Thrombophilia testing

a comprehensive approach on what and who to test

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BHS Educational course 2026

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Introduction

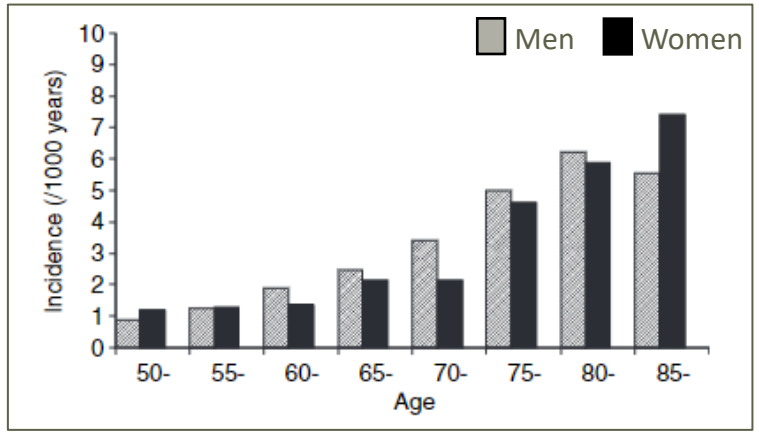
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VENOUS THROMBOEMBOLISM (VTE)

Venous thromboembolism: 1) Deep vein thrombosis (DVT)

2) Pulmonary embolism (PE)



F. Rosendaal, J Thromb Haemost 2007; 5 (Suppl 1): 310-7

Incidence: DVT: 0,1 to 0,2% per year

complicated by PE in 50%

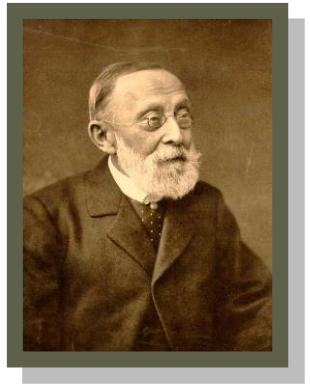
Mortality following PE: 10%

50% of all VTE occurs in risk situations

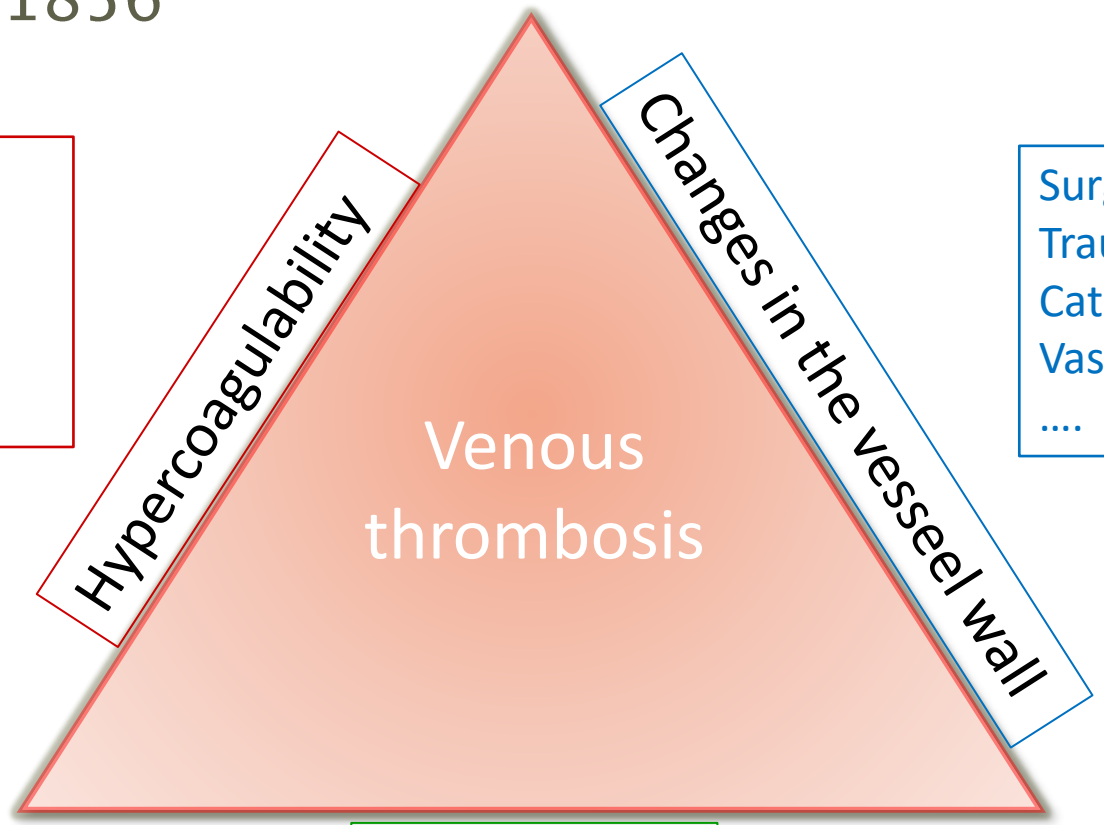
→ VTE is a multifactorial disease

PATHOGENESIS OF VENOUS THROMBOEMBOLISM

Virchow's triad – 1856



Acute phase
Cancer
Thrombophilia
Pregnancy/Post-partum
….



Surgery (esp. orthopeadic)
Trauma
Catheters
Vascular disease (atherosclerosis)
….

Immobilisation
Heart failure
Venous obstruction
Pregnancy ...

Thrombophilia testing: what to test?

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THROMBOPHILIA

Tendency of the blood to develop venous or arterial thrombosis

Inherited thrombophilia

- Deficiency of natural anticoagulants
  - ✓ Antithrombin
  - ✓ Protein S
  - ✓ Protein C
- APC resistance/ Factor V Leiden
- Prothrombin G20210A mutation

Acquired thrombophilia

- Surgery, trauma, immobilisation
- Pregnancy / Post-partum
- Antiphospholipid syndrome
- Cancer
- Paroxysmal nocturnal hemoglobinuria
- Myeloproliferative disorders
- Oral contraceptives/Hormone replacement θ
- Age
- Obesity

INHERITED THROMBOPHILIA

Clinical presentation

- ✓ Venous thromboembolic disease (DVT, PE, thrombophlebitis)
- ✓ Family history of thrombosis
- ✓ Early age of onset
- ✓ Recurrent events
- ✓ Unusual presentation (cerebral veins, mesenteric veins,…)
- ✓ Recurrent pregnancy loss, pregancy morbidity

INHERITED THROMBOPHILIA

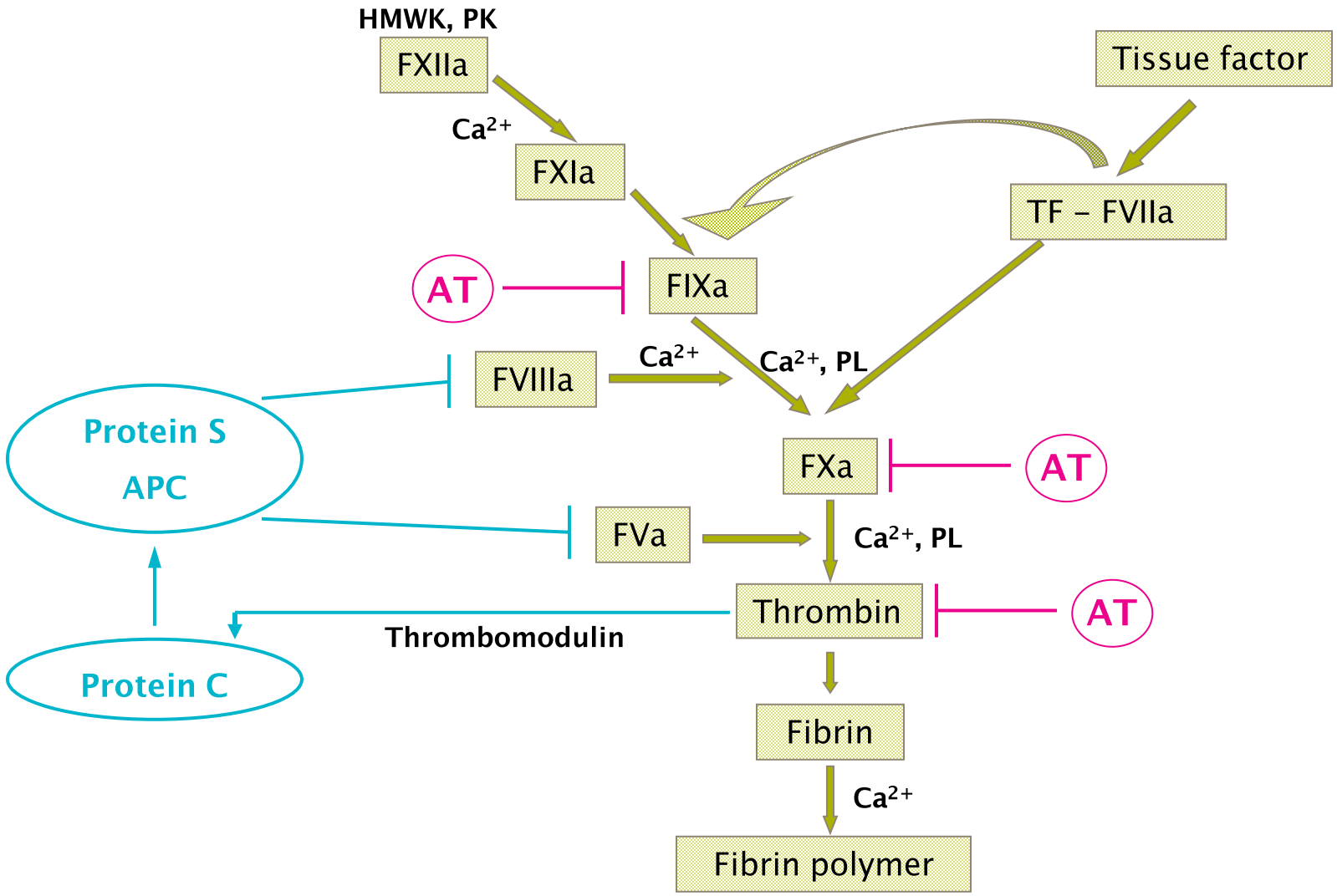
Prevalence and thrombotic risk

Table 1. Prevalence of thrombophilia and relative risk estimates for various clinical manifestations

	Antithrombin deficiency	Protein C deficiency	Protein S deficiency	Factor V Leiden	Prothrombin 20210A mutation	Lupus anticoagulant*	Anti-cardiolipin antibodies*	Anti-β2 GPI antibodies
Prevalence in the general population	0.02%	0.2%	0.03%-0.13%	3-7%	0.7%-4%	1%-8 %	5	3.4
Relative risk for a first venous thrombosis	5-10	4-6.5	1-10	3-5	2-3	3-10	0.7	2.4
Relative risk for recurrent venous thrombosis	1.9-2.6	1.4-1.8	1.0-1.4	1.4	1.4	2-6	1-6	
Relative risk for arterial thrombosis	No association	No consistent association	No consistent association	1.3	0.9	10	1.5-10	

Adapted from: S. Middeldorp, ASH Education Book 2011; 1: 150-155

COAGULATION CASCADE



INHERITED THROMBOPHILIA

What to test?

‘Loss of function’ mutations

Deficiencies of natural anticoagulants

- Antithrombin
- Protein S
- Protein C

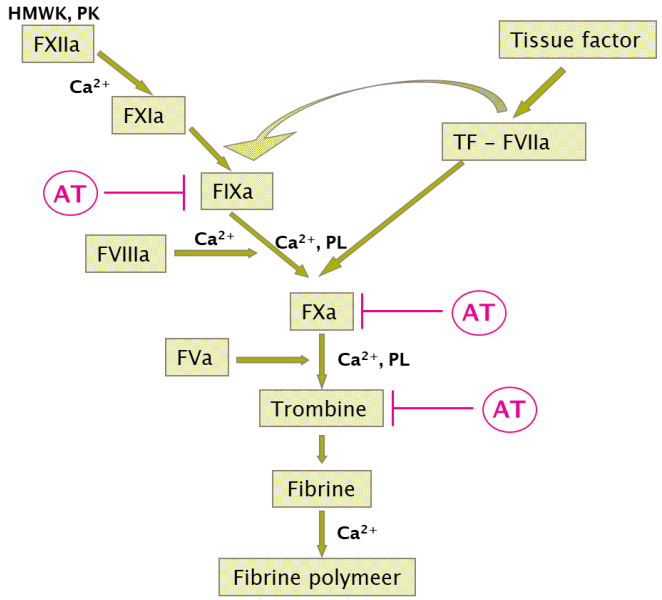
‘Gain of function’ mutations

APC resistance/ Factor V Leiden

Prothrombin G20210A variant

INHERITED THROMBOPHILIA

1. Antithrombin deficiency



Antithrombin (AT)

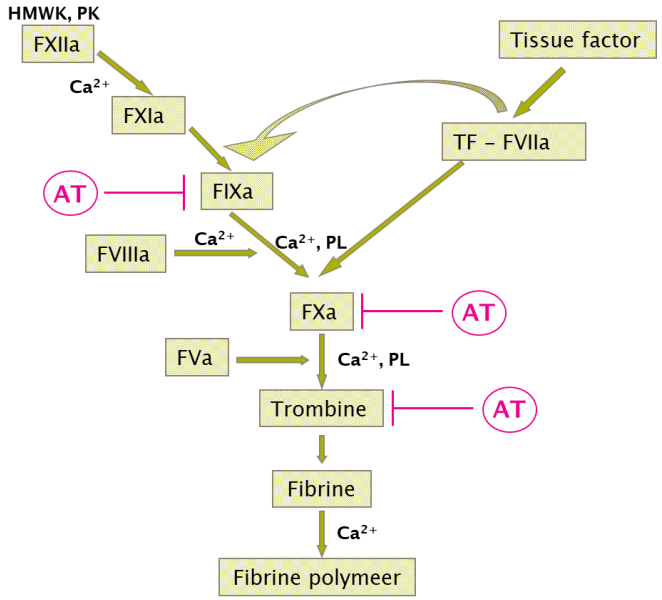
- serine protease inhibitor
- inhibits predominantly thrombin (FII), factor Xa and FIXa
- enhanced activity (x1000) after binding with heparin or heparan sulfates (on endothelium)
- encoded by *SERPINC1*, located on chromosome 1

Acquired AT deficiency:

heparin therapy, liver pathology, nephrotic syndrome, DIC...

INHERITED THROMBOPHILIA

1. Antithrombin deficiency



Inherited AT deficiency:

autosomal dominant inheritance
prevalence: 0.2 – 0.5% in general population

Laboratory assay:

antithrombin activity

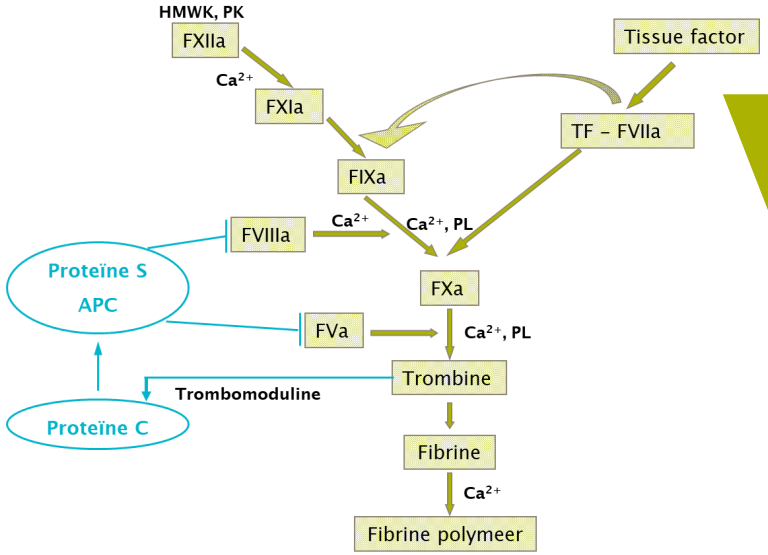
Classification:

- Type I: quantitative deficiency (homozygous type I –> probably lethal)
- Type II: qualitative deficiency Subtypes: Heparin Binding Site (HBS)
  Reactive Site
  Pleiotropic Effects

⇒ Thrombotic risk may depend on (sub)type of AT deficiency
Type II HBS variants < other (sub)types

INHERITED THROMBOPHILIA

2. Protein C deficiency



Protein C

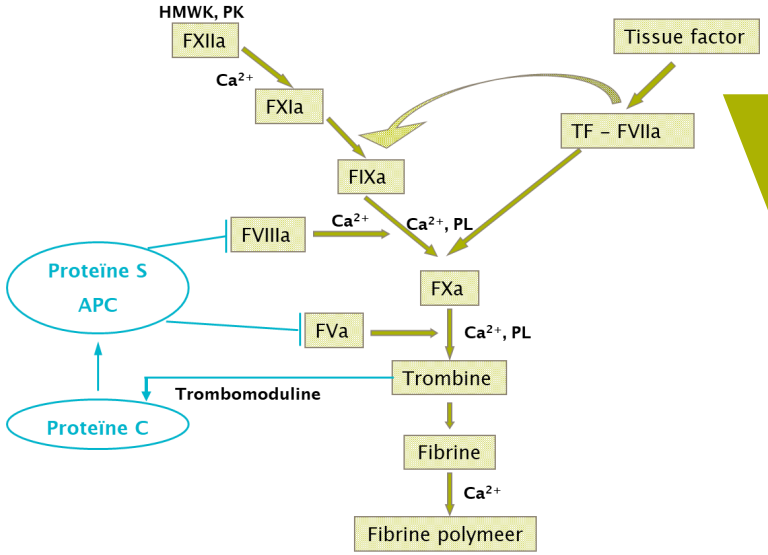
- vitamin K–dependent plasma glycoprotein
- needs conversion to activated protein C (APC)
- proteolytically inactivates FVa and FVIIIa
- encoded by *PROC*, located on chromosome 2

Acquired PC deficiency:

vitamin K–antagonists, liver pathology, DIC, vitamin K deficiency

INHERITED THROMBOPHILIA

2. Protein C deficiency



<u>Inherited protein C deficiency:</u>

autosomal dominant (! Neonatal purpura fulminans in homozygotes)

prevalence: ~0.3% in global population

<u>Laboratory assay:</u>

protein C activity

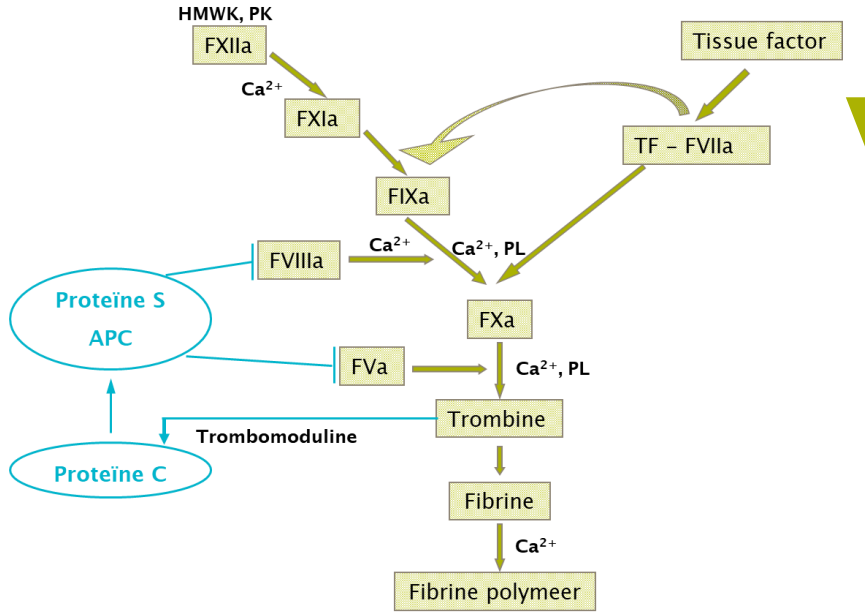
<u>Classification:</u>

Type I (quantitative): reduced production of the protein

Type II (qualitative): normal production of the protein but dysfunctional

INHERITED THROMBOPHILIA

3. Protein S deficiency



Protein S

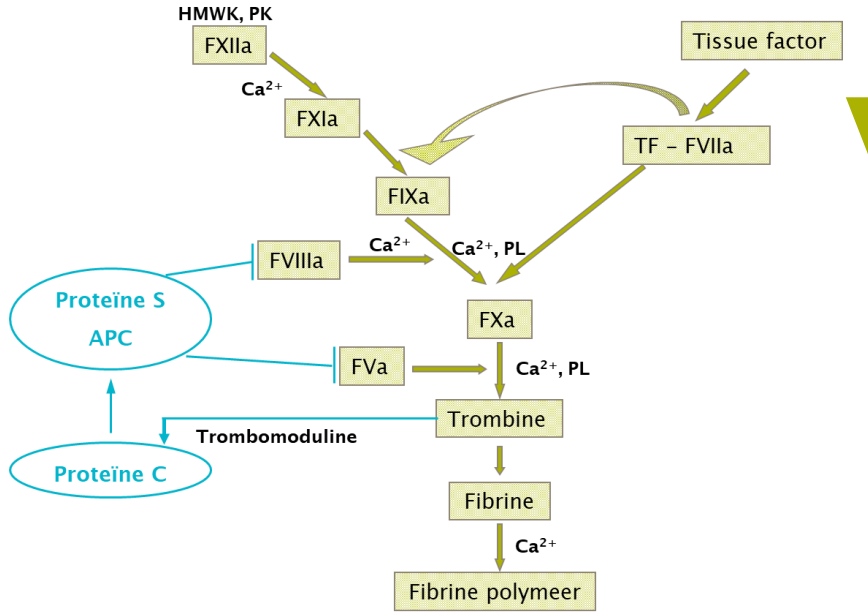
- vitamin K-dependent plasma glycoprotein
- 60% bound to C4b-binding protein and 40% free
- only free protein S exerts cofactor function of protein C
- encoded by *PROS1* gene, located on chromosome 3

Acquired protein S deficiency:

vitamin K-antagonists, liver pathology, asparaginase therapy, DIC, pregnancy, oral contraceptives,..

INHERITED THROMBOPHILIA

3. Protein S deficiency



Inherited protein S deficiency:

autosomal dominant

prevalence: 0.03 – 0.13% in global population

Laboratory assay:

free protein S antigen

Classification:

Classification	Total PS Antigen	Free PS Antigen	PS Activity
Type I	Low	Low	Low
Type II	Normal	Normal	Low
Type III	Normal	Low	Low

INHERITED THROMBOPHILIA

4. APC Resistance – Factor V Leiden

Acquired:

pregnancy, oral contraceptive use, hormonal substitution therapy, elevated FVIII, …

Mechanism?

Congenital:

Most frequent thrombophilia: 5% among Caucasians

Due to high prevalence: heterozygous and homozygous individuals!

INHERITED THROMBOPHILIA

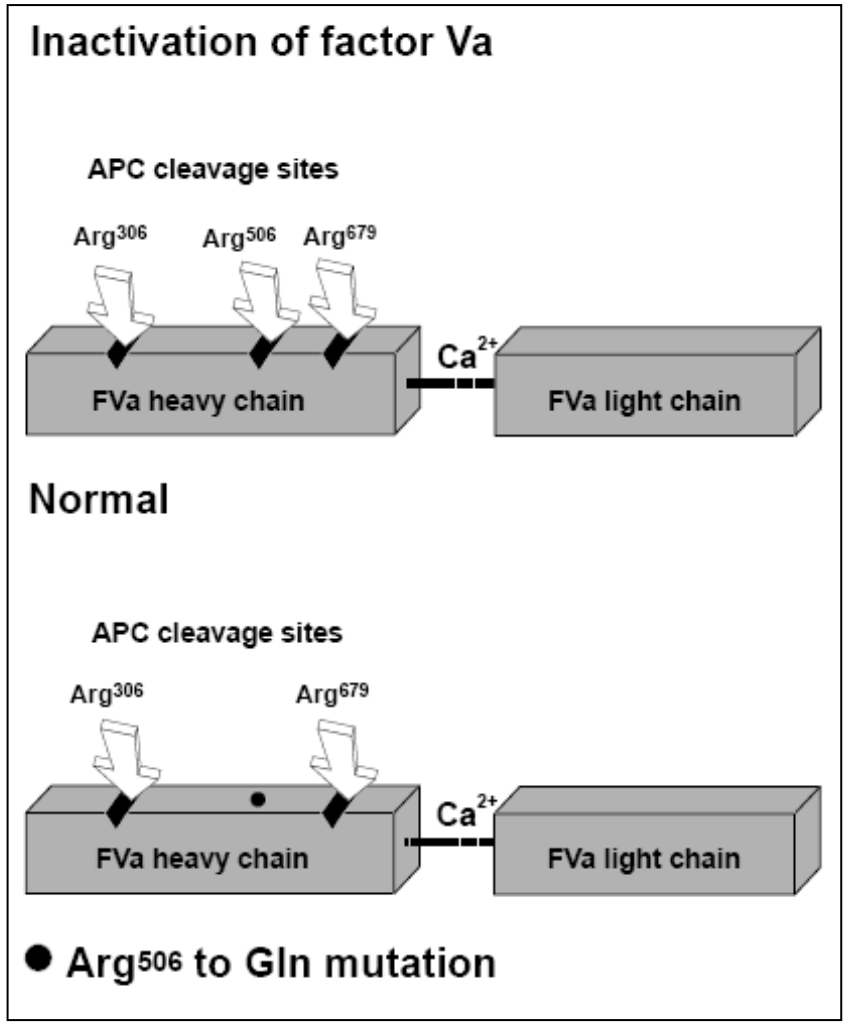
4. APC Resistance – Factor V Leiden

Molecular basis:

→ Factor V normally inactivated by APC by proteolytic degradation at 3 cleavage sites

→ Factor V Leiden (p.R506Q)

- Normal clotting activity
- Resistant to inactivation by APC



INHERITED THROMBOPHILIA

APC Resistance – Factor V Leiden

First-line assay:

modified APC-resistance assay (i.e. predilution in FV deficient plasma)

insensitive to acquired APC resistance

Second-line assay

molecular confirmation of positive APCR samples (hetero- vs. homozygous)

RIZIV/INAMI

'…enkel indien het opzoeken van geactiveerde proteïne C resistentie positief is met de specifieke gemodifieerde APC-R test'

'…uniquement en cas de recherche positive de la résistance à la protéine C activée par le test APC-R spécifique modifié'

INHERITED THROMBOPHILIA

5. Prothrombin G20210A

Prevalence:

1-4% among Europeans

More prevalent in Southern Europe

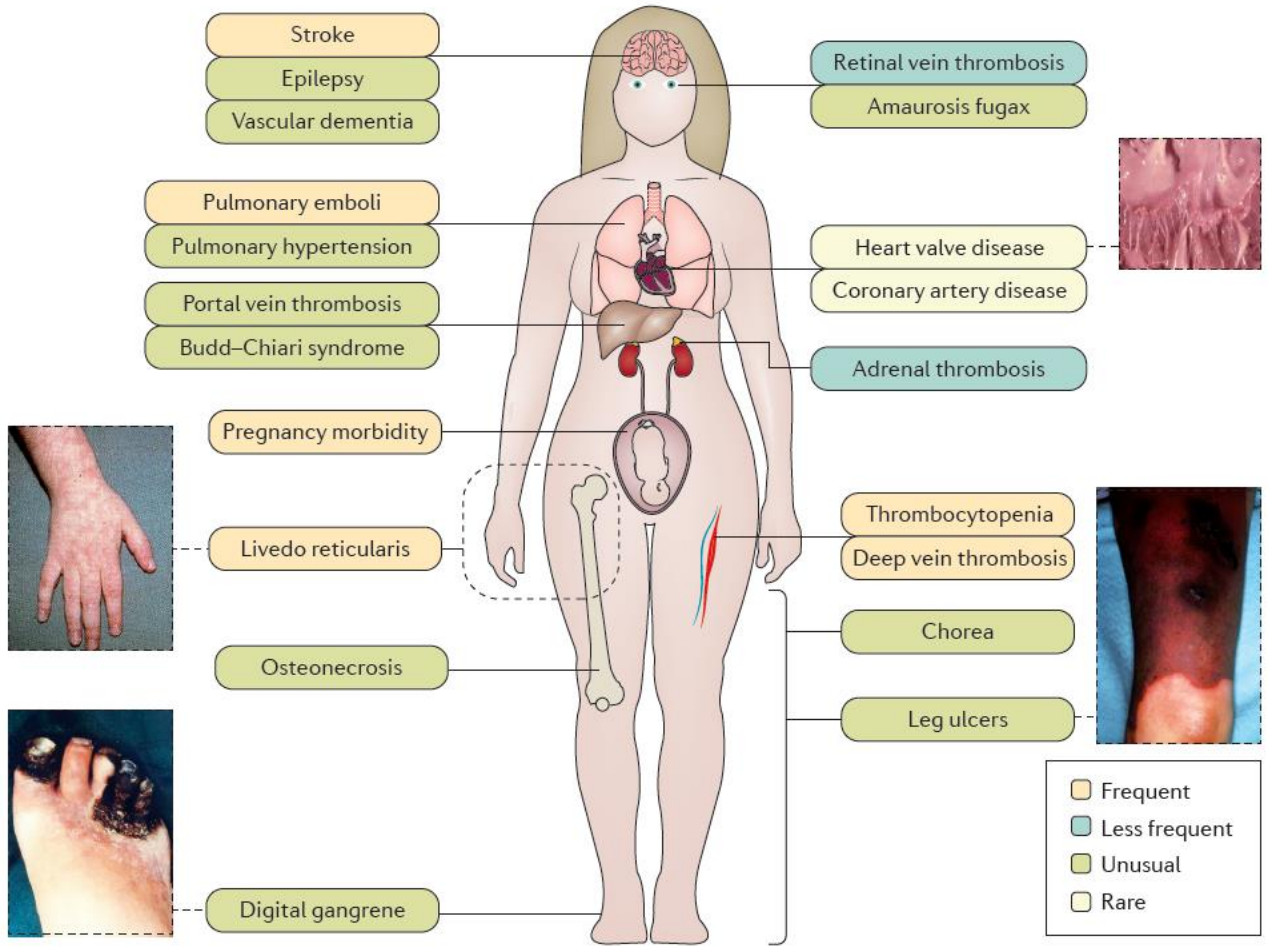
Molecular basis

Single point mutation in prothrombin gene, g.G20210A (3' untranslated region)

Associated with increased prothrombin levels but levels do not allow distinction between carriers and non-carriers

ACQUIRED THROMBOPHILIA

Antiphospholipid syndrome (APS)



Schreiber, K. et al. Antiphospholipid syndrome. Nature Reviews Disease Primers 4, 2018, Jan 11;4: 17103. doi: 10.1038/nrdp.2017.103.

ACQUIRED THROMBOPHILIA

Antiphospholipid syndrome

1. Clinical criteria:

- Venous/arterial thrombosis
- Pregnancy complications

  premature birth due to (pre)eclampsia or placental insufficiency, miscarriages (>1 after10 wks or >3 before 10 wks)

2. Laboratory criteria:

Persistent positivity for

- Lupus anticoagulant
- and/or anticardiolipin Ab (IgG/IgM)
- and/or anti-β2-glycoprotein I Ab (IgG/IgM)

Box 1 | **The classification criteria for definite APS**

The revised classification criteria for antiphospholipid syndrome (APS) are referred to as the Miyakis criteria[2]. A patient has to fulfil at least one clinical criteria and at least one laboratory criteria.

Clinical criteria

Vascular thrombosis

≥1 clinical episode of arterial, venous or small-vessel thrombosis. Thrombosis must be objectively confirmed. If histopathological confirmation is used, thrombosis must be present without inflammation of the vessel wall.

Pregnancy morbidity

- ≥1 unexplained death of a morphologically normal fetus ≥10 weeks of gestation
- ≥1 premature delivery of a morphologically normal fetus <34 weeks gestation because of severe pre-eclampsia or eclampsia (defined according to standard definitions) or recognized features of placental insufficiency
- ≥3 unexplained consecutive miscarriages at <10 weeks of gestation, with maternal and paternal factors (such as anatomical, hormonal or chromosomal abnormalities) excluded

Laboratory criteria

The presence of antiphospholipid antibodies on ≥2 occasions at least 12 weeks apart and <5 years before clinical manifestations, as demonstrated by ≥1 of the following:

- Presence of lupus anticoagulant in plasma
- Medium titre to high titre of anticardiolipin antibodies (>40 GPL* or MPL*, or >99th percentile‡) of immunoglobulin G (IgG) or IgM isotypes
- Anti-β2-glycoprotein 1 antibodies of IgG or IgM isotypes present in plasma

*GPL and MPL are arbitrary units; 1 GPL or MPL refers to 1 µg of IgG or IgM antibody, respectively. ‡Exact value depends on the assay.

Schreiber, K. et al. Antiphospholipid syndrome. Nature Reviews Disease Primers 4, 2018, Jan 11;4: 17103. doi: 10.1038/nrdp.2017.103.

THROMBOPHILIA

What not to test?

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DOI: 10.1002/rth2.12739

FORUM

A call to action: MTHFR polymorphisms should not be a part of inherited thrombophilia testing

Thomas G. Deloughery MD, MACP, FAWM[1] | Beverley J. Hunt OBE[2] | Geoffrey D. Barnes MD, MSc[3] | Jean M. Connors MD[4] | The WTD Steering Committee

Thrombophilia testing: who and when to test

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ACQUIRED THROMBOPHILIA

When to test for antiphospholipid syndrome?

- Younger patients (<50 years) with unprovoked VTE
- VTE at unusual sites
- Younger patients (<50 years) with ischemic stroke or transient ischemic attack
- Arterial thrombosis in other sites in younger patients (< 50 years)
- Microvascular thrombosis
- Recurrent VTE unexplained by subtherapeutic anticoagulation, nonadherence or malignancy
- Pregnancy morbidity

  fetal loss after 10 weeks, recurrent 1st trimester miscarriage, prematurity (<34 weeks' gestation) associated with severe (pre)eclampsia, HELLP syndrome, placental insufficiency (fetal growth restriction), stillbirth
- Systemic Lupus Erythematosus (part of SLE diagnosis guidelines)

Devreese KMJ et al. Update of the guidelines for lupus anticoagulant detection and interpretation. Guidance from the Scientific and Standardization Committee for lupus anticoagulant/antiphospholipidantibodies of the International Society on Thrombosis and Haemostasis. J ThrombHaemost 2020; 18:2828–2839

INHERITED THROMBOPHILIA

Important considerations before testing

- ✓ Explanation for thrombotic event?

  -> thrombophilic trait only in 50% of cases!
- ✓ Prediction of recurrence?
- ✓ Educate and inform asymptomatic family members?

  -> to prevent first thrombotic event?
- ✓ To guide treatment?

INHERITED THROMBOPHILIA

Prediction of recurrence after first VTE?

- EPCOT study (European Prospective Cohort on Thrombophilia)

  Inclusion criteria: patients with inherited thrombophilia and 1 VTE

TABLE 2. Incidence Rates (%/year) of a Recurrent DVT or PE in Patients Who Did Not Receive Long-Term Anticoagulant Treatment During Prospective Follow-Up

Patients	n	Time Between 1st Event and Study Entry, Mean Years (Range)	DVT/PE, n	Person Years, n	Incidence Rate, %/Year (95% CI)	Incidence Rate Men, %/Year (95% CI)	Incidence Rate Women, %/Year (95% CI)
All	180	6.4 (0–38)	44*	881.7	5.0 (3.6–6.7)	9.6 (6.3–13.9)	2.8 (1.7–4.5)
PC deficiency	37	6.3 (0–27)	10	195.0	5.1 (2.5–9.4)	10.8 (4.0–23.4)	2.9 (0.8–7.4)
PS deficiency	25	5.3 (0–16)	8	122.4	6.5 (2.8–11.8)	10.5 (3.9–22.9)	3.1 (0.4–11.0)
AT deficiency	11	9.3 (1–24)	6	57.4	10.5 (3.8–22.8)	11.6 (2.4–33.9)	9.5 (2.0–27.8)
FVL	79	5.3 (0–38)	13 (1 AA)	366.3	3.5 (1.9–6.1)	7.2 (2.9–14.9)	2.2 (0.8–4.8)
Multiple defects	28	9.4 (1–30)	7†	140.6	5.0 (2.0–10.3)	10.7 (3.5–24.9)	2.1 (0.3–7.7)

⇒ Highest risk for recurrence in patients with deficiency of AT/PC/PS or combined thrombophilia

C. Vossen et al. Artherioscler Thromb Vasc Biol 2005; 25: 1992-1997

INHERITED THROMBOPHILIA

Reasons not to test

- ✓ Psychological impact of positive test result
- ✓ False reassurance in case of negative test result
- ✓ High costs
- ✓ Pitfalls in laboratory diagnosis!
  - Sensitivity of commercial assays
  - Interference by anticoagulant drugs (VKA, DOAC's,…)
  - ….

INHERITED THROMBOPHILIA TESTING

Guidelines – often conflicting!

Do not test:

- Provoked VTE
- Arterial thrombosis

Consider testing:

- Unprovoked VTE in young patients and positive family history of VTE
- Recurrent VTE
- Unusual site thrombosis (cerebral or splanchnic veins)
- 1st degree (female) relatives of patients with deficiency of AT/PC/PS

  -> may influence choice of oral contraception, VTE prophylaxis during pregnancy

1) Connors JM, NEJM 2017; 377: 1177-87
2) Carroll B et al, Vasc Med 2018; 23: 388-399
3) Arachchillage D et al, Br J Haematol 2022; 198: 443-458

Thrombophilia testing: practical considerations

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THROMBOPHILIA TESTING

Practical considerations

- Avoid blood sampling during acute phase of thrombotic event
- Avoid blood sampling when patient is treated with anticoagulant therapy (vitamin K-antagonist, direct oral anticoagulants, heparin)
- Confirm deficiencies of antithrombin, protein C and protein S on a 2nd sample and consider genetic testing (*SERPINC1 – PROC – PROS1*)
- Consult your laboratory for special cases!

Take home messages

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TAKE HOME MESSAGES

Testing for inherited thrombophilia can be *considered* in *selected* patients

- Young patients with VTE > risk of recurrence (debatable!)
- VTE at unusual sites
- Neonatal purpura fulminans: urgency!
- Asymptomatic (female) family members of patients with deficiency of antithrombin, protein C or protein S

TAKE HOME MESSAGES

Testing for antiphospholipid syndrome should be performed (according to guidelines)

- Young patients with venous/arterial thrombosis
- Women with pregancy morbidity

Collaboration between physician and laboratory is crucial!

- Physician: needs to know limitations of laboratory assays
- Laboratory: needs clinical information for correct interpretation of results

Some people with inherited thrombophilia may never develop thrombosis

Others suffer from thrombosis whithout any (known) risk factor





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