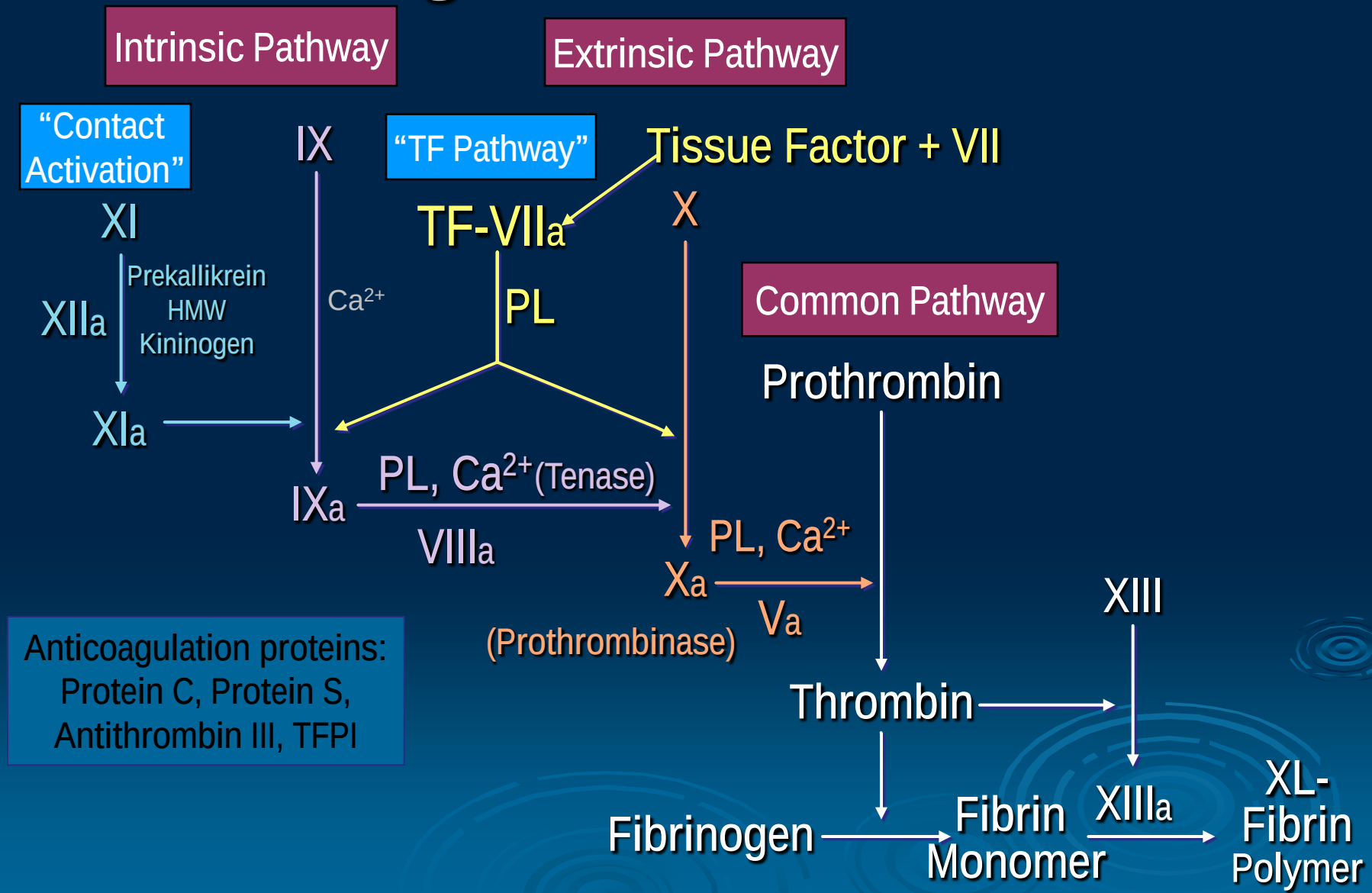
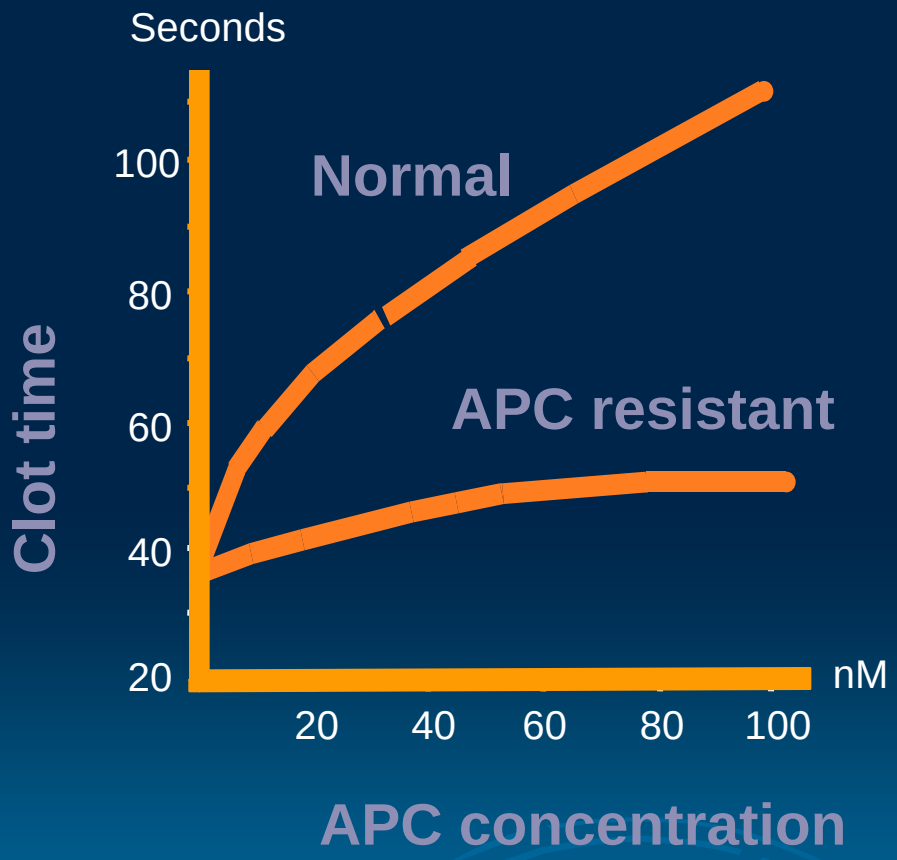
Coagulation Cascade



APC Resistance

- Common in the general population
- Most common cause of hereditary thrombophilia
- Can be hereditary or acquired
- APC Resistance alone is not a significant risk factor. Having APC Resistance combined with other risk factors, however, greatly increases risk of thrombosis

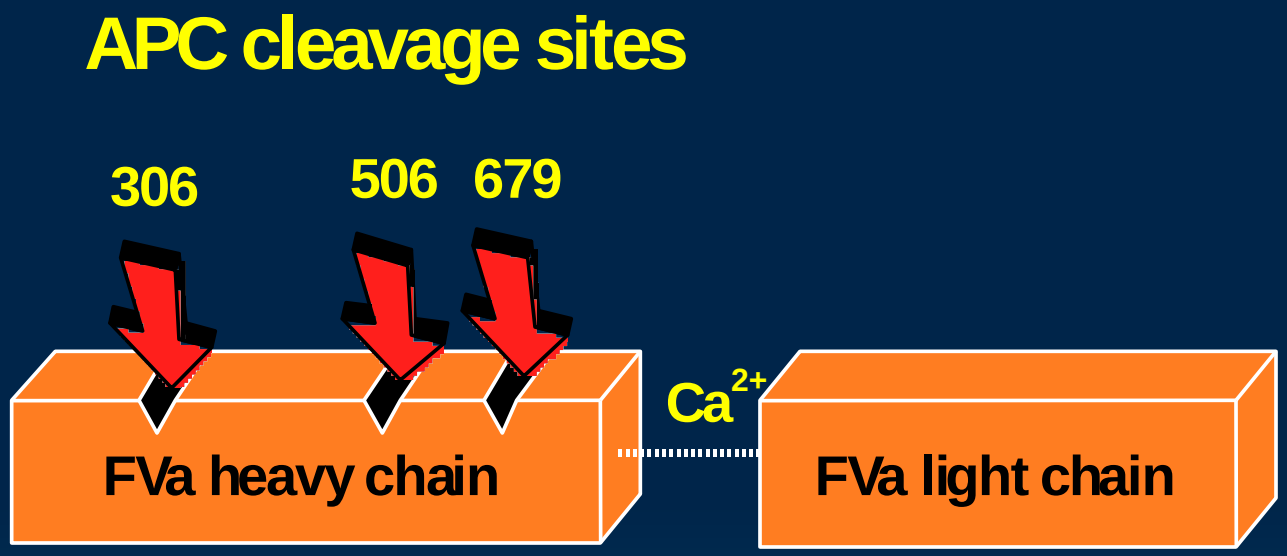
ANTICOAGULANT RESPONSE TO APC



APC resistance phenotype

- A poor anticoagulant response to activated protein C (APC).
- In an APC R patient, there is not as much inactivation of coagulation

INACTIVATION OF NORMAL FVa



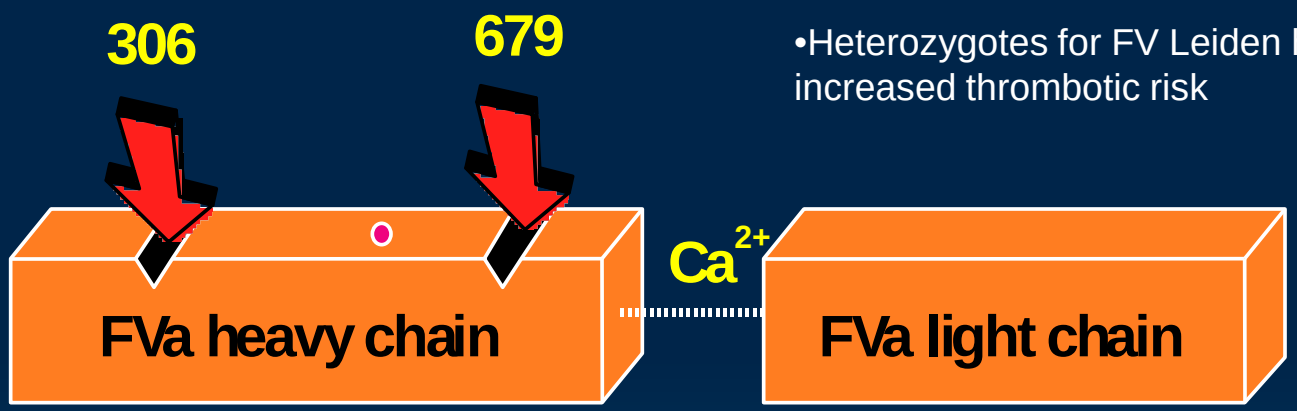
Normal

- APC cleaves sites on the heavy chain, inactivating FVa and helping to prevent too much thrombin activation.
- Cleaves at the 306, 679 and **506** positions.

INACTIVATION OF MUTANT FVa:Q^{506}

FV Leiden Mutation

- Accounts for approx. 90% of APC Resistance
- Prevalent in about 2 – 13% of general population
- Accounts for about 20 – 60% of VTE cases
- Heterozygotes for FV Leiden have 2 – 5 fold increased thrombotic risk



- **Mutant**

Arg to Glu Mutation results in a 10-fold lower inactivation rate of FVa

i.e. FVa molecule isn't allowing APC to do its job of inactivating FVa and ultimately inhibiting thrombin generation.

GENETIC AND ACQUIRED RISKS

Genetic risk factors:

APC resistance (FV:Q^{506}, FV Leiden)

Acquired risk factors:

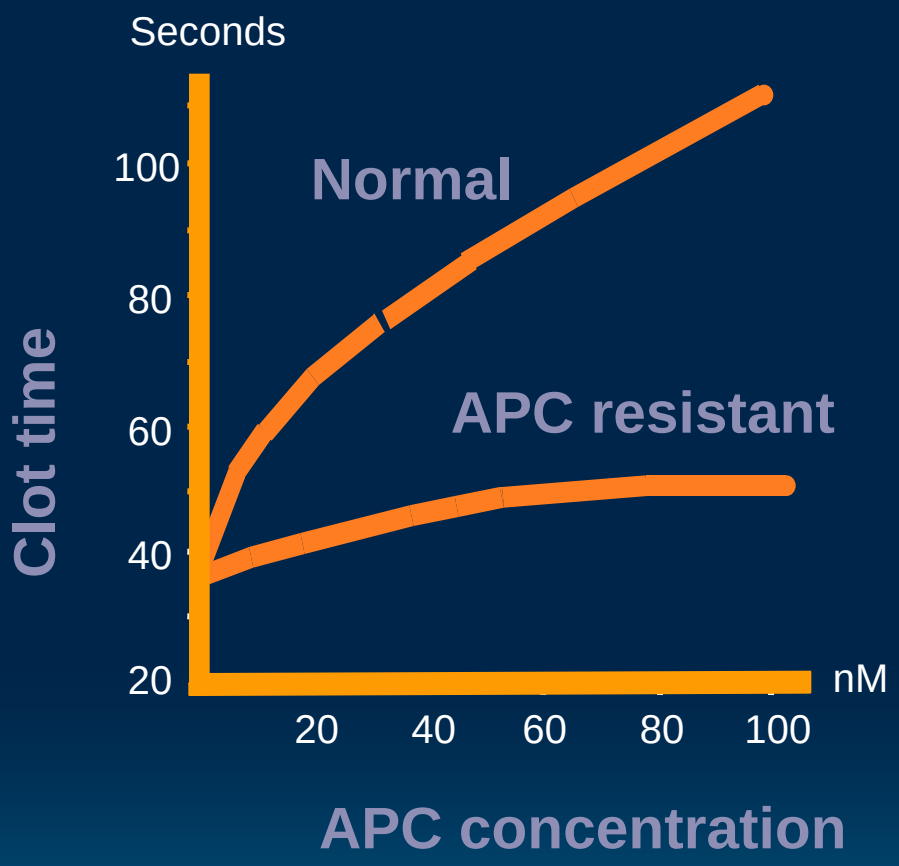
Surgery, Pregnancy and Oral Contraceptive Pills / Patch

Account for about 5 – 10% of APC resistance

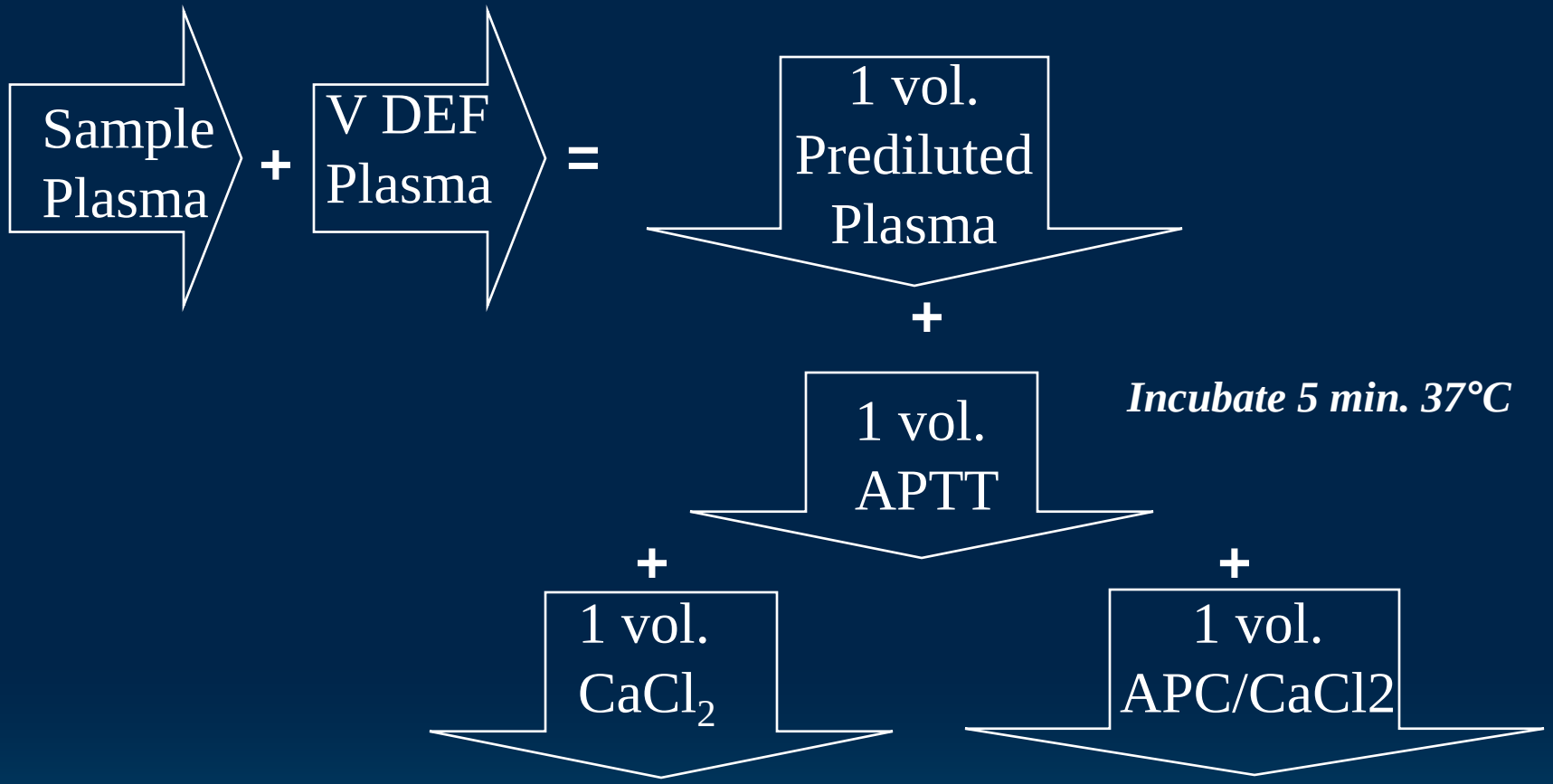
TESTING FOR APC RESISTANCE

- "Gold standard" is an APTT based clotting assay.
- Two APTT tests are run: one with CaCl2 ("Baseline clotting time") and one with an excess of APC and CaCl2 ("Activated clotting time").
- Record the clotting times and calculate the ratio.
- In a normal patient, this excess APC will cause inactivation of FVa at a higher rate, meaning less thrombin generation, prolonged clotting time, and higher ratio between basal and APC clotting times.
- In an abnormal patient, however, even if you add that excess APC, FVa is not being inactivated as much, so you don't see that prolongation of APC clotting time.
- *Therefore, the ratio between basal and APC clotting times is not as high as it would be in a normal patient.*

- By diluting the sample 1:4 in FV-deficient plasma, you test for FV Leiden. This also allows testing of samples containing heparin or warfarin.


Seconds
100
80
60
40
20
Normal
APC resistant
Clot time
nM
20
40
60
80
100
APC concentration

APTT-based APC Resistance Assays



Record time for clot formation

APC RESISTANCE: INTEPRETATION OF RESULTS

- APC- ratio = $\frac{\text{Clot time APC/CaCl}_2}{\text{Clot time CaCl}_2}$

- APC Resistance is indicated when the APC ratio is below or equal to the calculated cut-off value.

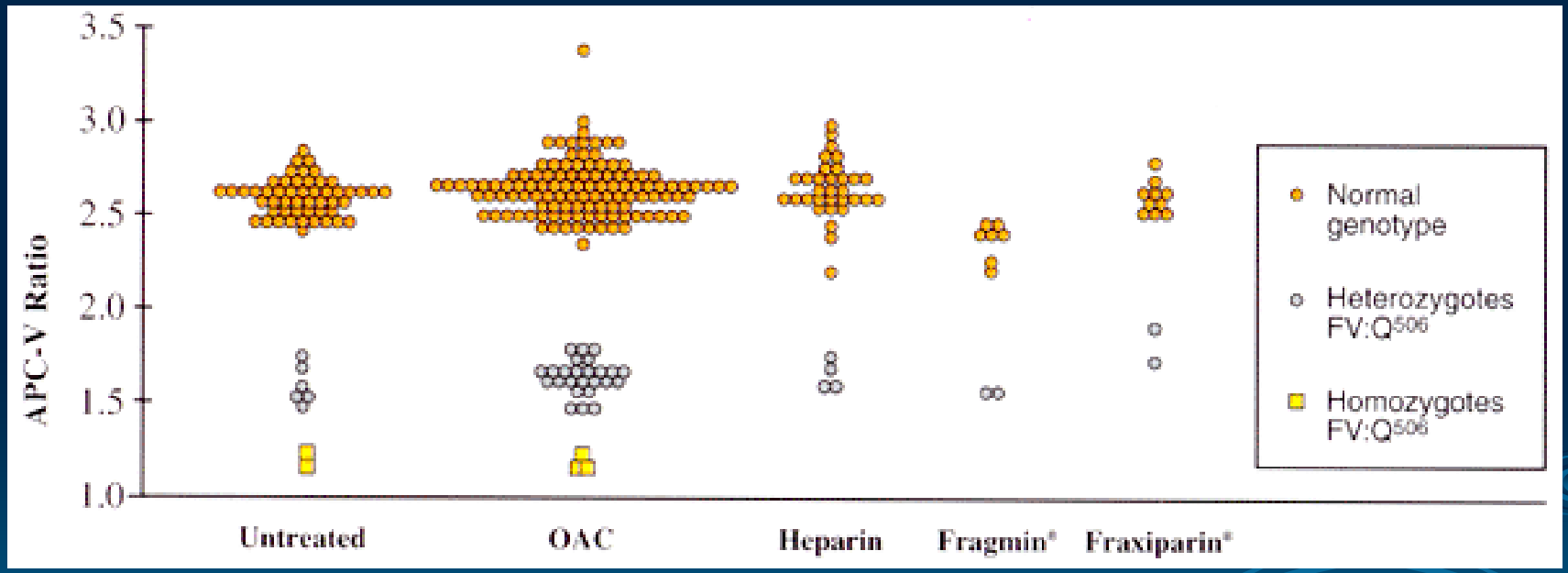
- APC R V ratio below the calculated cut-off is due to presence of the factor V:Q506 mutation

APTT-based APC Resistance Assays

- Benefit:
  - Offers genotypic information for clinical decision-making
- Utility:
  - For factor V:Q^{506} mutation *screening*
  - Ratio at or below cut-off may be confirmed with genetic test
- Features:
  - Unsurpassed sensitivity for the factor V:Q^{506} mutation and close to 100% specificity
  - Applicable to anticoagulant treated patients
  - Economical alternative to genetic testing

APC Resistance

Clear discrimination between normals, heterozygotes, and homozygotes is achieved with the APTT-based screening assay.



COATEST® APC RESISTANCE V

The Gold Standard for APC Resistance Testing!