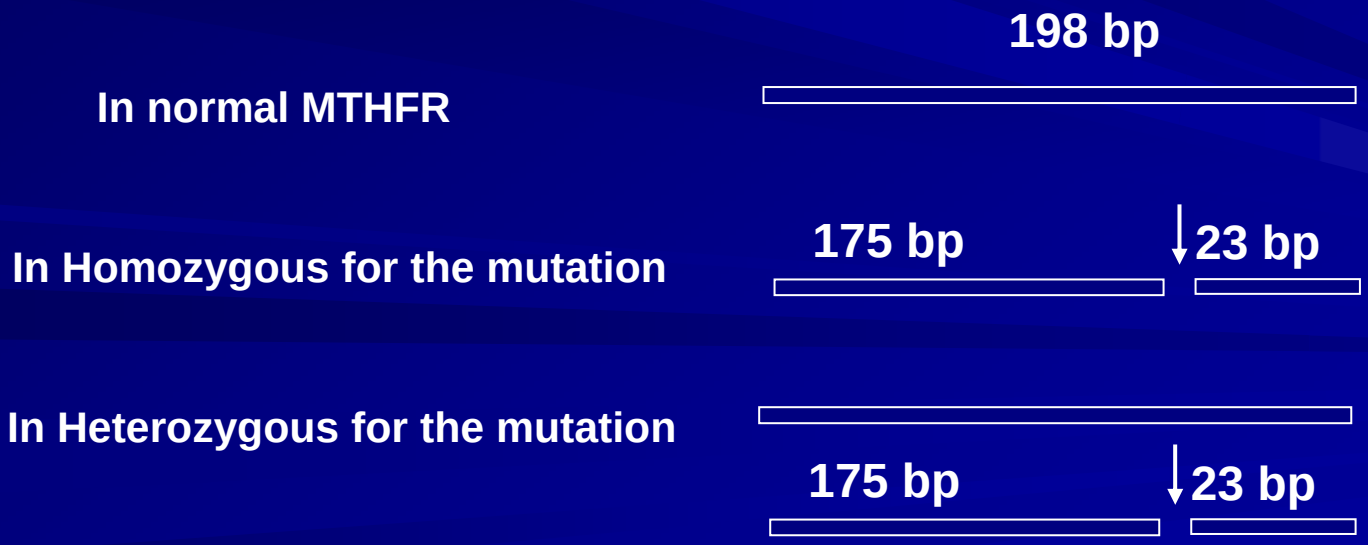
198bp

Method Used for MTHFR Mutation Detection

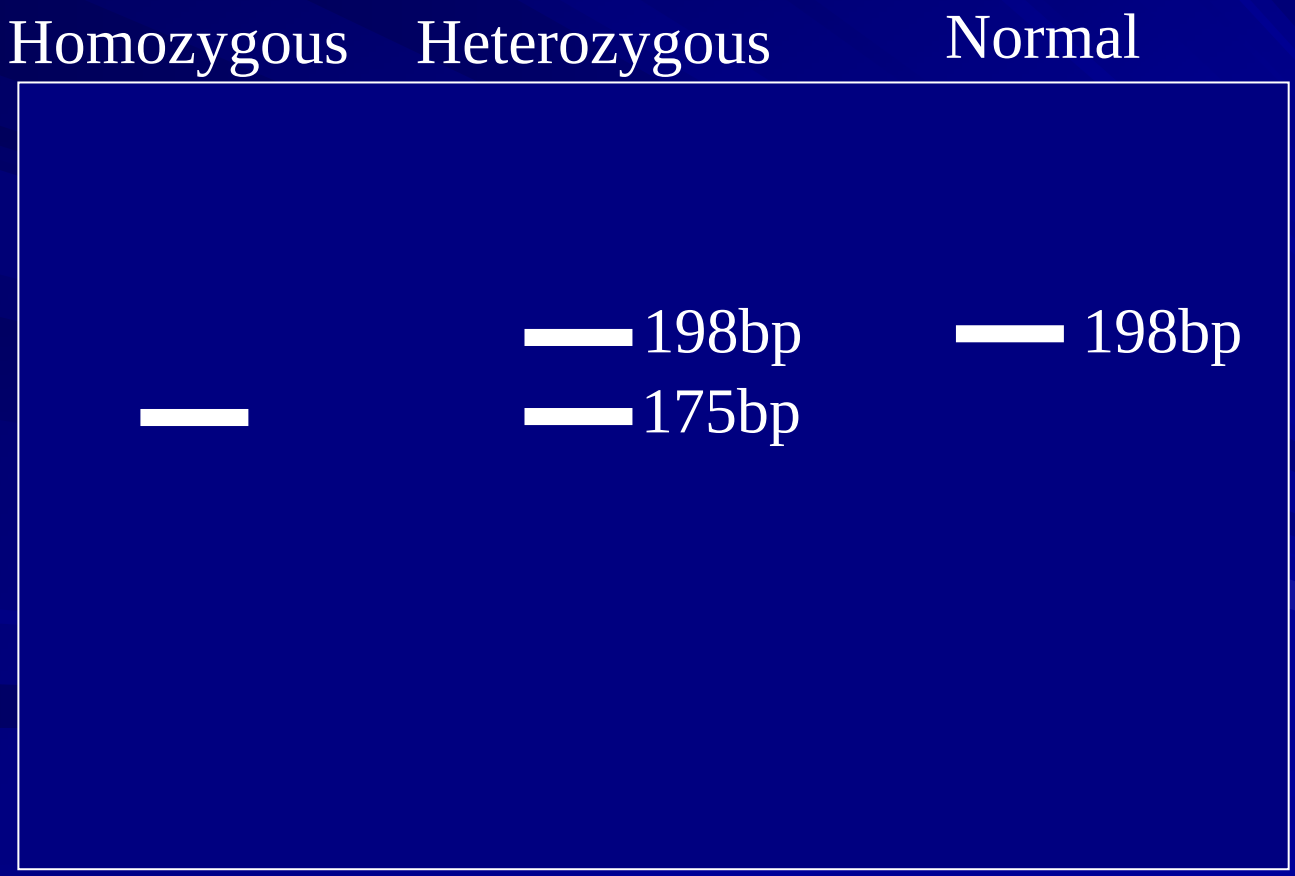
MTHFR Gene



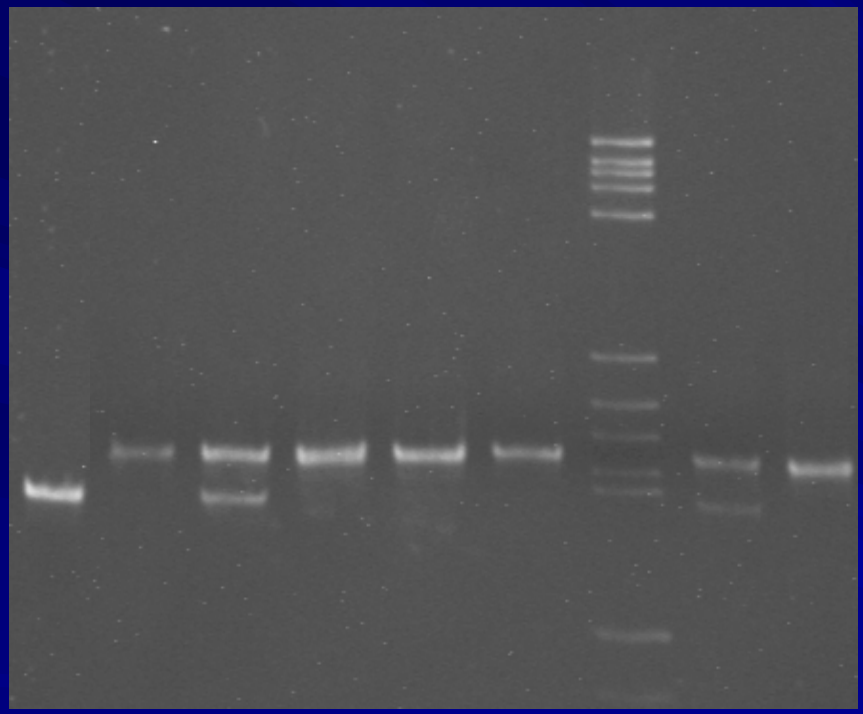
PCR product is digested with *HinfI* restriction enzyme



Diagrammatic representation *HinfI* Analysis



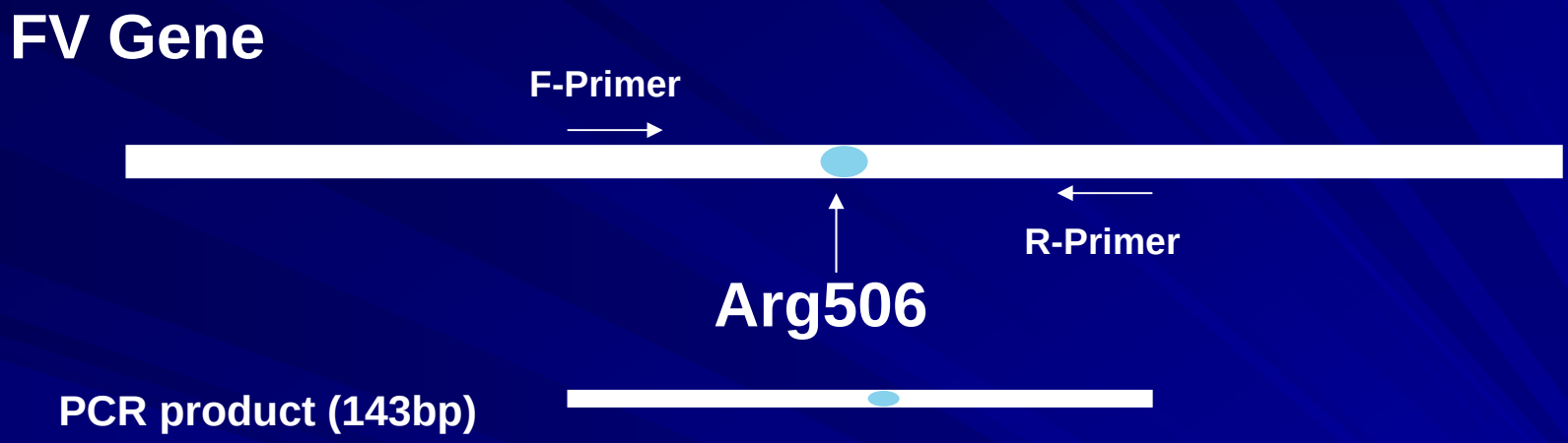
Restriction Enzyme Analysis for MTHFR Mutation



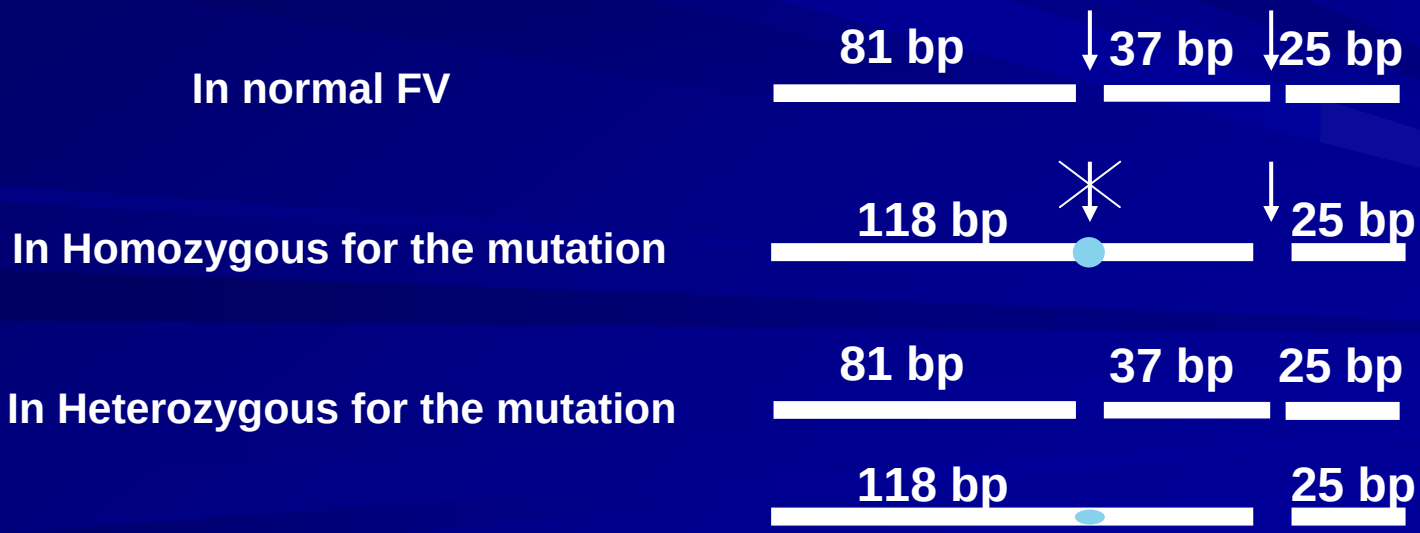
175bp

198bp

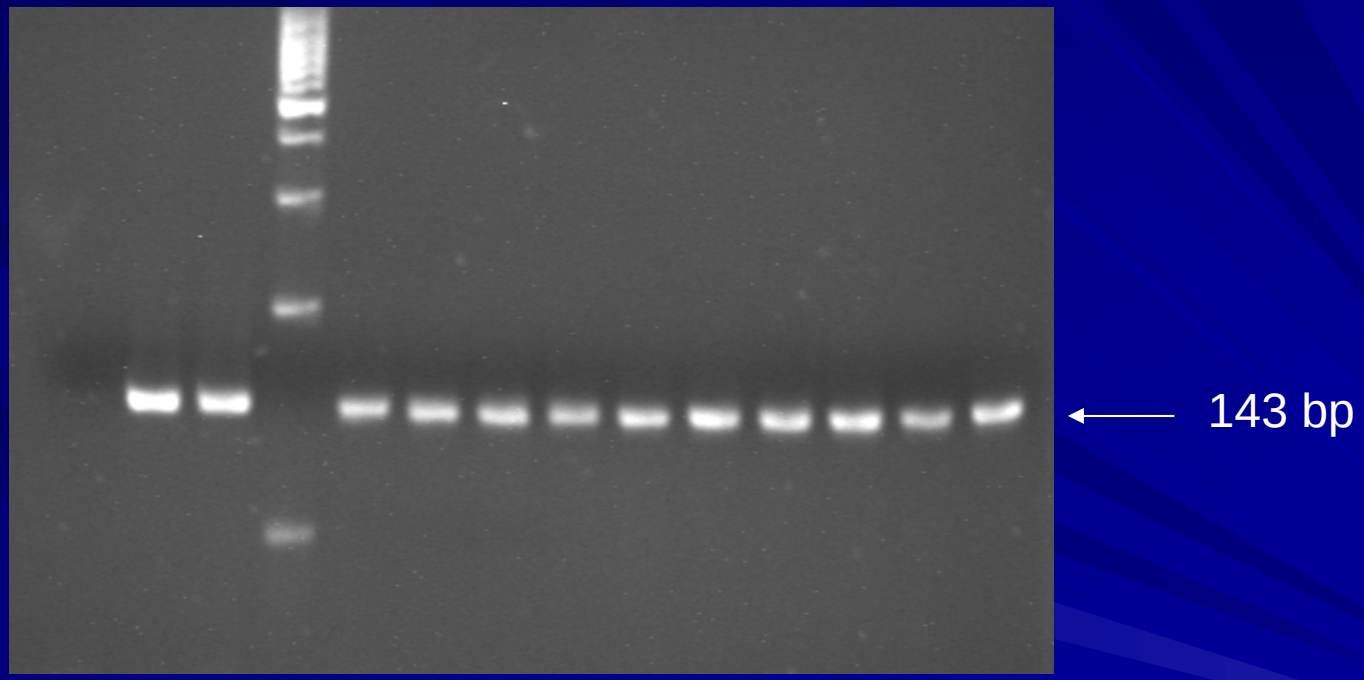
Method Used for FV Leiden Mutation Detection



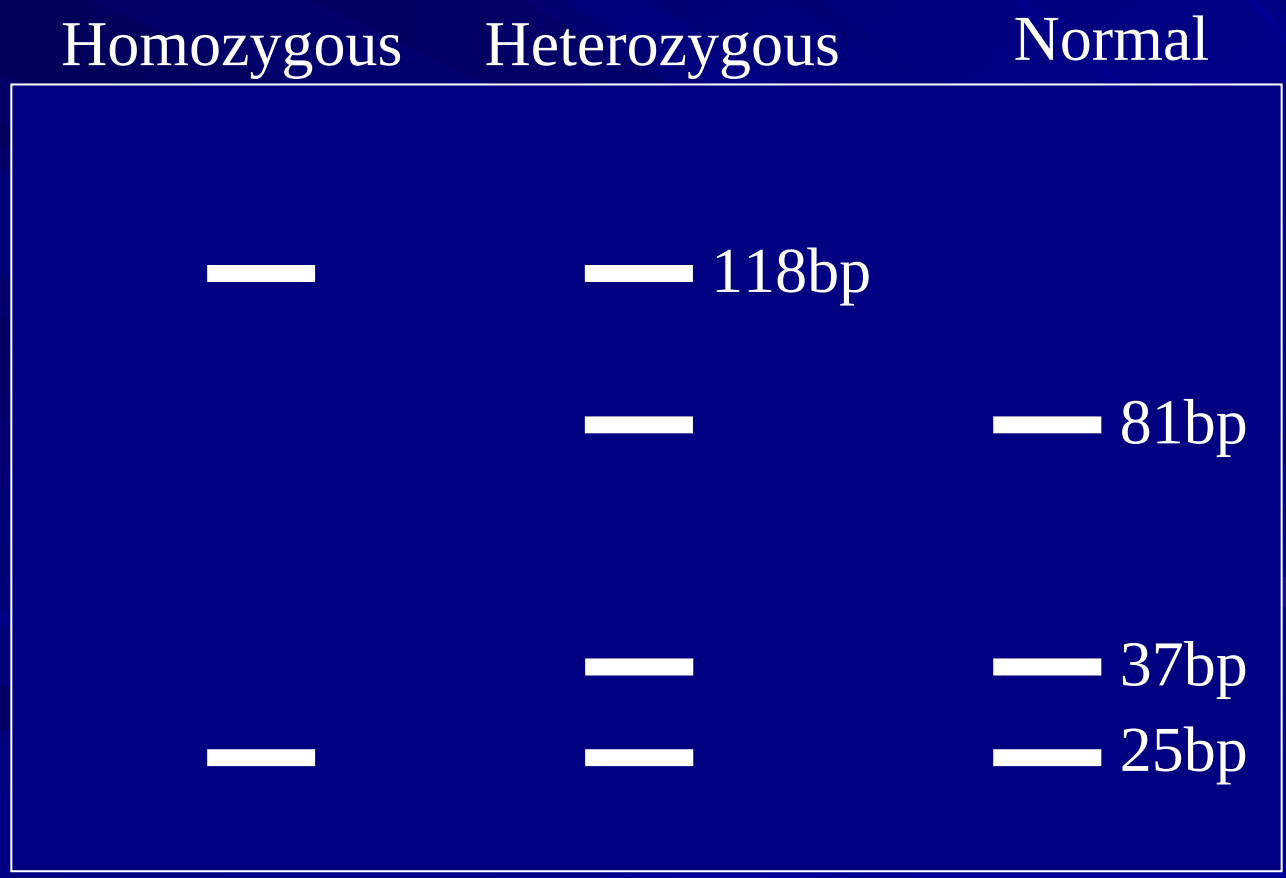
PCR product is digested with *Mnl*I restriction enzyme



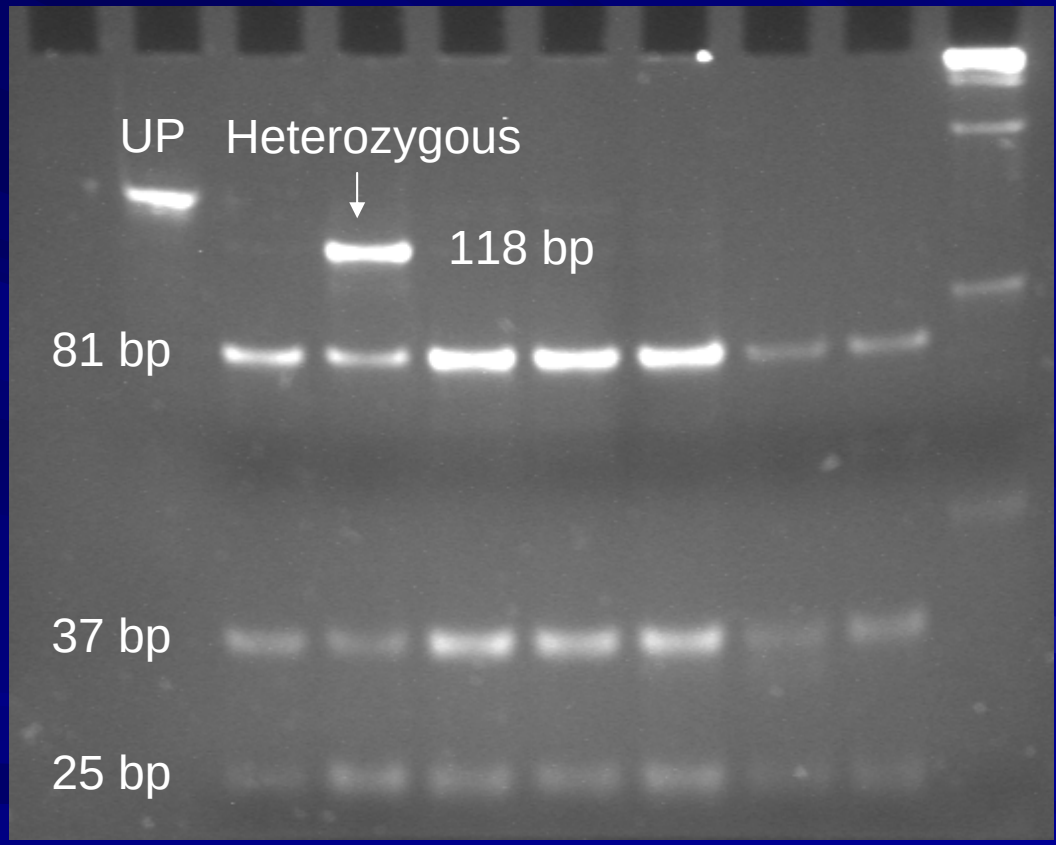
PCR Amplification for FV Leiden



Diagrammatic representation MnlI Analysis



Restriction Enzyme Analysis for FV Leiden Mutation



Thank You