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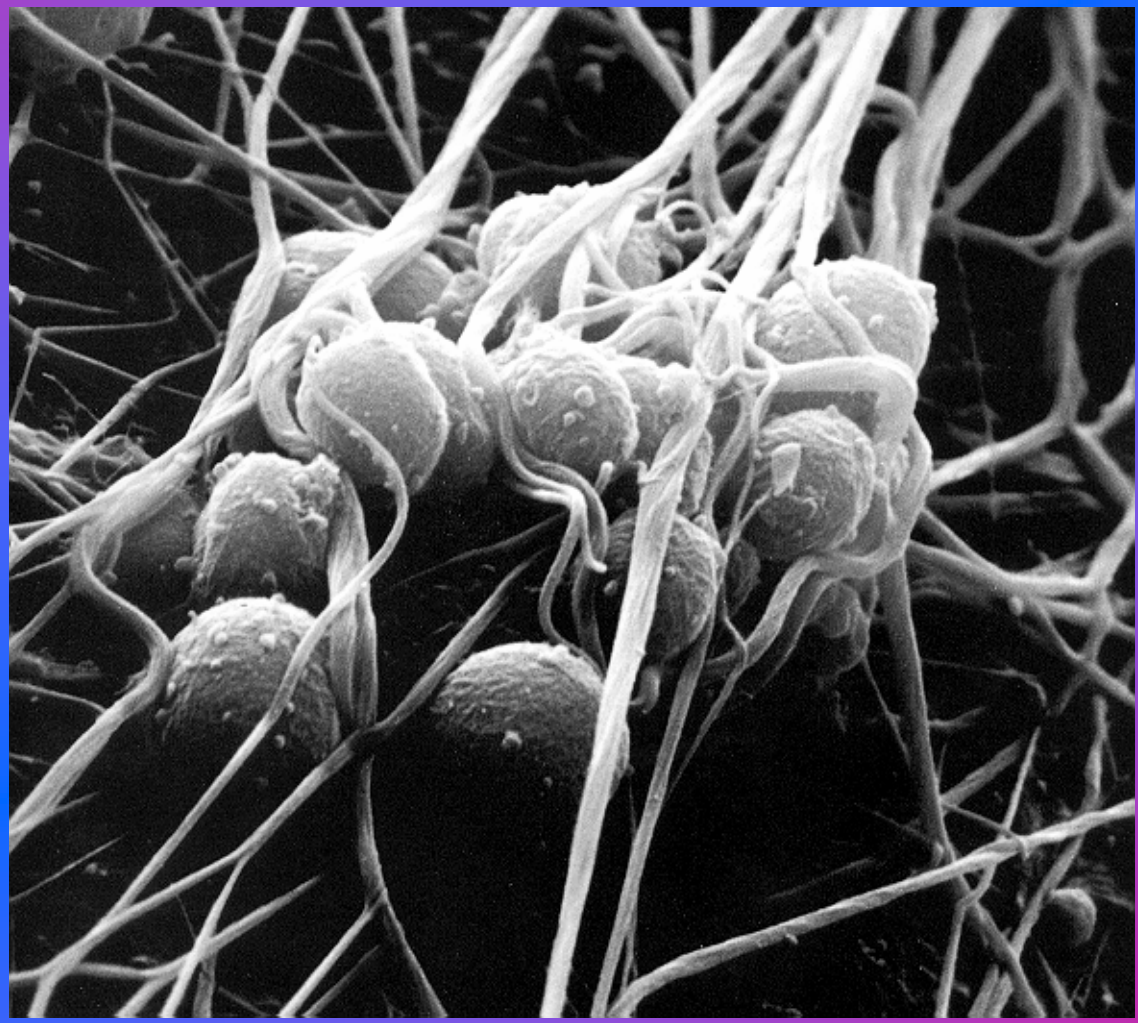
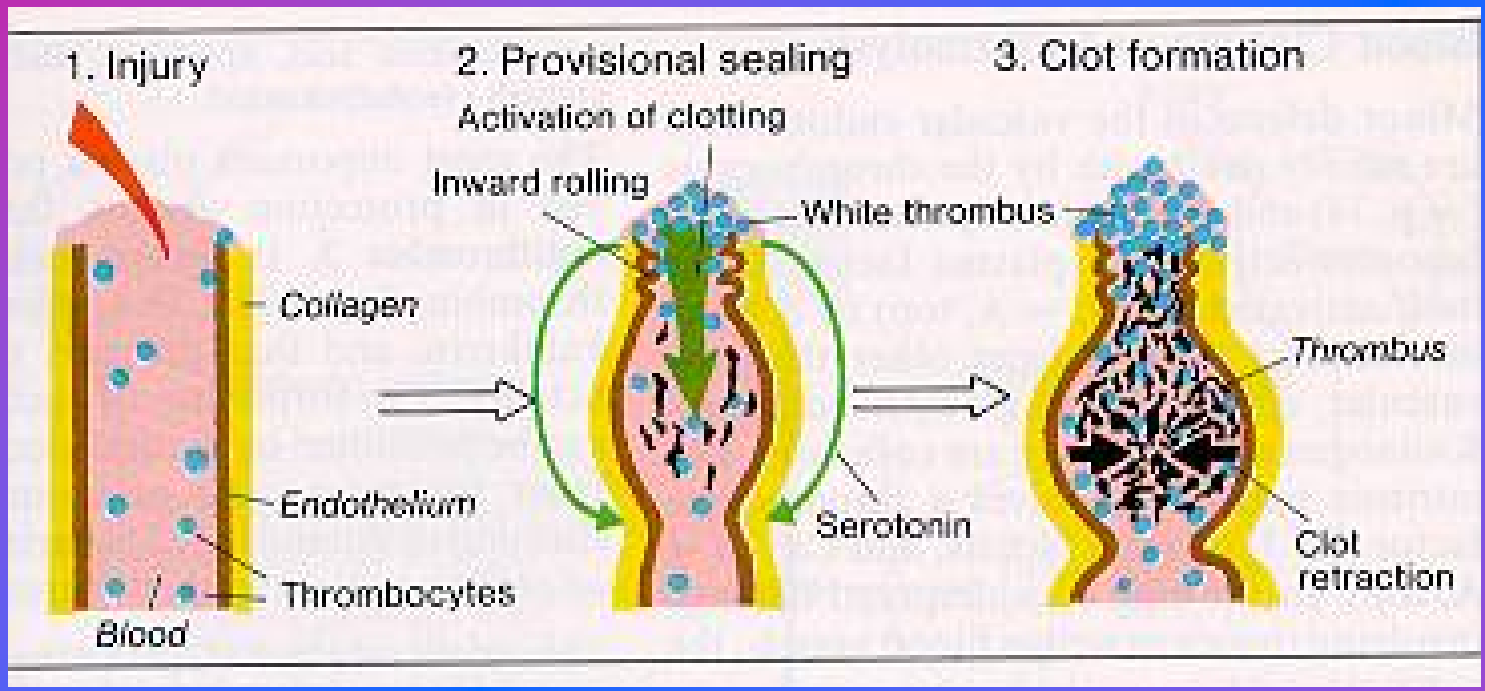
Blood clotting

Platelets emprisoned in a fibrin net.

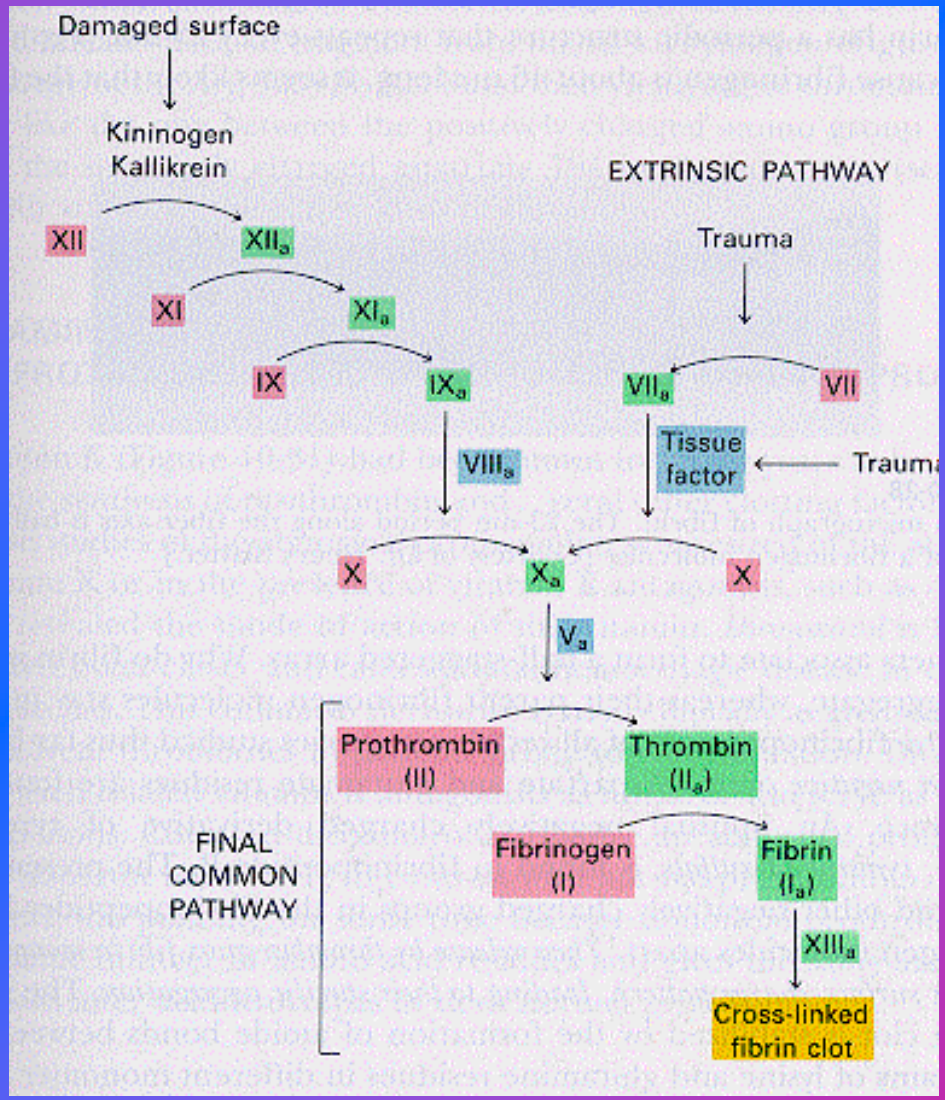


Hemostasis...how it works.



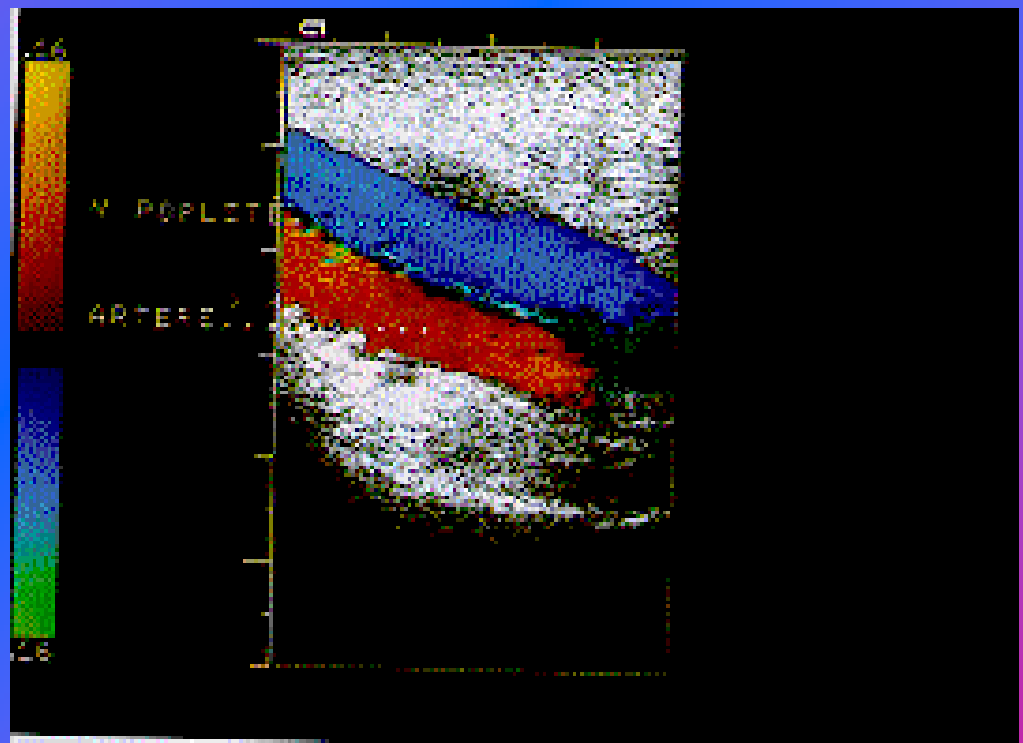
- When the endothelium is defective the blood comes into contact with collagen fibers
- Thrombocytes adhere to the site of injury (adhesion) and activete themselves to secrete substances (serotonin, PDGF, thromboxane A2 and PAF leading to aggregation.
- This thrombocyte plug (white thrombus) leads to a provisional stopping of the leak.

Blood clotting



Visualizing blood easily flowing through a vessel.

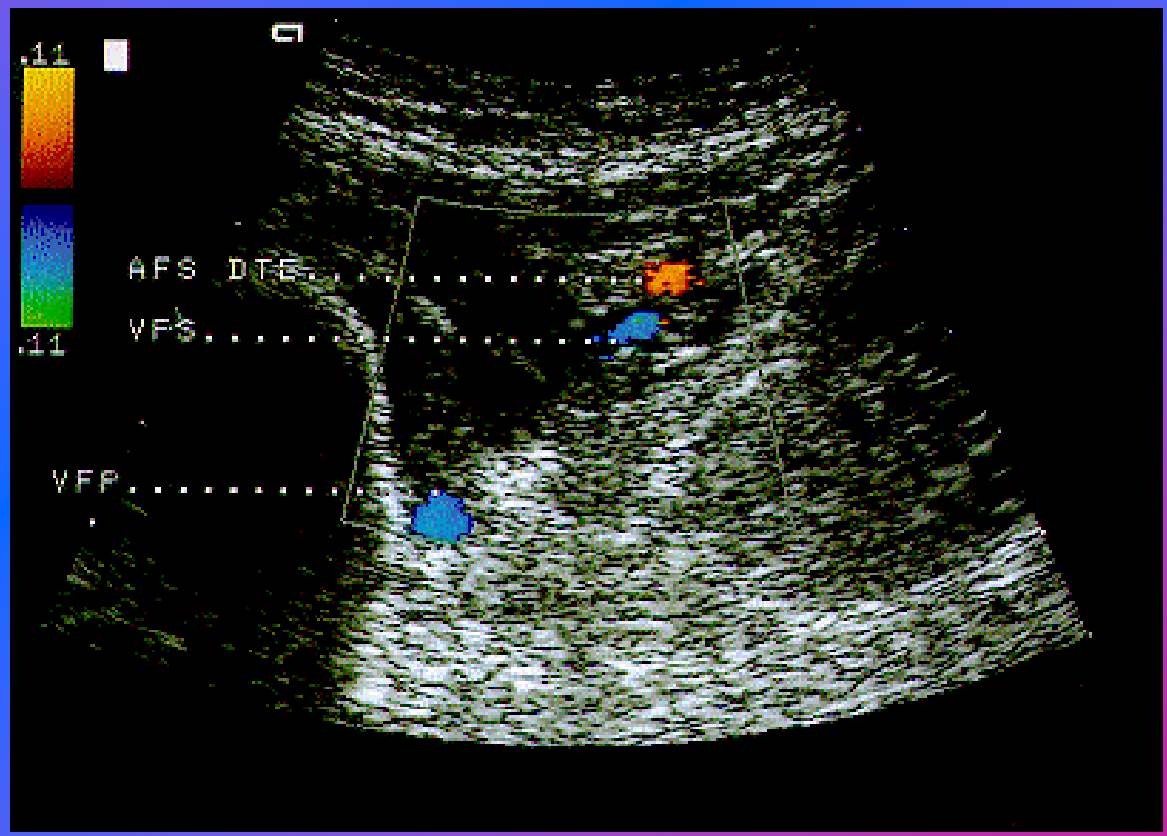
- Doppler analysis of two parallel normal blood vessels



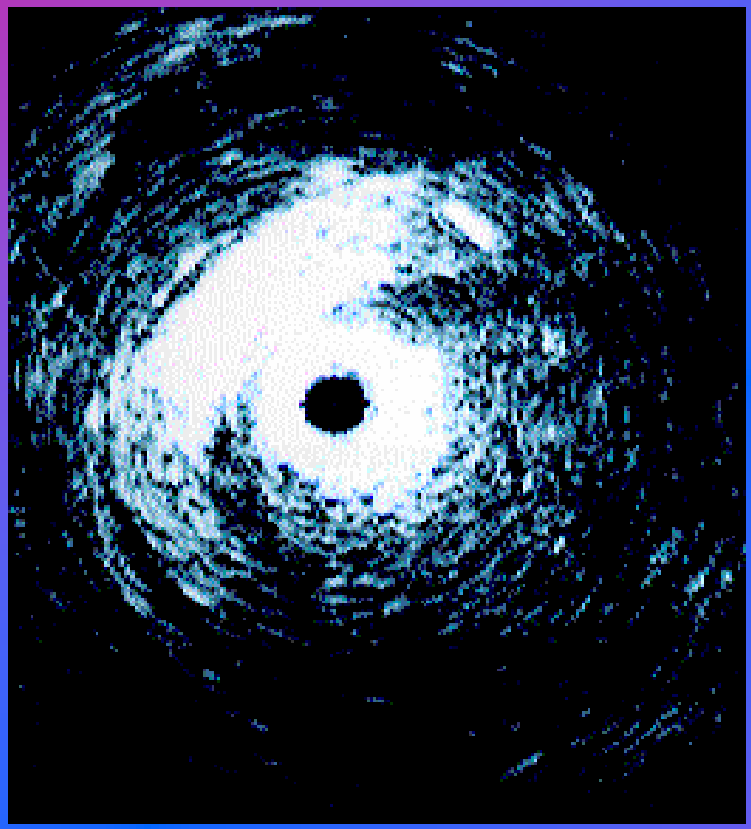
ig. 11 : artère et veine poplitées en coupe longitudinale (écho-Doppler couleur).

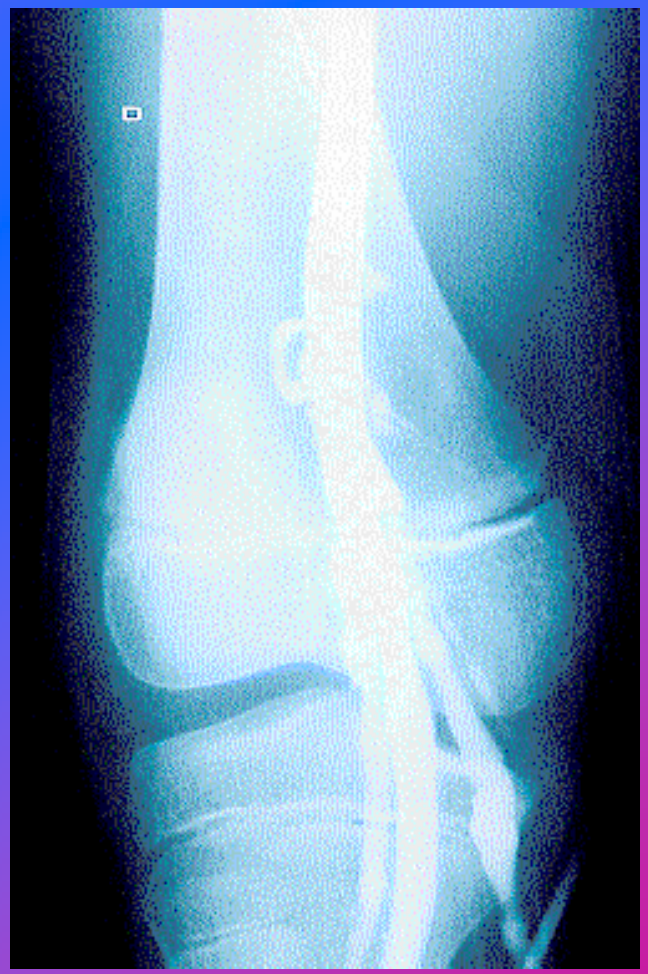
And a transversal view

Transversal cut at 1/3 of the right leg.
VFS:superficial femoral vein
AFS: superficial femoral artery
VFP: deep femoral vein

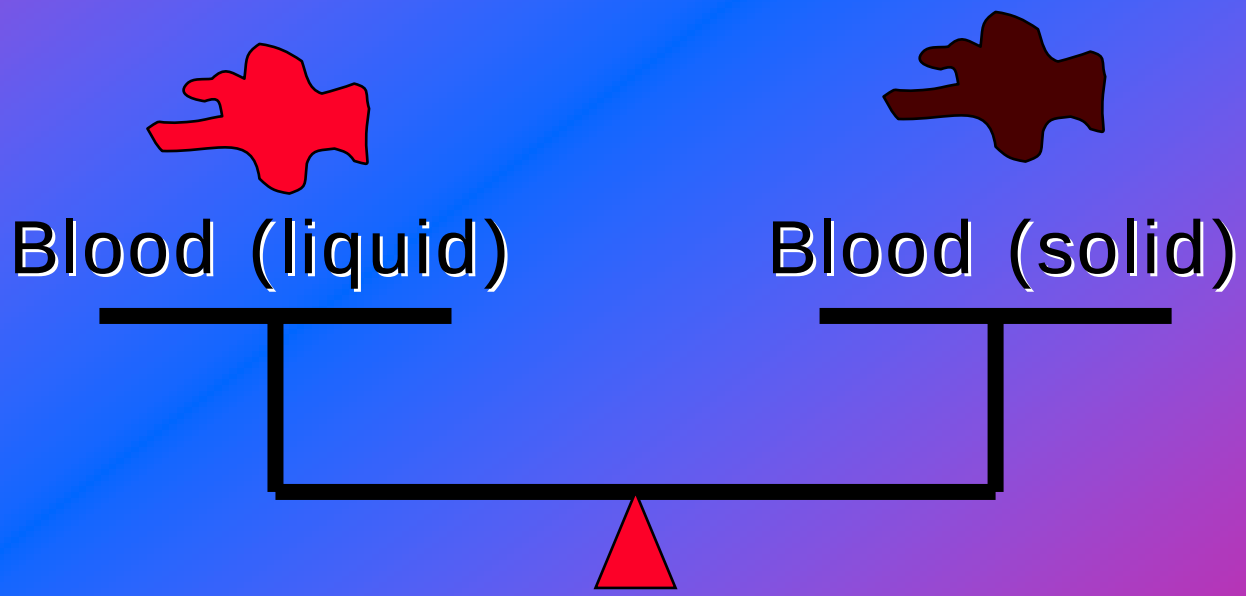


Inside view of a vein and its flow by phlebography



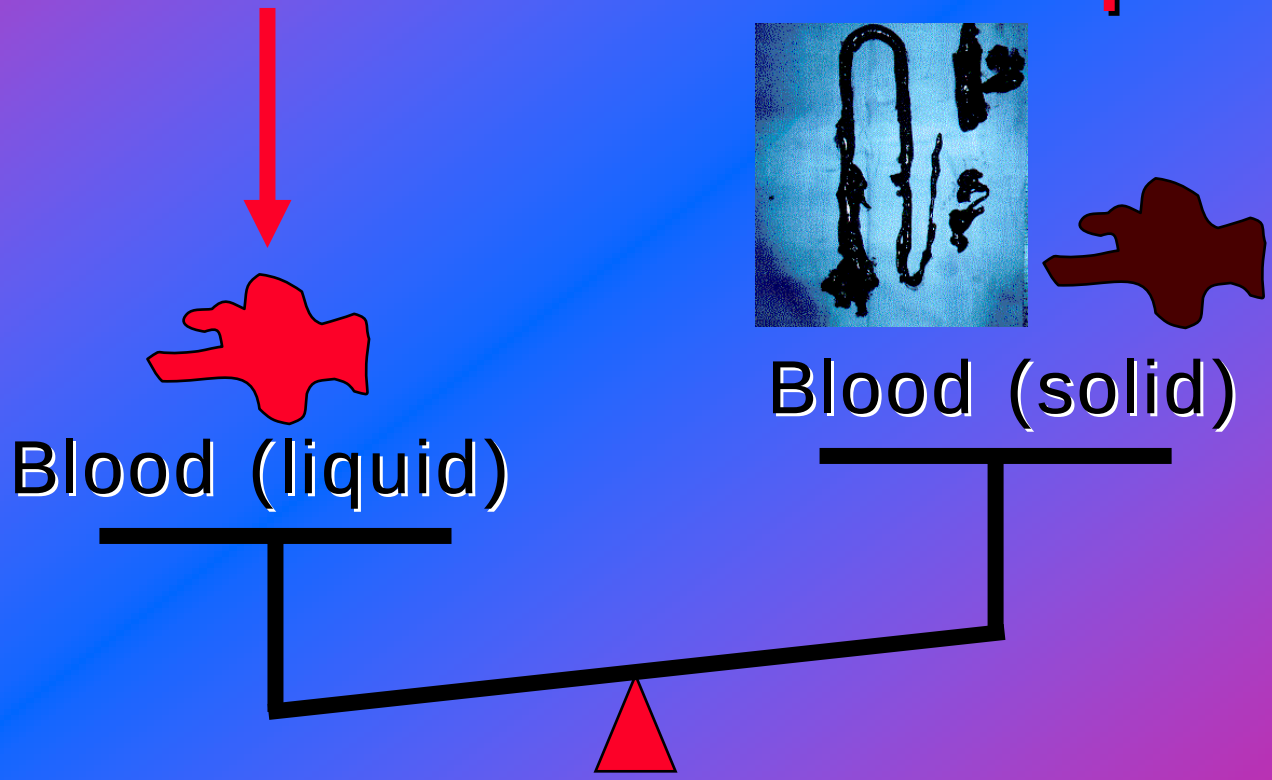


Blood clotting like everything in our body, is the result of an equilibrium

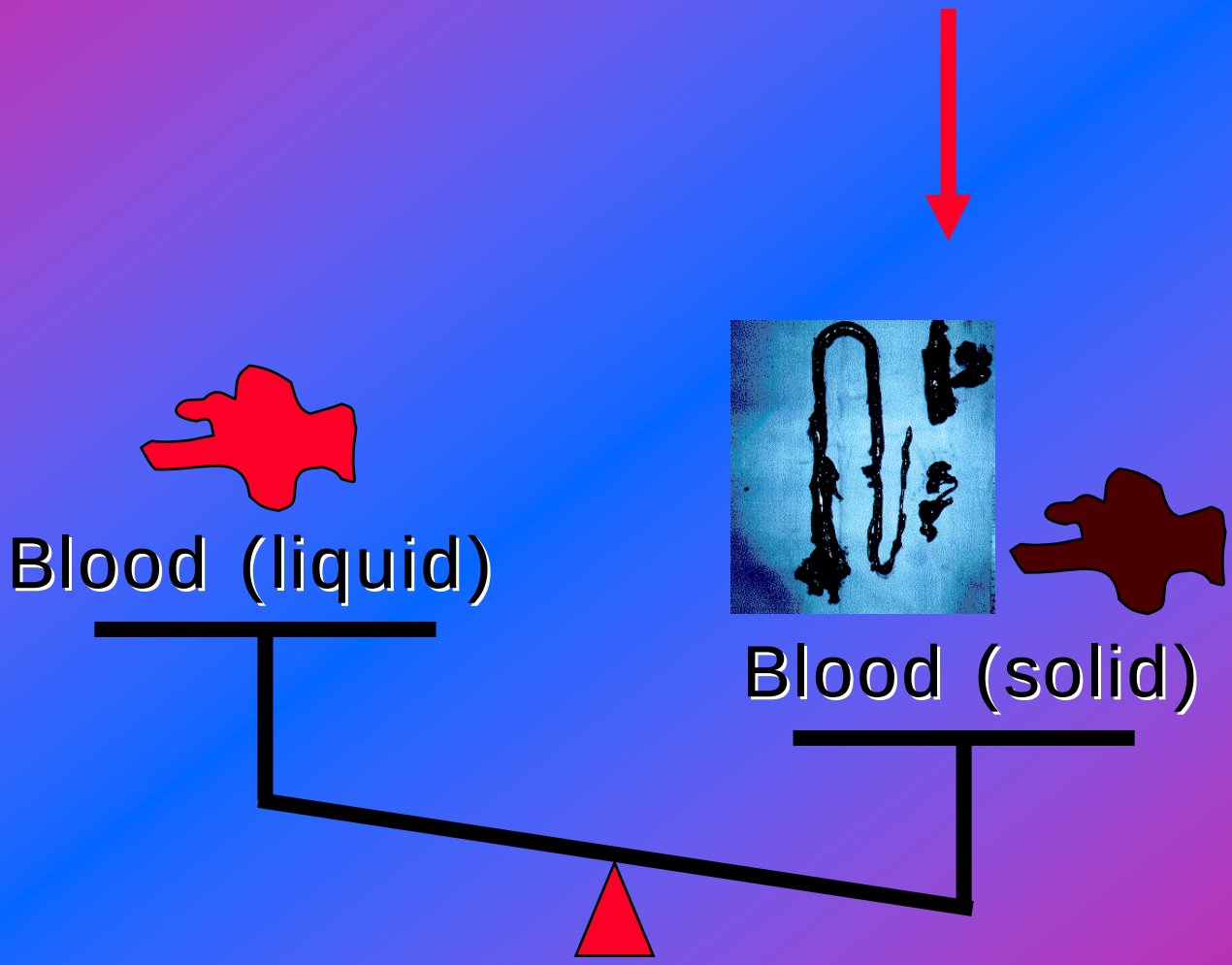


hemostasis

But if the equilibrium is in some way disturbed the result could be hemophilia

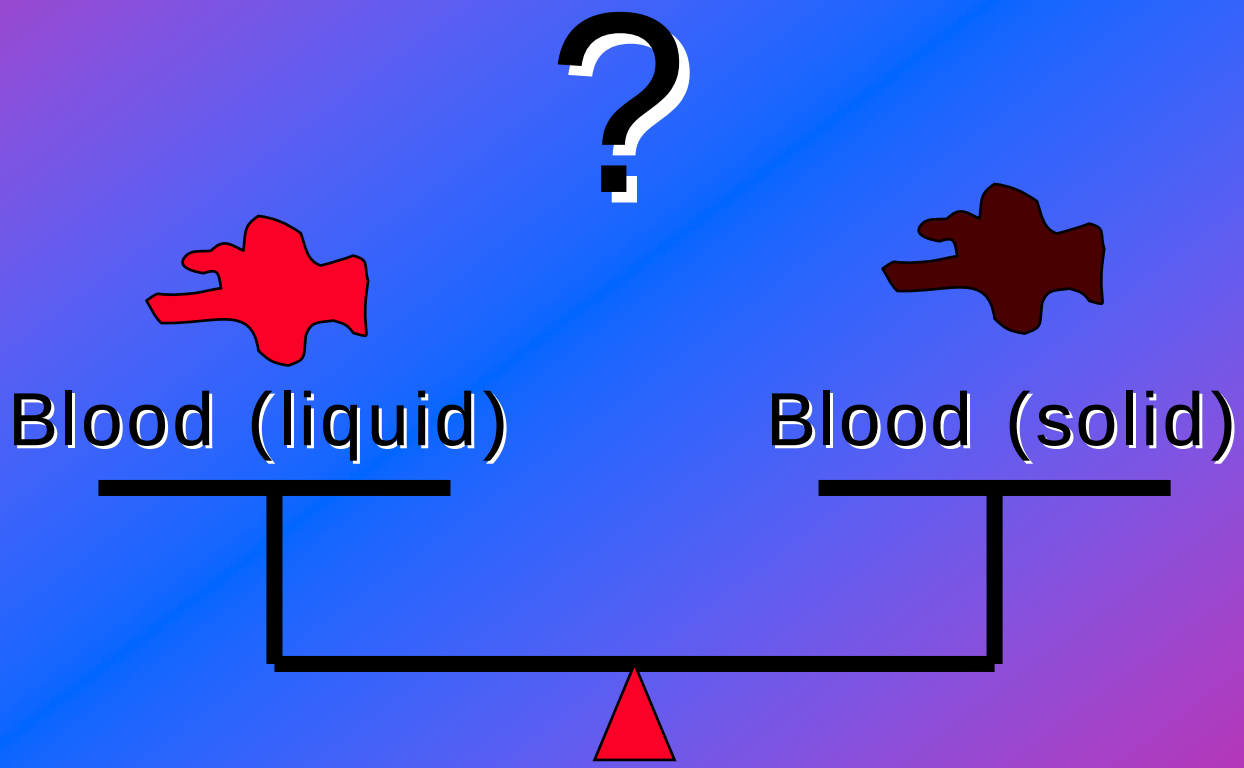


or thrombosis

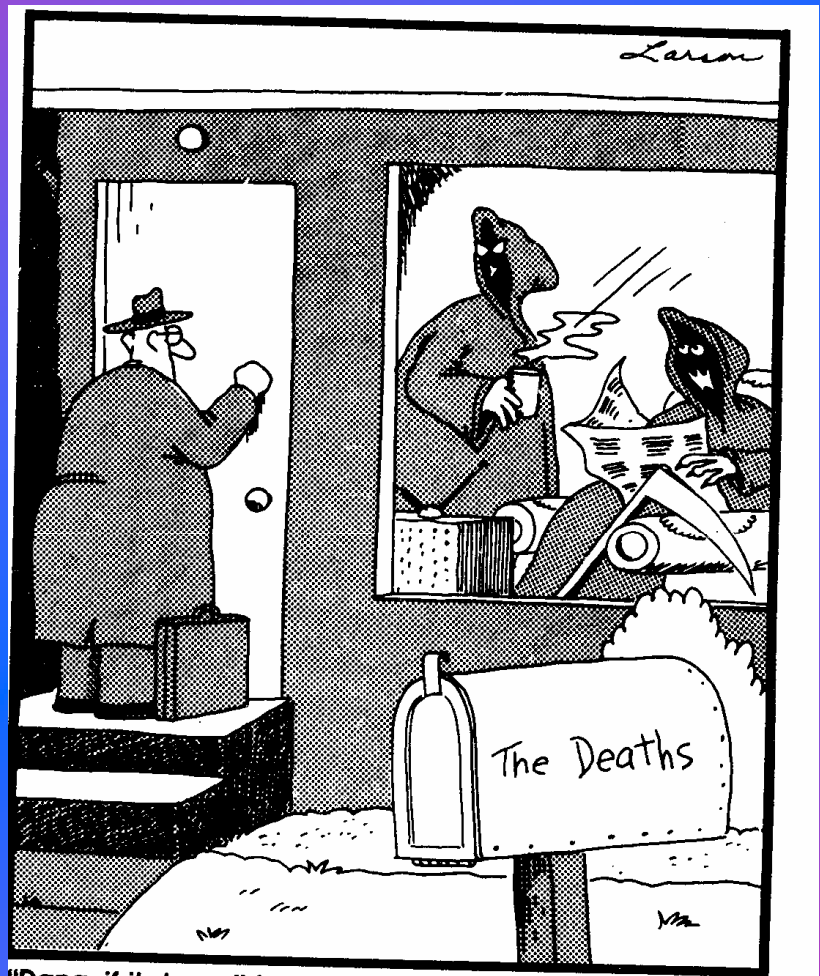


hemostasis

But how to derange this equilibrium?



In the industrilized countries the 50% of deaths are related to cardiovascular diseases



"Dang, if it doesn't happen every time! ... We just sit down to relax and someone's knockin' at the door."

Cardiovascular risks are "classical" risks

- Smoke
- Alcool
- Hypertension
- Cholesterol Tot/HDL
- Lpa
- Sex
- Left Ventricular hypertrophy

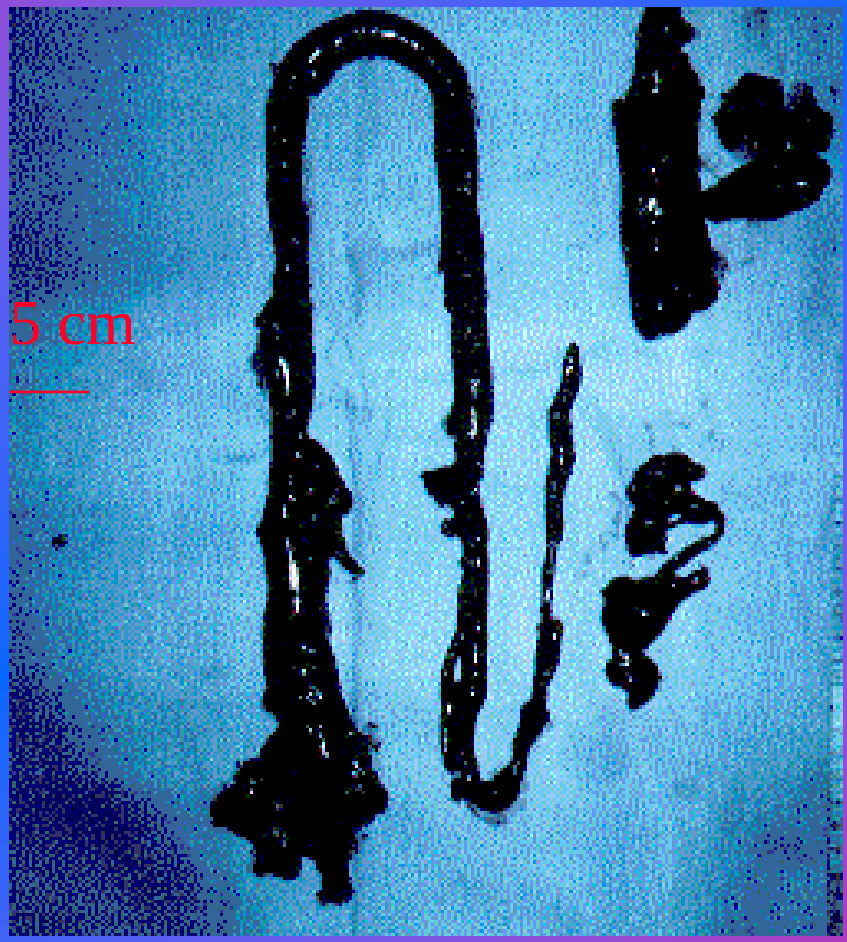
But also genetic risks

- Hereditary (homozygous or heterozygous; dominant or recessive);
- Confer an independent risk, different from classical
- The genetic risk is additive to the classical risks
- Generally is a loss of function or a strong reduction of function

Many genetic risks have been found in relation to cardiovascular pathologies. Here three of them related to thrombophilia.

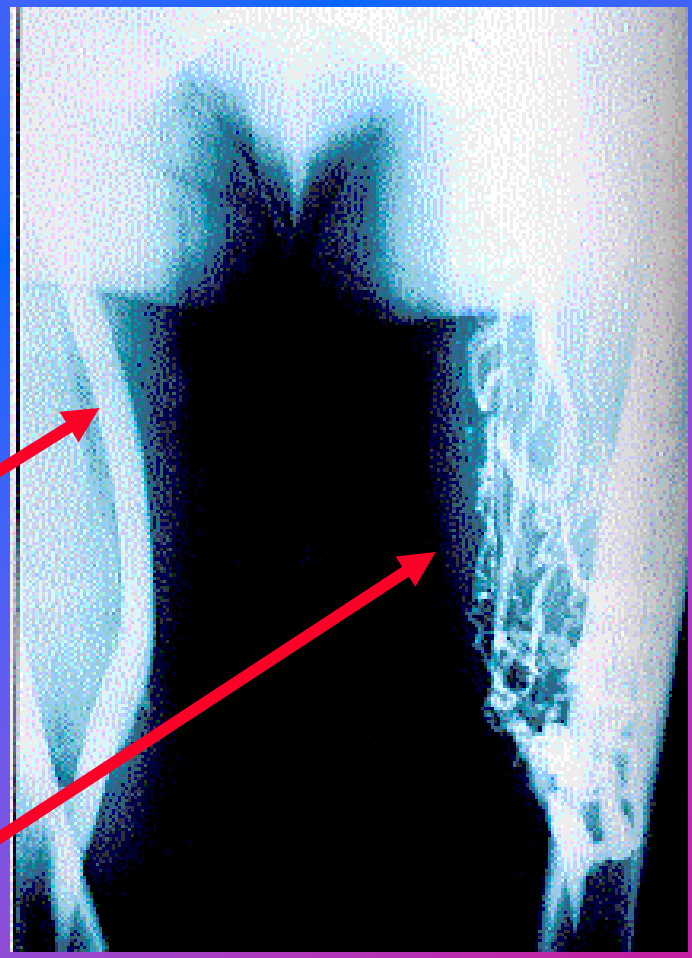
- The “Leiden” mutation on the Factor V gene of clotting
- Mutation on the Prothrombin gene
- Mutation on the gene coding for the enzyme Methylene tetrahydrofolate reductase (homocysteine metabolism)

Here is the result of a thrombotic process: the thrombus.



Thrombus occlude veins and stop blood flow

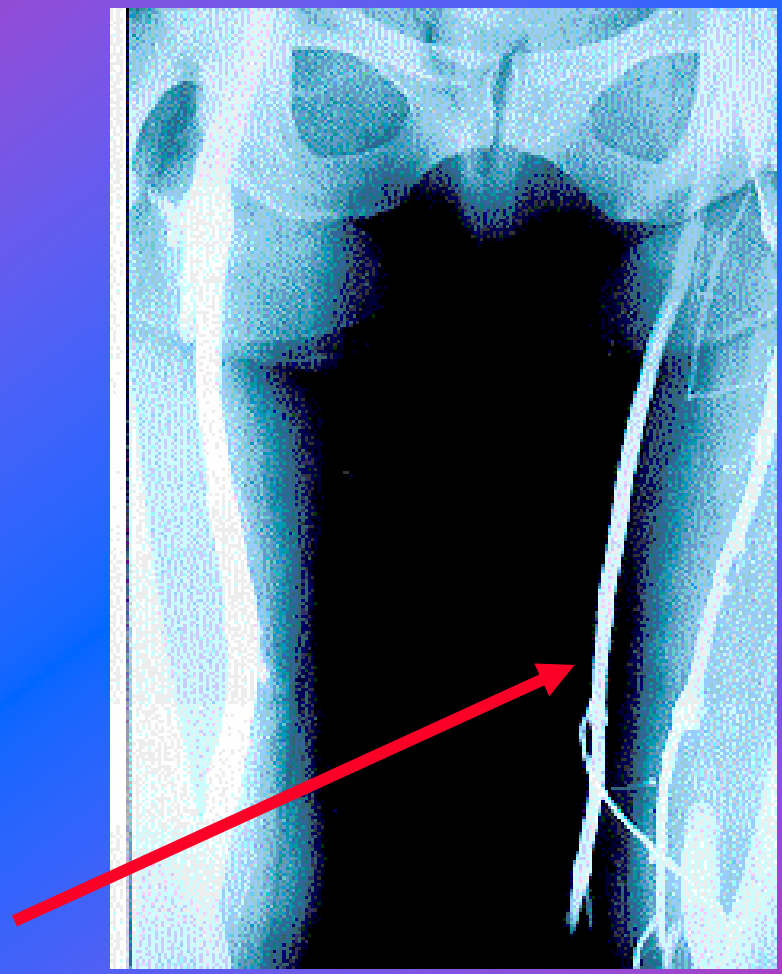
When a thrombus stops blood flow the system supply by generating a new network of blood vessels



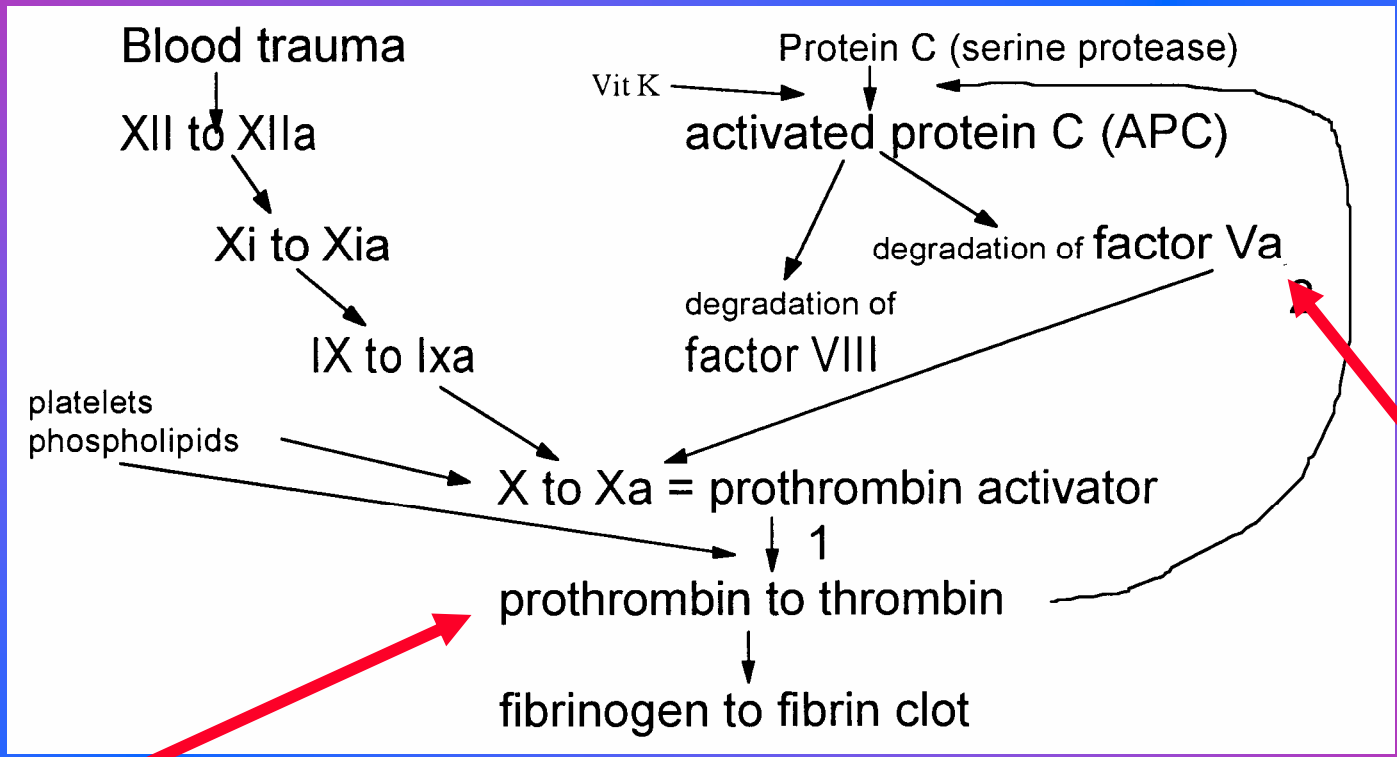
Normal blood flow

Occlusion by a thrombus stops blood flow

Usually blood find new ways to circumvent the thrombus



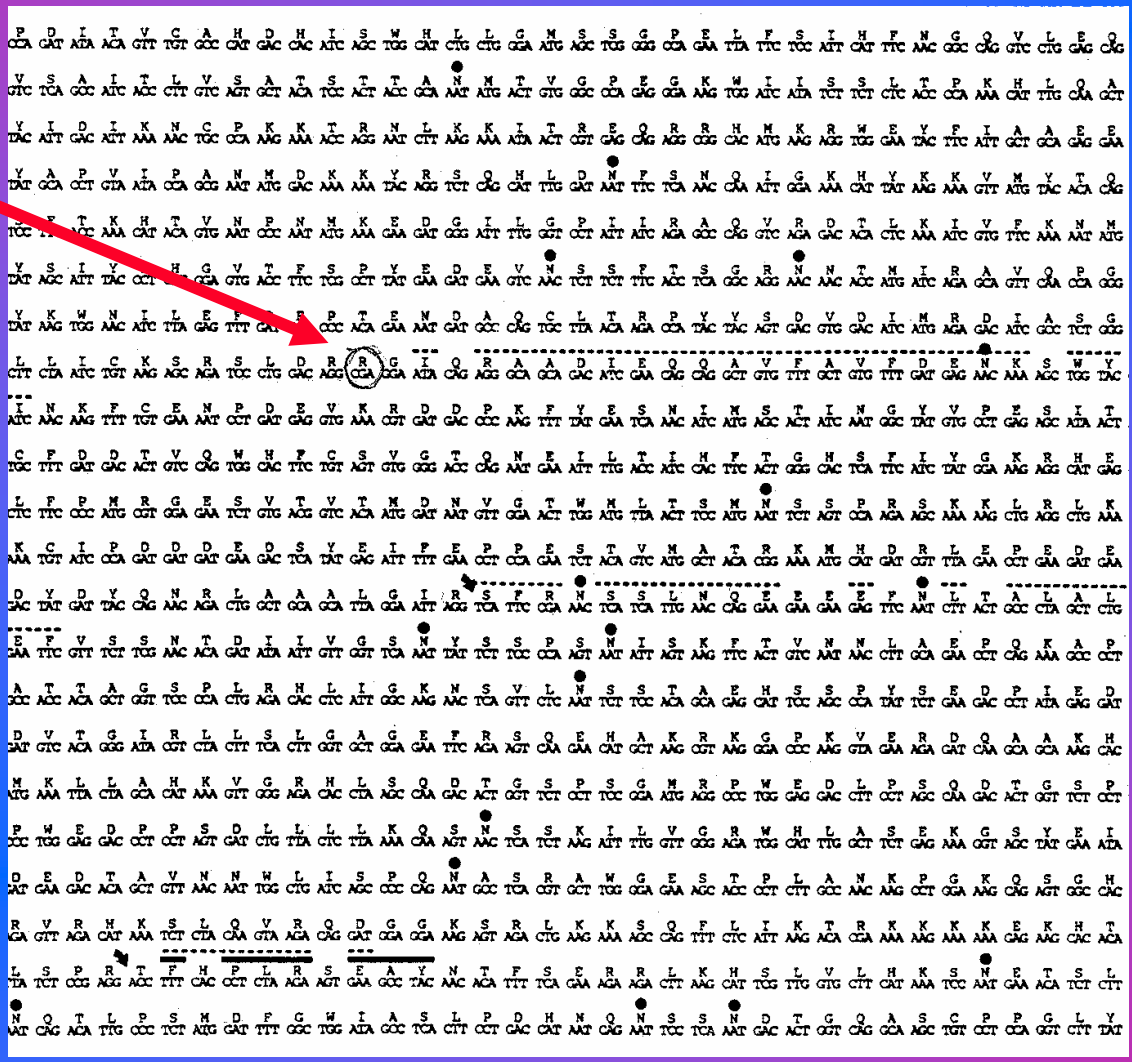
The clotting pathway.



The Factor V protein…

- is a 330 Kda protein
- is activated by factor Xa to form an heterodimer of 105/220 Kda
- is activated by thrombin to form an heterodimer of 105/74 Kda
- APC (activated protein C) binds to activated factor V (Va) and cut the Va at position Arg506
- in APC-resistent patients Arg506 is replaced by Gln (Leiden mutation)

The sequence of Factor V.



“Leiden”

Epidemiology:

- Frequency in the general population: 2-5%
- The thrombotic risck in healthy women (wt) taking the pill is 2-8 fold, in women w/m (heterozygous) is 35 higher, and in homozygous women is 150-500 fold
- The 60% of cases of thrombosis is found in pregnant women (APC resistence), of them 90% bear the “Leiden” mutation
- In presence of the “Leiden” mutation, a post-operatory profilaxis reduces of 50% the risk of thrombosis

“Leiden”

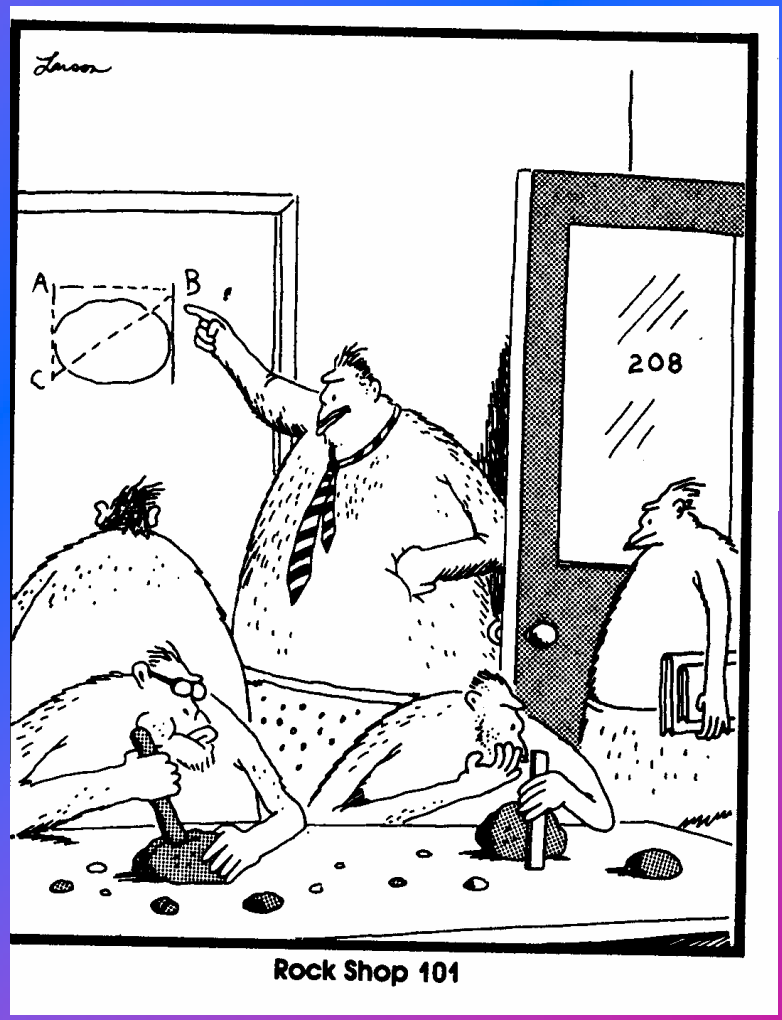
Indications

- Familial thrombotic events
- Anti-conceptionals
- Immobilization causing venous stasis
- Previous thrombotic events

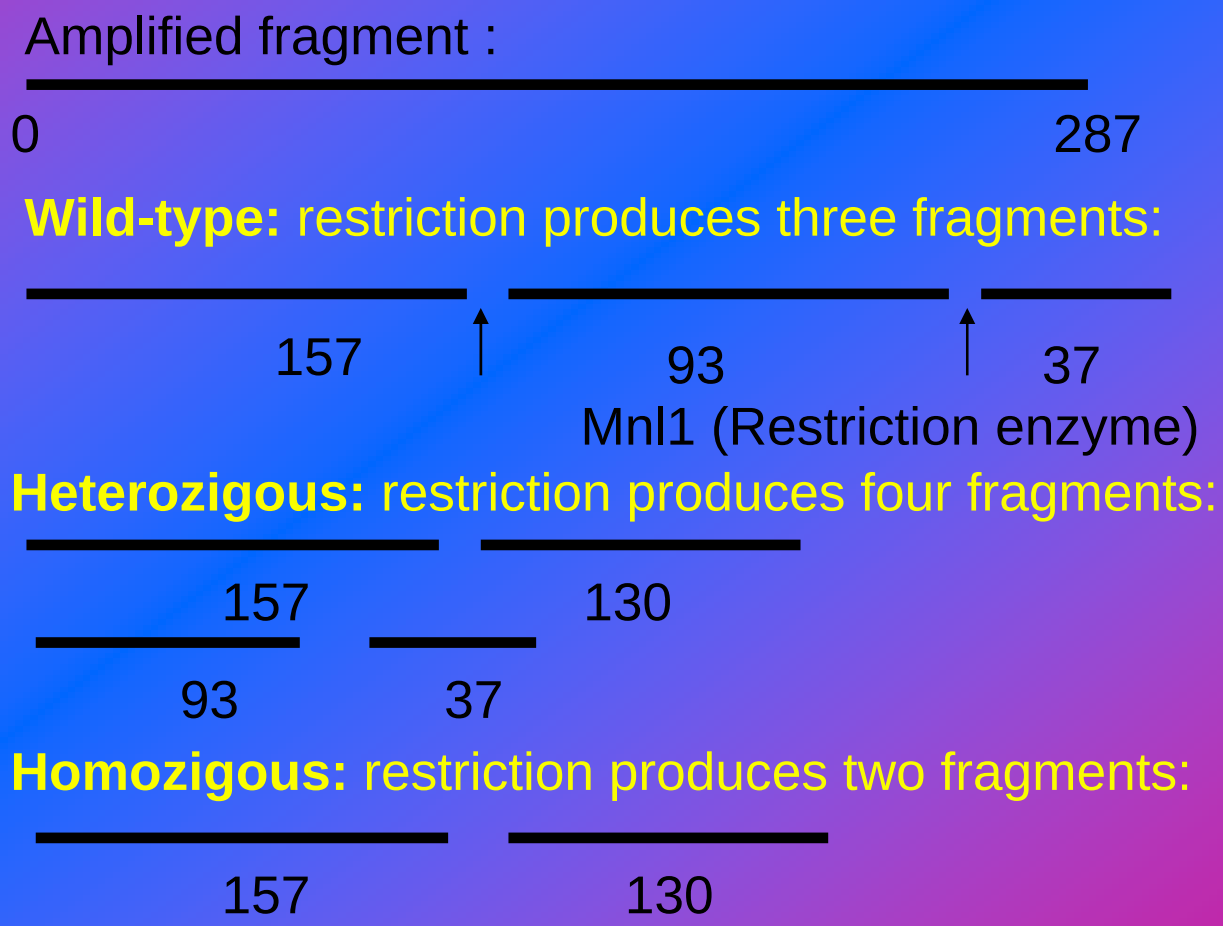
“Leiden”

Technical aspects

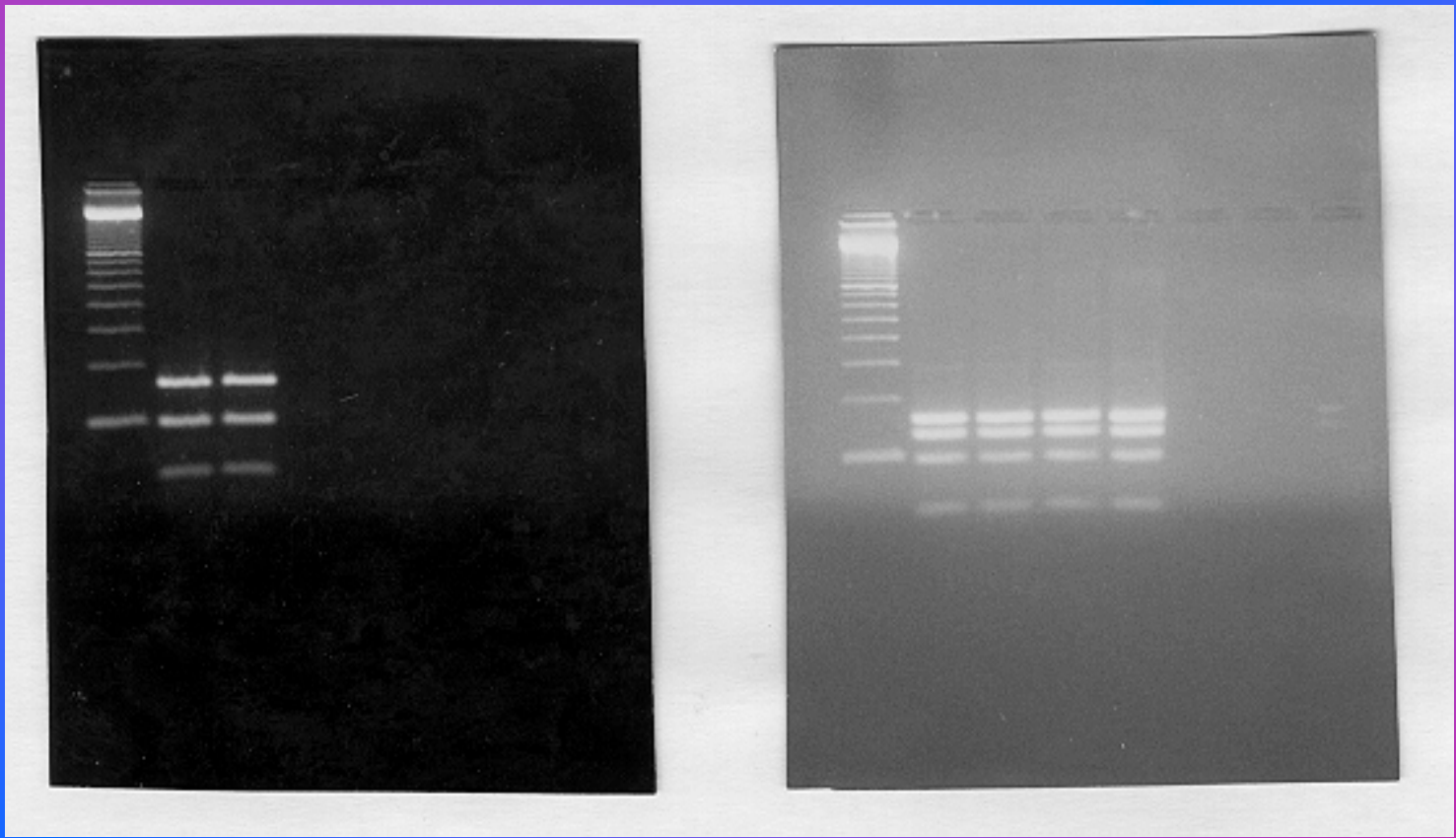
- DNA extraction from peripheral blood (EDTA/ACD)
- PCR amplification
- Restriction analysis
- Results: wild-type, heterozygous, homozygous



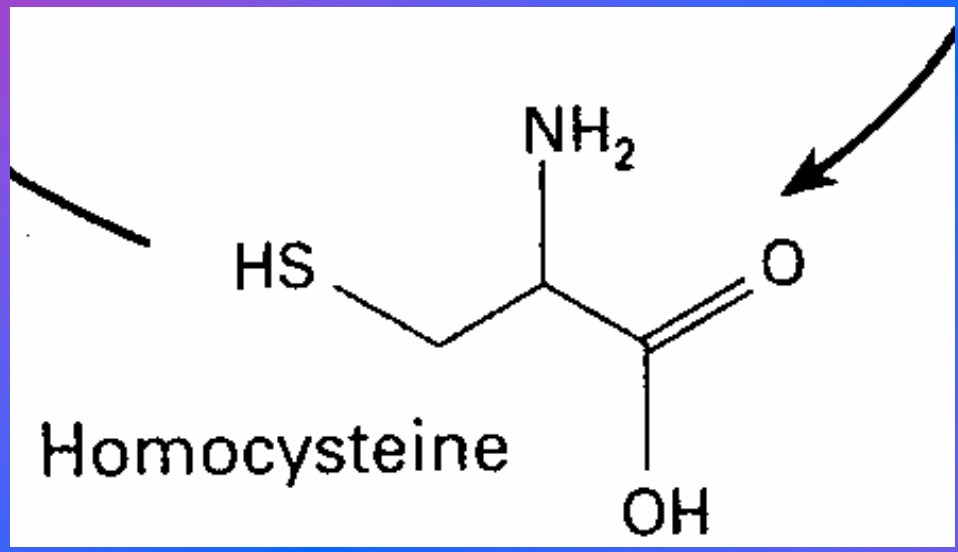
“Leiden” with Mnl1 restriction



Factor V (Leiden) results.



Homocysteine

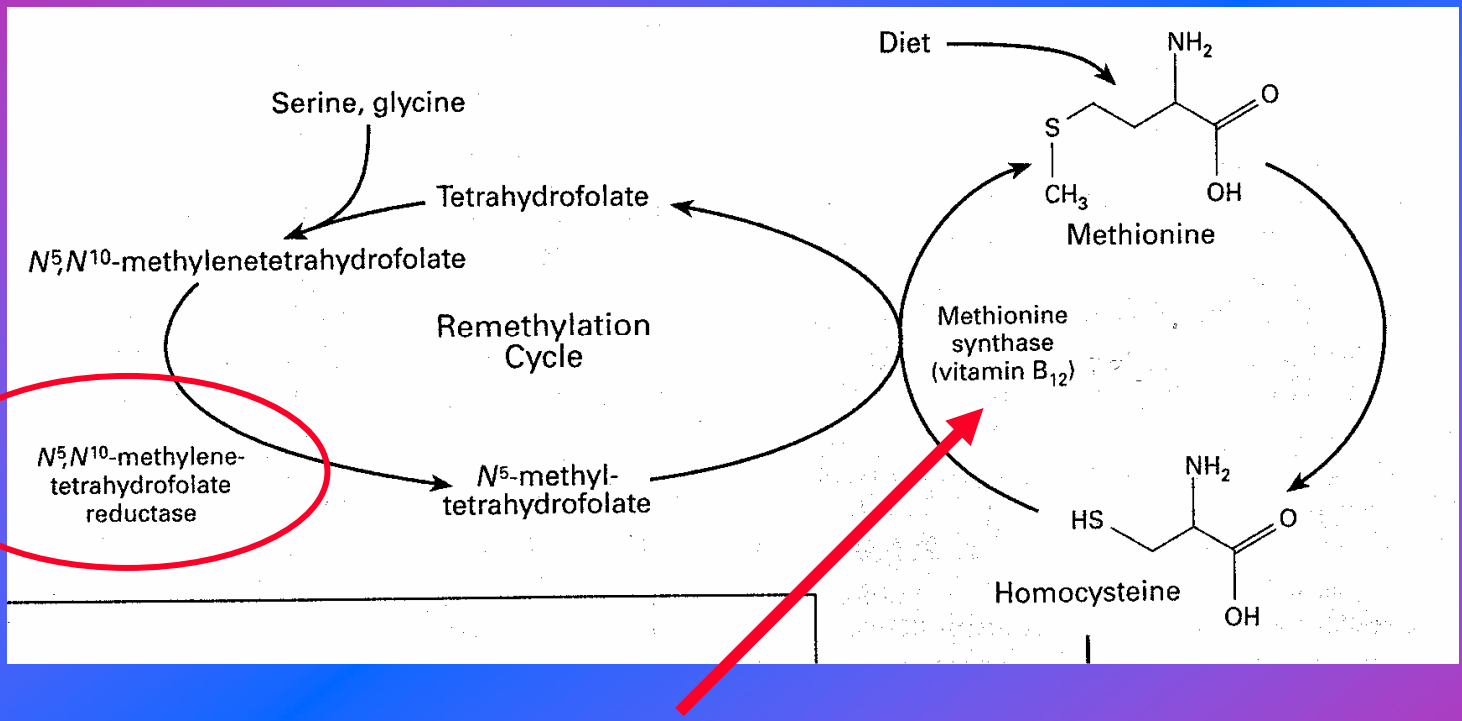


Intermediate aminoacid formed during the metabolism of methionine

It is metabolized by one of two pathways:

- remethylation
- transsulfuration

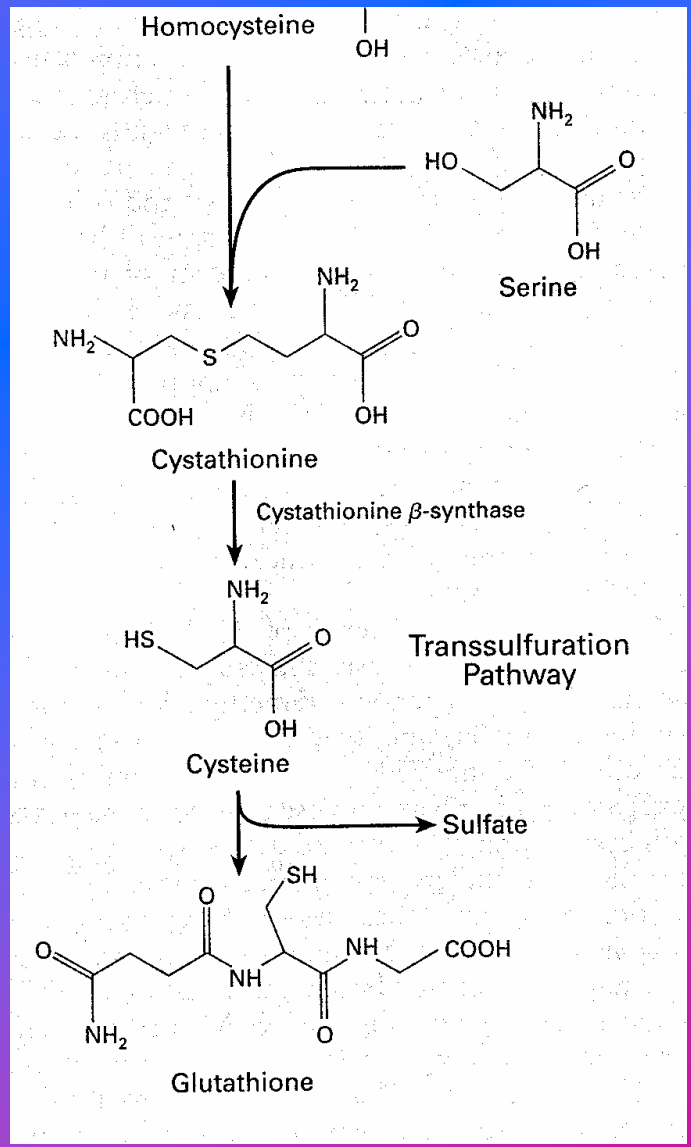
Homocysteine metabolism



In the remethylation cycle homocysteine is salvaged by the acquisistion of a methyl group in a reaction catalyzed by the enzyme methionine synthase.

Homocysteine metabolism

In conditions of methionine excess or cysteine requirement, homocysteine enters the transsulfuration pathway to end with the formation of glutathione or further secreted metabolized to sulfate and excreted in the urine.



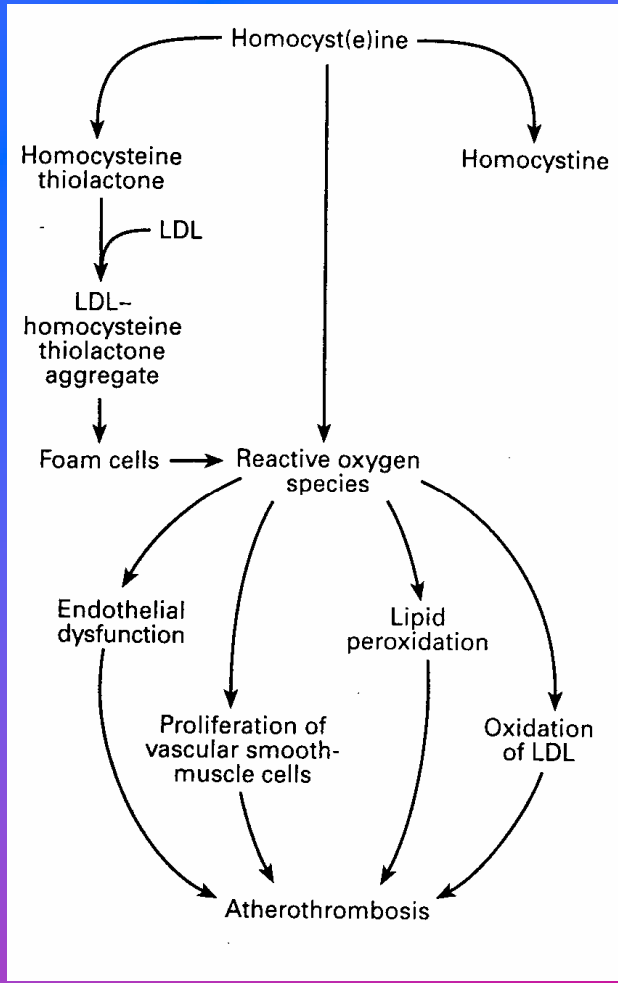
Homocysteine physiopathology

1.Homocysteine is rapidly auto-oxidized in plasma, forming homocystine and homocysteine thiolactone

2. Potent reactive oxygen species including superoxide and hydrogen peroxide are produced during the auto-oxidation process and hydrogen peroxide has been implicated in the vascular toxicity of hyperhomocysteinemia

3.Oxydative damage to vascular endothelial cells induces platelet accumulation, platelet-rich thrombus formation and smooth-muscle cell proliferation in areas of endothelial injury

4. Consequent platelet and leukocyte activation



Homocysteine physiopathology (2)

Cytotoxic reactive oxygen species, including superoxide anion radicals initiate lipid peroxidation, occurring at the endothelial plasma membrane level and within lipoprotein particles resulting with:

- enhancement of the activity of factor XII and factor V and
- depression of the activity of protein C
- induction of the expression of the tissue factor

facilitating ultimately to the formation of thrombin and creating a prothrombotic environment

Methylenetetrahydrofolatoe reductase (MTHFR)

Epidemiology

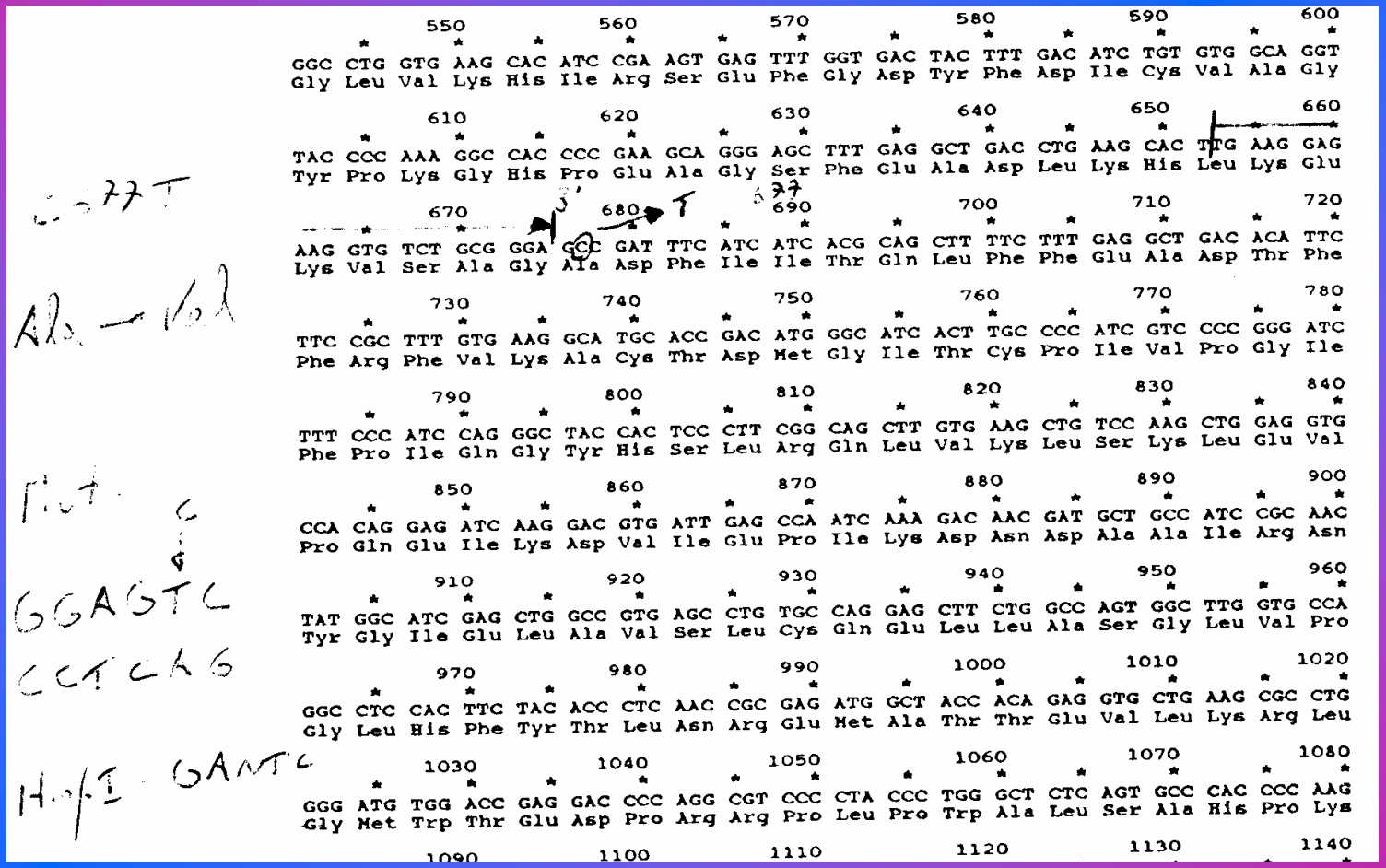
- the C677T mutation is independent of other classical risk factors
- the homozigous form of the mutation is found 5-18% of the general population (16.2% in Switzerland) and in 19% of patients with CAD

Methylenetetrahydrofolate reductase (MTHFR)

Indications

- Homocysteine is armful to epithelial cells which lay in internal blood vessels (cytotoxic)
- elevated levels of homocysteine is found in patients with angina pectoris, MI and ictus
- if homocysteine is >15 µM in patients with coronaric disease the mortality risk increases 5 times compared to a patient with normal homocysteine
- the presence of an elevated amount of homocysteine correlates with a mutation on the MTHFR gene (C677T)

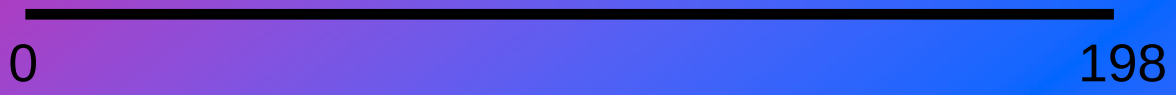
The sequence



```
          550         560         570         580         590         600
          *           *           *           *           *           *
GGC CTG GTG AAG CAC ATC CGA AGT GAG TTT GGT GAC TAC TTT GAC ATC TGT GTG GCA GGT
Gly Leu Val Lys His Ile Arg Ser Glu Phe Gly Asp Tyr Phe Asp Ile Cys Val Ala Gly

          610         620         630         640         650         660
          *           *           *           *           *           *
TAC CCC AAA GGC CAC CCC GAA GCA GGG AGC TTT GAG GCT GAC CTG AAG CAC TTG AAG GAG
Tyr Pro Lys Gly His Pro Glu Ala Gly Ser Phe Glu Ala Asp Leu Lys His Leu Lys Glu

          670         680         690         700         710         720
          *           *           *           *           *           *
AAG GTG TCT GCG GGA GCC GAT TTC ATC ATC ACG CAG CTT TTC TTT GAG GCT GAC ACA TTC
Lys Val Ser Ala Gly Ala Asp Phe Ile Ile Thr Gln Leu Phe Phe Glu Ala Asp Thr Phe

          730         740         750         760         770         780
          *           *           *           *           *           *
TTC CGC TTT GTG AAG GCA TGC ACC GAC ATG GGC ATC ACT TGC CCC ATC GTC CCC GGG ATC
Phe Arg Phe Val Lys Ala Cys Thr Asp Met Gly Ile Thr Cys Pro Ile Val Pro Gly Ile

          790         800         810         820         830         840
          *           *           *           *           *           *
TTT CCC ATC CAG GGC TAC CAC TCC CTT CGG CAG CTT GTG AAG CTG TCC AAG CTG GAG GTG
Phe Pro Ile Gln Gly Tyr His Ser Leu Arg Gln Leu Val Lys Leu Ser Lys Leu Glu Val

          850         860         870         880         890         900
          *           *           *           *           *           *
CCA CAG GAG ATC AAG GAC GTG ATT GAG CCA ATC AAA GAC AAC GAT GCT GCC ATC CGC AAC
Pro Gln Glu Ile Lys Asp Val Ile Glu Pro Ile Lys Asp Asn Asp Ala Ala Ile Arg Asn

          910         920         930         940         950         960
          *           *           *           *           *           *
TAT GGC ATC GAG CTG GCC GTG AGC CTG TGC CAG GAG CTT CTG GCC AGT GGC TTG GTG CCA
Tyr Gly Ile Glu Leu Ala Val Ser Leu Cys Gln Glu Leu Leu Ala Ser Gly Leu Val Pro

          970         980         990         1000        1010        1020
          *           *           *           *           *           *
GGC CTC CAC TTC TAC ACC CTC AAC CGC GAG ATG GCT ACC ACA GAG GTG CTG AAG CGC CTG
Gly Leu His Phe Tyr Thr Leu Asn Arg Glu Met Ala Thr Thr Glu Val Leu Lys Arg Leu

          1030        1040        1050        1060        1070        1080
          *           *           *           *           *           *
GGG ATG TGG ACC GAG GAC CCC AGG CGT CCC CTA CCC TGG GCT CTC AGT GCC CAC CCC AAG
Gly Met Trp Thr Glu Asp Pro Arg Arg Pro Leu Pro Trp Ala Leu Ser Ala His Pro Lys

          1090        1100        1110        1120        1130        1140
```

Methylenetetrahydrofolatoe reductase (MTHFR)

Amplified fragment :

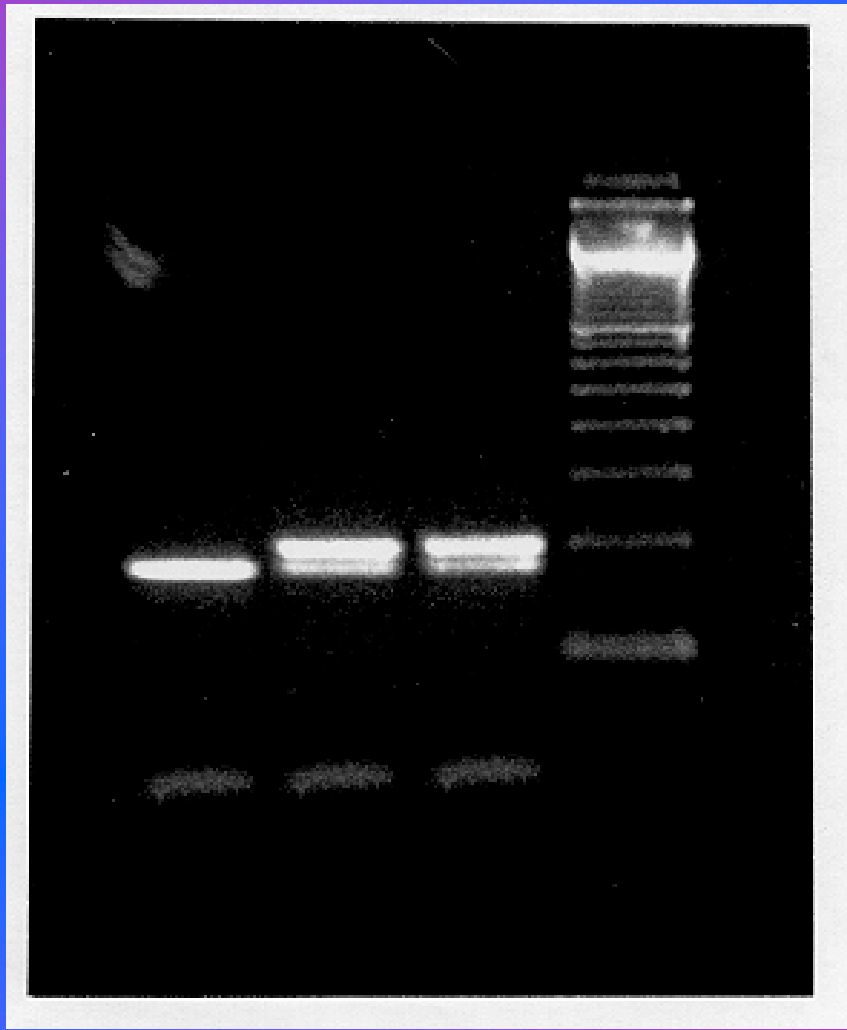


Wild-type: the fragment is not cut :

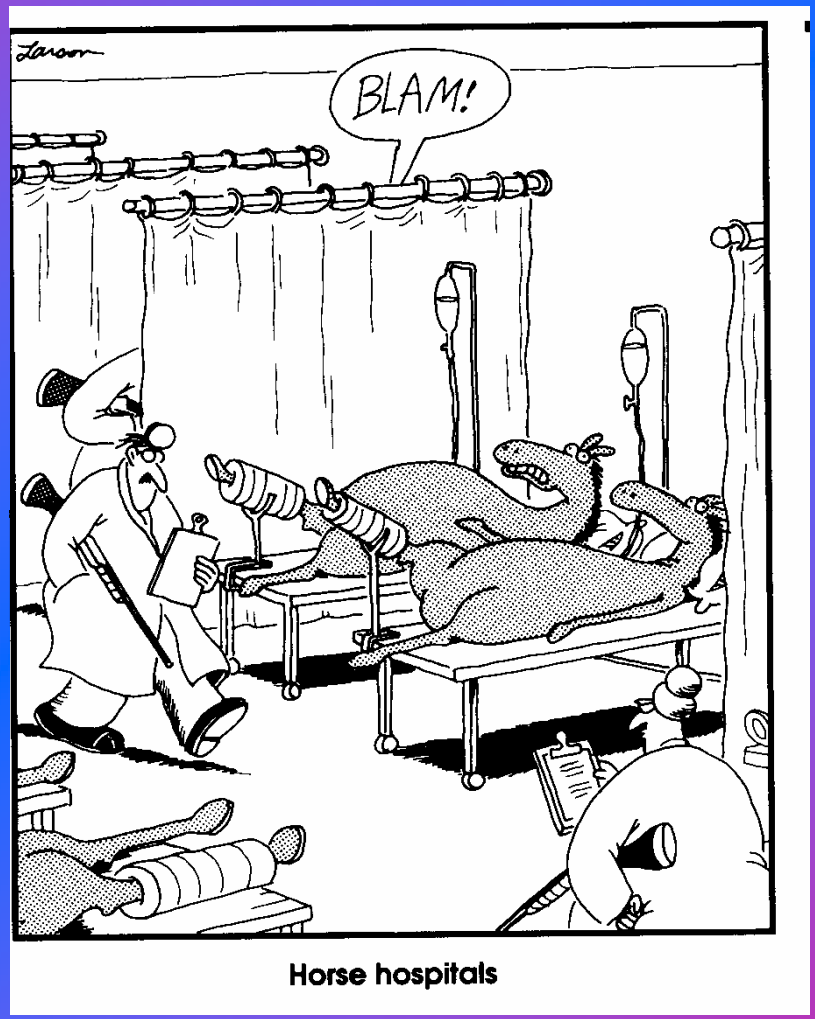
Heterozigous: restriction produces three fragments:

Homozigous: restriction produces two fragments:

The results are here.

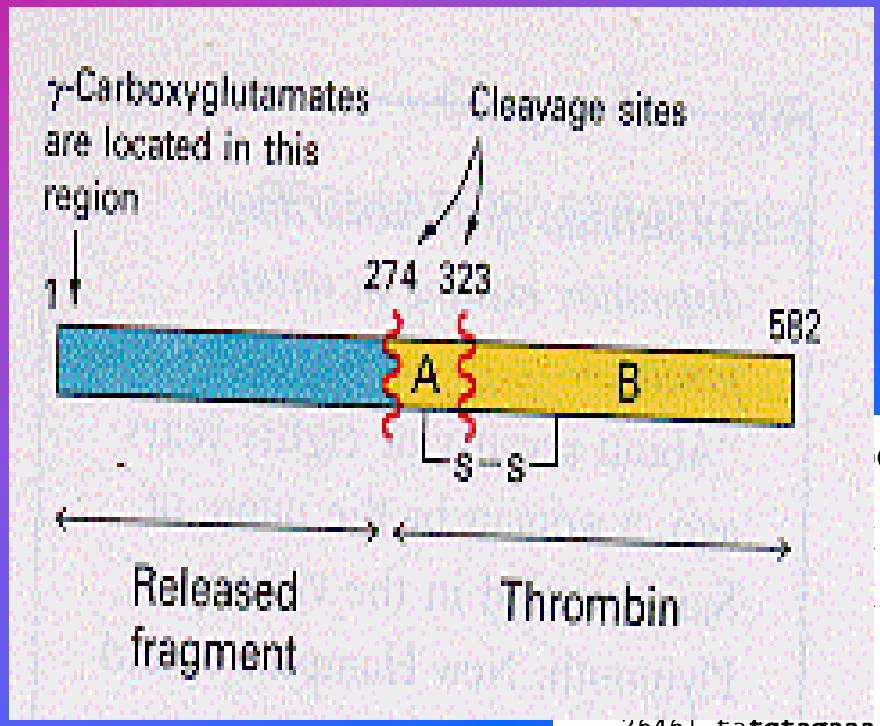


Prothrombin, another same old story.



Horse hospitals

What is Prothrombin?



e codifica la protrombina (estratto da GeneBank, Internet)

```
                                                ctgttgggtg gagaatagac tgtaggtggg caaagaatga aggaaactag
                                                agctcgagct agaagtggtg agaagggttt ggatttgggg tctatgctga
                                                gacaagattt gctaggattg gatgtgtagg gtgaggaagt ggggacagca
                                                gaggggtaag tggactctca ccagctgtgt ctcgtgaagg ggcgtggctg
                                                tatgctcctg agcacagacg gctgttctct ttcaaggtta caagcctgat
                                                gaggggatgc ctgtgaaggt gacagtgggg gacccttttgt catgaaggta
                                                aagcccaggg cctggtgaac acatcttctg ggggtgggga gaaactctag
26461 tatctagaaa cagttgcctg gcagaggaat actgatgtga ccttgaactt gactctattg
26521 gaaacctcat ctttcttctt cagagcccct ttaacaaccg ctggtatcaa atgggcatcg
26581 tctcatgggg tgaaggctgt gaccgggatg ggaaatatgg cttctacaca catgtgttcc
26641 gcctgaagaa gtggatacag aaggtcattg atcagtttgg agagtagggg gccactcata
26701 ttctgggctc ctggaaccaa tcccgtgaaa gaattatttt tgtgtttcta aaactatggt

26761 tcccaataaa agtgactctc agcGagcctc aatgctccca gtgctattca tgggcagctc
26821 tctgggctca ggaagagcca gtaatactac tggataaaga agacttaaga atccaccacc
26881 tggtgcacgc tggtagtccg agcactcggg aggctgaggt gggaggat
```

"**a**" nelle persone con rischio trombotico

Prothrombin

- 34 KDal mass and contains two chains
- cleaves only certain arginine-glycine bonds
- synthesized as a zymogen called prothrombin
- Proteolytic cleavage of the Arg274-Thr275 bond releases a 32 Kdal fragment from the 66 Kdal zymogen
- Cleavage at the Arg323-Ile324 bond yields active thrombin
- The vitamin K-dependent carboxylation reaction converts glutamate, a weak chelator of Ca++, into gamma-carboxyglutamate a much stronger chelator.
- The binding of Ca++ by prothrombin anchors it to phospholipid membranes derived from blood platelets bringing prothrombin in close proximity to factor Xa and Factor V, two proteins that mediate its conversion to thrombin

The sequence of the prothrombin gene

Sequenza del gene che codifica la protrombina (estratto da GeneBank, Internet)

```
....26041 attattctgc ctgttgggtg gagaatagac tgtaggtggg caaagaatga aggaaactag
    26101 tgggttcagg agctcgagct agaagtggtg agaagggttt ggatttgggg tctatgctga
    26161 aggtagagcc gacaagattt gctaggattg gatgtgtagg gtgaggaagt ggggacagca
    26221 agaatgactg gaggggtaag tggactctca ccagctgtgt ctcgtgaagg ggcgtggctg
    26281 ggctatgagc tatgctcctg agcacagacg gctgttctct ttcaaggtta caagcctgat
    26341 gaagggaaac gaggggatgc ctgtgaaggt gacagtgggg gacccttttgt catgaaggta
    26401 agcttctcta aagcccaggg cctggtgaac acatcttctg ggggtgggga gaaactctag
    26461 tatctagaaa cagttgcctg gcagaggaat actgatgtga ccttgaactt gactctattg
    26521 gaaacctcat ctttcttctt cagagcccct ttaacaaccg ctggtatcaa atgggcatcg
    26581 tctcatgggg tgaaggctgt gaccgggatg ggaaatatgg cttctacaca catgtgttcc
    26641 gcctgaagaa gtggatacag aaggtcattg atcagtttgg agagtagggg gccactcata
    26701 ttctgggctc ctggaaccaa tcccgtgaaa gaattatttt tgtgtttcta aaactatggt
    26761 tcccaataaa agtgactctc agcgagcctc aatgctccca gtgctattca tgggcagctc
    26821 tctgggctca ggaagagcca gtaatactac tggataaaga agacttaaga atccaccacc
    26881 tggtgcacgc tggtagtccg agcactcggg aggctgaggt gggaggat
```

"**a**" nelle persone con rischio trombotico

The amplification produces a 345 bp fragment and the transition G to A creates a restriction site for Hind III.

Mutation on the prothrombin gene (G20210A)

Epidemiology

- The prevalence in the general population is about 1-2%
- The incidence of deep venous thrombosis (DVT) in the general population is 1/1000
- The mutation on the prothrombin gene gives a 8-fold greater risk to develop a DVTand a 4-fold risk to develop a MI
- The mutation is found in 18% of patients with a personal history or familial of DVT (2.3% in healthy)
- 87% of patients with the polymorphism are in the higher quartile of prothrombin levels

Mutation on the prothrombin gene (G20210A) and more...

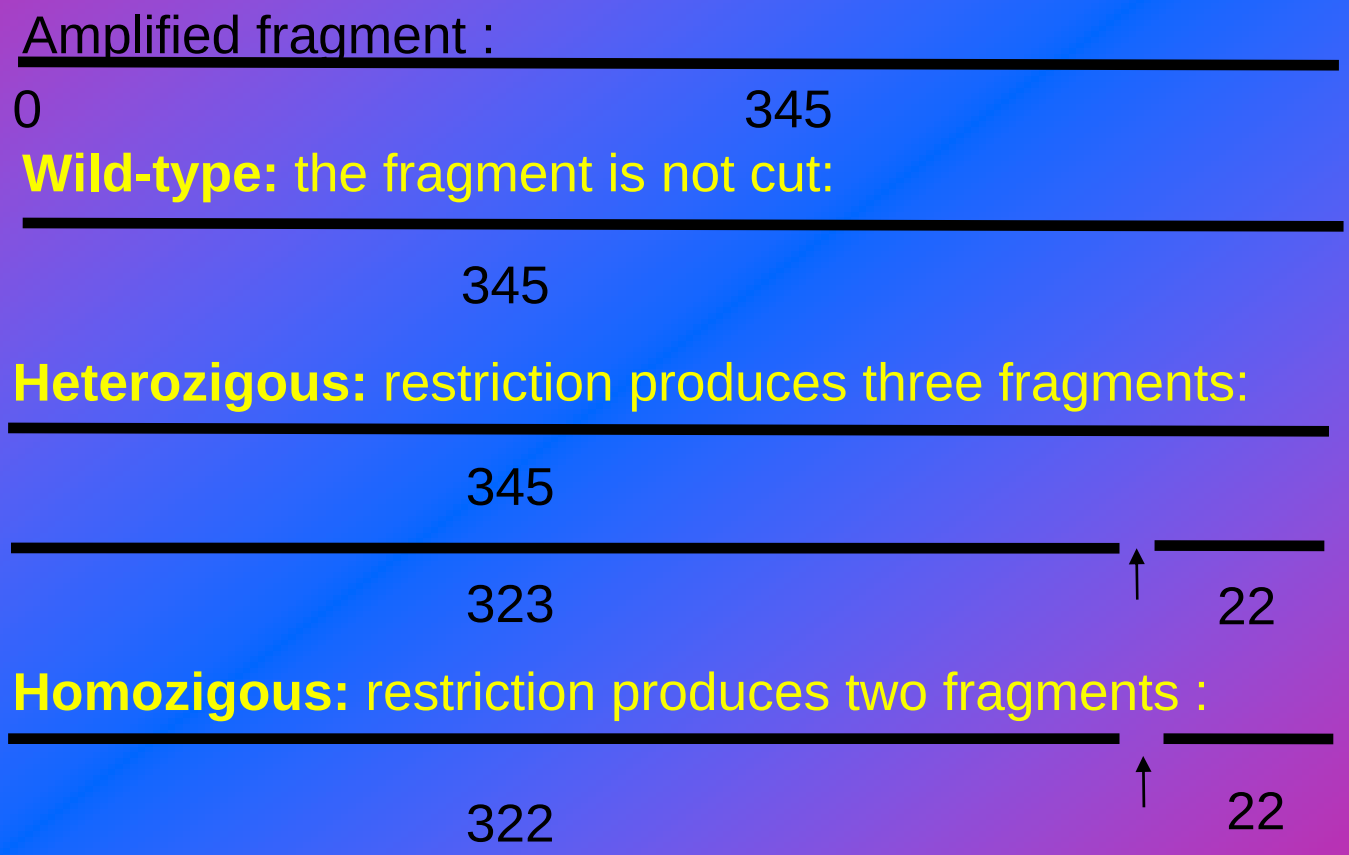
- The G20210A transition is more frequent in women which had a first MI (5.1%) than controls (1.6%)
- The relative risk is much higher if another risk factor like smoke is present. The odds ratio goes up from 4.0 to 43.3.

Mutation on the prothrombin gene (G20210A)

Indications

- Familial thrombotic events
- Anti-conceptionals
- Immobilization causing venous stasis
- Previous thrombotic events

Mutation on the prothrombin gene (G20210A)



Which looks like this.