Risk factors for venous thrombosis: first episode and recurrence

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ISTH Educational Course on Thrombosis, Thrombophilia, Thrombolysis and DIC

Moscow, 17-19 September 2014

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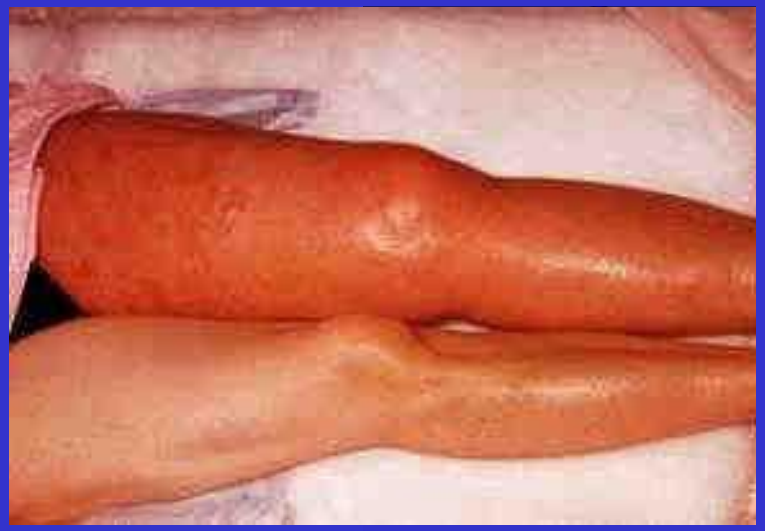
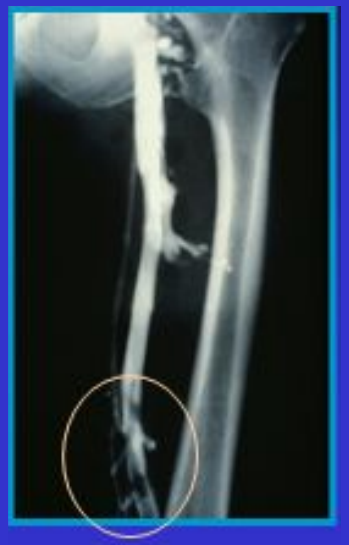
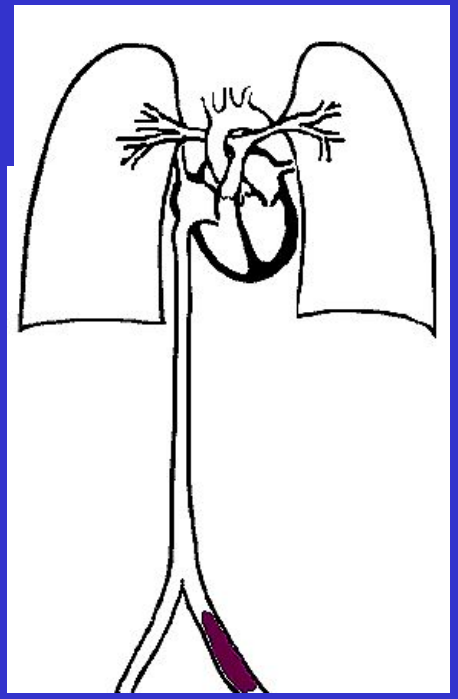
Deep vein thrombosis and pulmonary embolism







Deep vein thrombosis

- incidence 1-2 per 1000 per year
- pulmonary embolism in 35%
- postthrombotic syndrome in 25%
- fatalities 6% acute, 20% after one year

(Oger, Thromb Haemost 2000; Naess, J Thromb Haemost 2007)

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Rudolf Virchow

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Virchow: clots and thrombosis

Rudolf Virchow (1821-1902)

- autopsy studies that showed clots in legs and lungs of patients who died of pulmonary embolism (1846)

- theory on the pathogenesis of thrombosis ("Virchow's triad")
  - stasis
  - blood components
  - vessel wall

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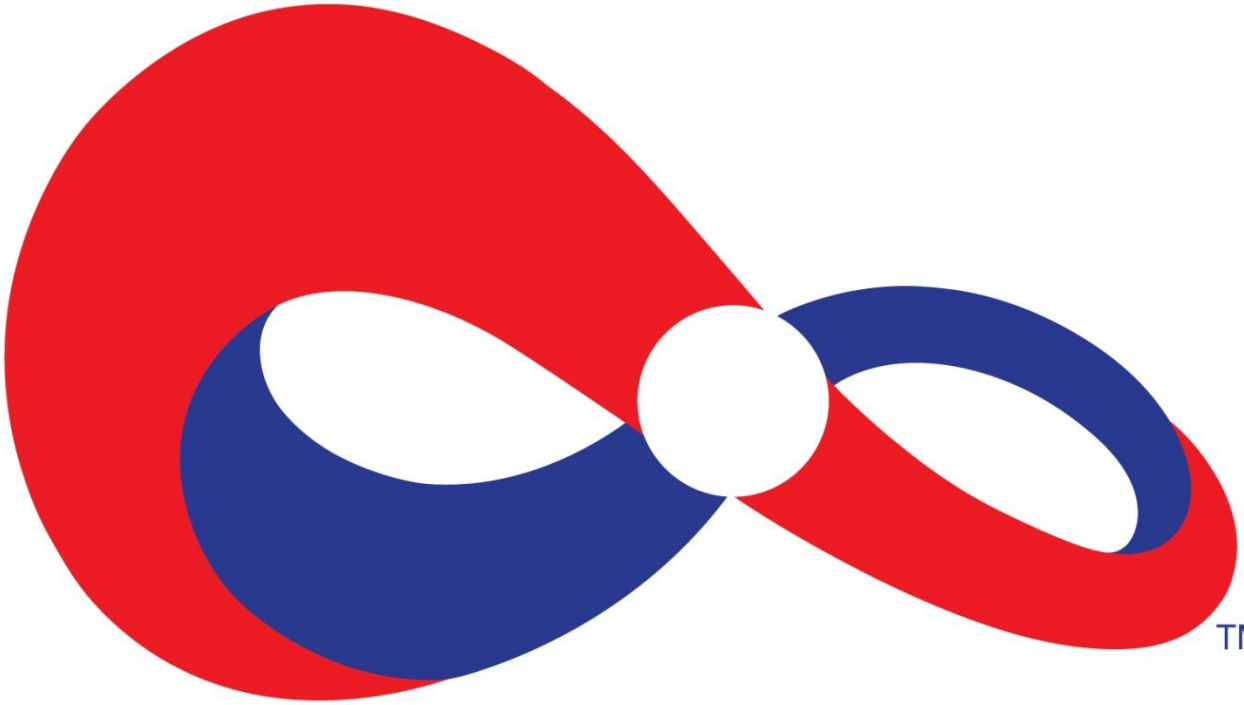

WORLD THROMBOSIS DAY

OCTOBER 13

Causes of thrombosis - Virchow

- Die marantische Thrombose
  - Krebs, Typhen, Geschwächten Herzkraft, Gangraena senilis, Tuberkulose
- Die Compressions-Thrombose
  - Tuberkulose, Dislocation von Knochen, Druck von Geschwülsten
- Die Dilatations-Thrombose
  - Aneurysmen, Varices
- Die traumatische Thrombose
  - Amputations-Thrombose, Aderlass-Thrombose
- Die Thrombose der Neugeborenen

- Die puerperalen Thrombosen

- Entzündung der Gefässwand; Eindringen von Eiter in das Gefässlumen

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Causes of thrombosis - today

age
major surgey
neurosurgery
orthopaedic surgery
prostatectomy
trauma
prolonged bed rest
central venous catheter
plaster cast
malignancy
chemotherapy
psychotropic drugs
myeloproliferative disease
obesity
smoking
no alcohol

thalidomide
oral contraceptives
hormone therapy
long haul travel
heparin induced thrombopenia
hyperthyroid disease
Cushing syndrome
high FVIII
high VWF
high FIX
high FXI
high prothrombin
lupus anticoagulant
dysfibrinogenaemia
low TFPI
high PCI

high TAFI
hypofibrinolysis
hyperhomocysteinaemia
hypercysteinemia
non-0 blood group
antithrombin deficiency
protein C deficiency
protein S deficiency
factor V Leiden
prothrombin 20210A
factor XIII val34leu
SERPINC1 (rs2227589)
FXI (rs2289252)
FXI (rs2036914)
GP6 (rs1613662)
FV (rs4524)

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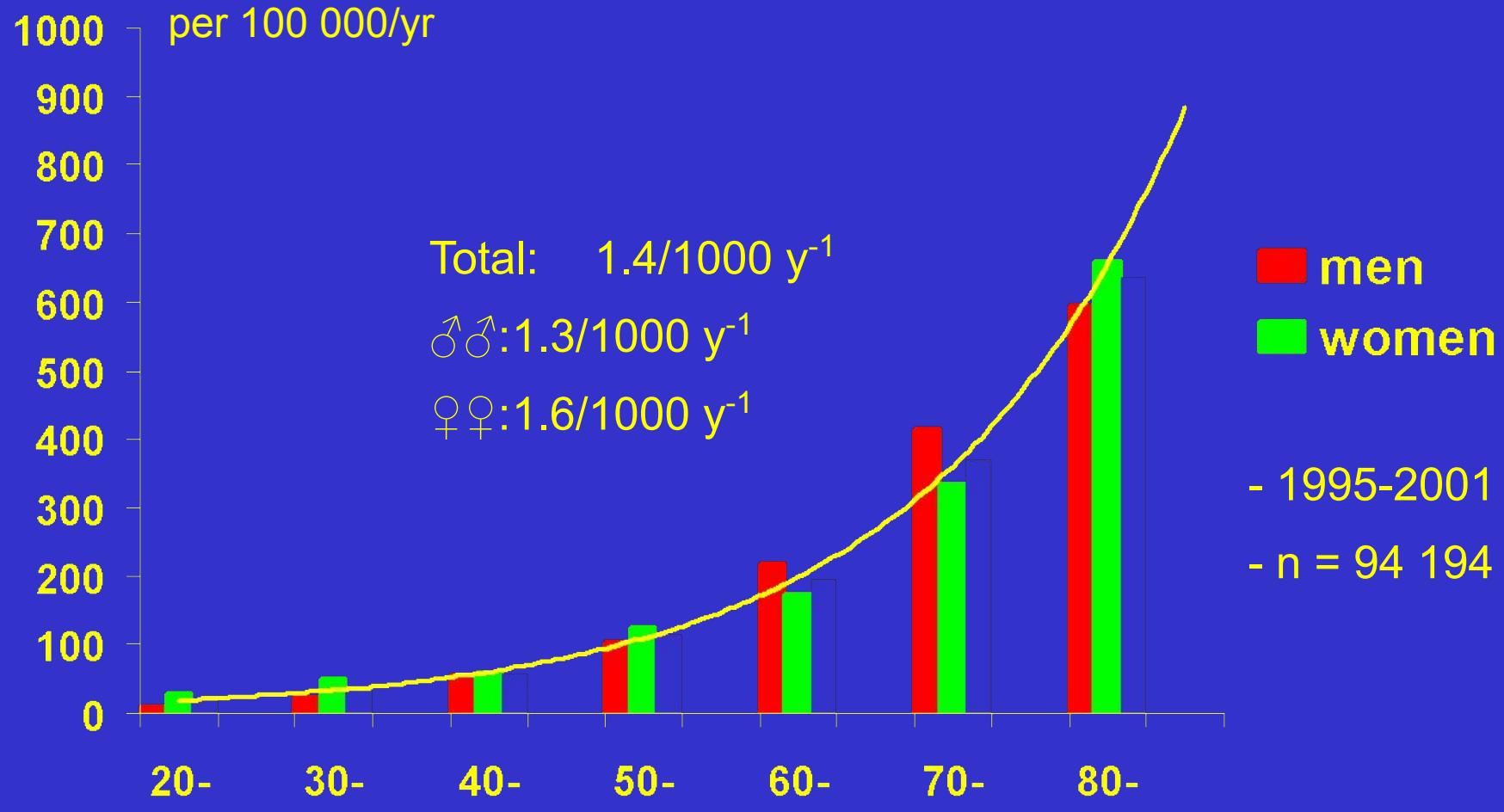
Venous thrombosis by age



(Naess, J Thromb Haemost 2007)

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Ten unresolved questions

Unresolved question 1

- Why the steep age-increase?
  - note: 2/3 of patients > 65 yrs
  - virtually no studies including elderly people!

Candidate explanations

- higher prevalence of risk factors with age
  - co-morbidity
  - immobilisation
- age-specific risk factors
  - frailty
- vessel wall changes
- increasing prevalence of history of asymptomatic events
  - ‘new’ events are recurrent events

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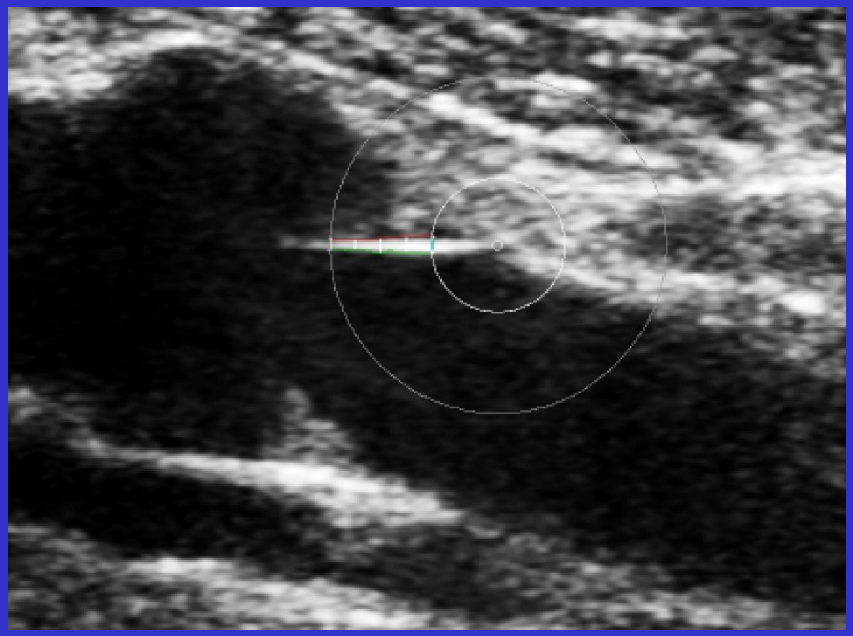
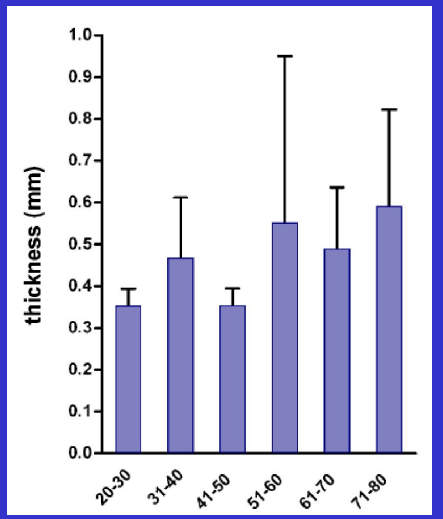
Venous valves



PEDLAR study

- venous valve thickness with ultrasound
- 77 healthy individuals
- mild increase with age



(van Langevelde, ATVB 2010)

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Age-specific factors

Age-specific risk factor	Highest quartile (> 35.5 kg) OR (95% CI)	Lowest quartile(<22 kg) OR (95% CI)
Handgrip strength	1 (ref)	2.8 (1.1-6.9)
	No OR (95% CI)	**Yes** OR (95% CI)
ADL (Dependent)	1 (ref)	2.0 (1.1-3.4)
Venous stasis		
	No OR (95% CI)	**Yes** OR (95% CI)
Varicosis	1 (ref)	1.6 (1.0-2.7)
Leg edema	1 (ref)	4.6 (2.3-8.7)
Compr. stockings	1 (ref)	2.6 (1.4-5.0)

AT AGE study

- 500 VT patients > 70 yrs
- healthy controls

(Engbers, ms submitted)

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Causes of thrombosis

major surgey
prostatectomy
neurosurgery
orthopaedic surgery
trauma
prolonged bed rest
life style
central venous catheter
plaster cast
malignancy
chemotherapy
psychotropic drugs
myeloproliferative disease
obesity

thalidomide
oral contraceptives
hormone therapy
long haul travel
psychotropic drugs
hyperthyroid disease
non-0 blood group
high FVIII
high VWF
high FIX
high FXI
high prothrombin
lupus anticoagulant
dysfibrinogenaemia

low TFPI
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hyperhomocysteinaemia
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antithrombin deficiency
protein C deficiency
protein S deficiency
factor V Leiden
prothrombin 20210A
factor XIII val34leu
SERPINC1 (rs2227589)
CYP4V2 (rs13146272)
GP6 (rs1613662)

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Unresolved question 2

- too many risk factors

.....And 3

- what is the use of finding more and more risk factors (with marginal odds ratios)?

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Too many risk factors

- Suppose we did not know the cause of reproduction, and we did a genome and sociome scan. Positive associations for:
  - age
  - having a partner
  - previous children
  - mild alcohol intake
  - free of severe co-morbid states
  - spending a weekend in Paris
  - no trombophilia
  - etc
  - ethnicity
  - educational level
  - religion (but not priests)
  - cold winters
  - absence of crime-enhancing genes
  - no gross chromosomal abnormalities
  - many SNPs

- many risk factors represent the same mechanism

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Risk factors for thrombosis

- genes
- environment
- behaviour (including life style)
- combinations

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Risk factors for thrombosis

- genes
- environment
- behaviour (including life style)
- combinations

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Causes thrombosis

Stasis	Blood
age	anticoagulant defects
immobilisation	procoagulant defects
	hormones
	cancer

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When to believe?

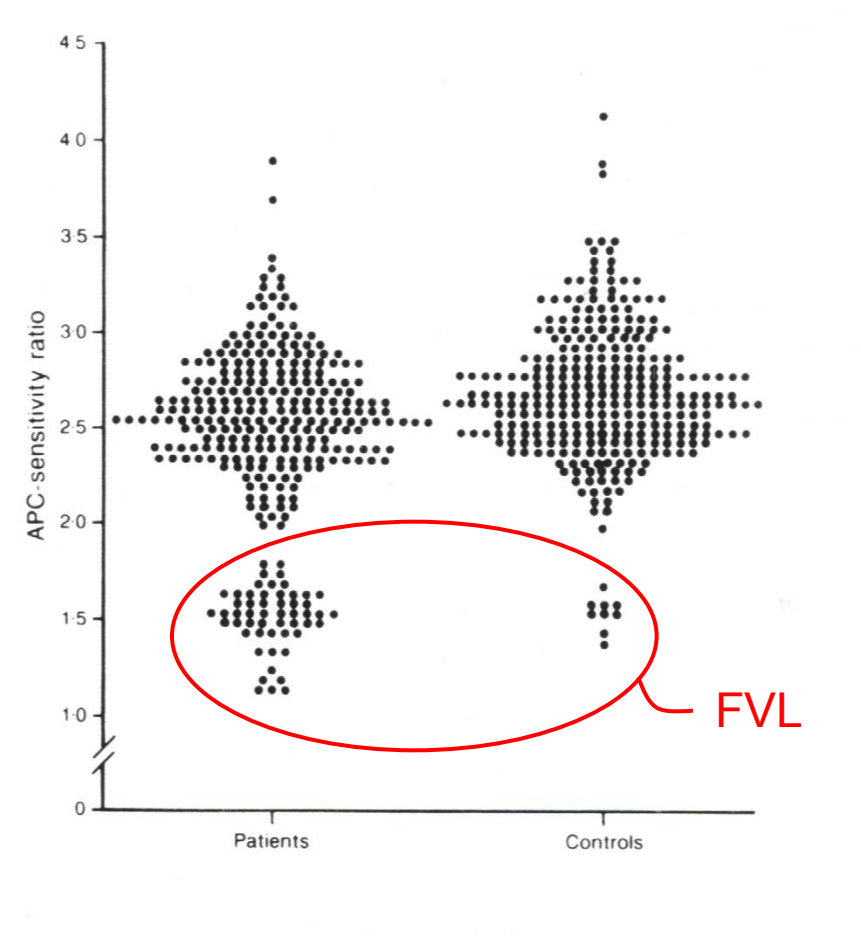
Two premises of Lane:

The first is fundamental. In order for a gene change to have an effect, it must be mediated through a phenotype. In studies that report consistent relationships linking polymorphisms, phenotype and clinical effect, there can be some confidence that the genetic variation is influencing disease.

The second related premise to be used is that if a polymorphism is producing an important contribution to disease, then it will be observed consistently in studies designed to minimise bias.

(Simmonds, Thromb Haemost 2001)

Intermediate phenotype



Factor V Leiden > APC-resistance

Factor V Leiden > risk

(Mendelian randomisation)

APC-resistance > risk

(intermediate phenotype)

(Dahlbäck, PNAS 1993; Bertina, Nature 1994; Rosendaal, Blood 1995)

Established genetic risk factors

	pop.(%)	RR	
• protein C deficiency	0.2	10	family studies
• protein S deficiency	0.1	10	
• antithrombin deficiency	0.02	20	
• ABO blood group (non-O)	50	2	association studies
• factor V Leiden	3-5	3-8	
• prothrombin 20210A	2	3	

(Heijboer, NEJM 1990; Koster, Blood 1995; Jick, Lancet 1969; Bertina, Nature 1994; Rosendaal, Blood 1995; Poort, Blood 1996)

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Conundrum

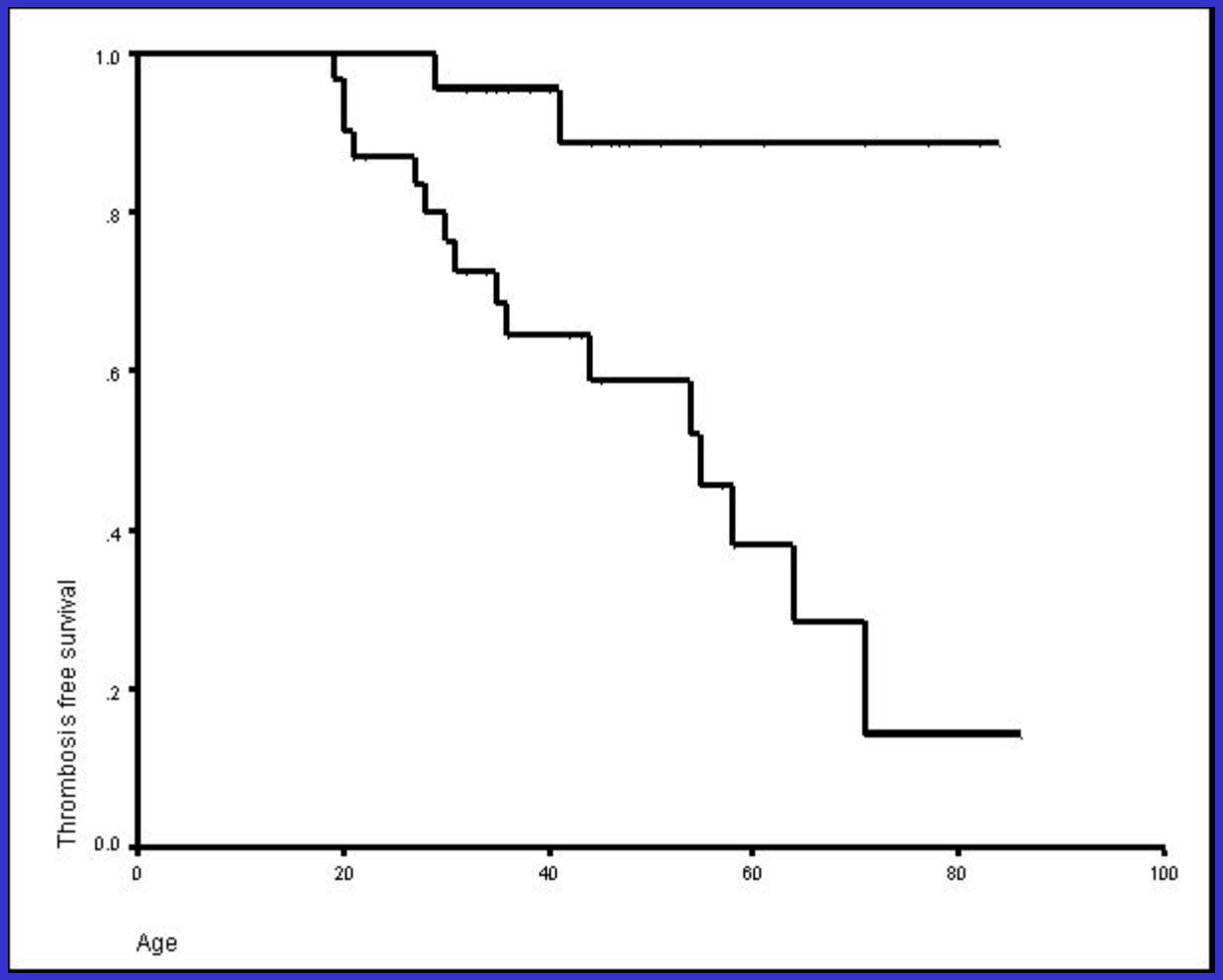
- deficiencies of PC, PS, AT in the population not impressive

LETS study (n=1000)
low PC (<55U/dl) RR=4.0
low total (<67U/dl) and free PS (<57U/dl) RR=1.7
low antithrombin (<80U/dl, 2x) RR=5.0
MEGA study (n=5000):
low PS (<67 U/dl) RR=0.9

- some misclassification (low levels vs deficiency)
- true deficiencies really rare?
- all families have multiple defects?
- all unhappy families unique (but true)
- consequences for medical practice unclear

(Koster, Blood 1995, Pintao Blood 2013)

Protein C deficiency: 1993 view



no defect

defect

24 families
161 individuals

(Allaart, Lancet 1993)

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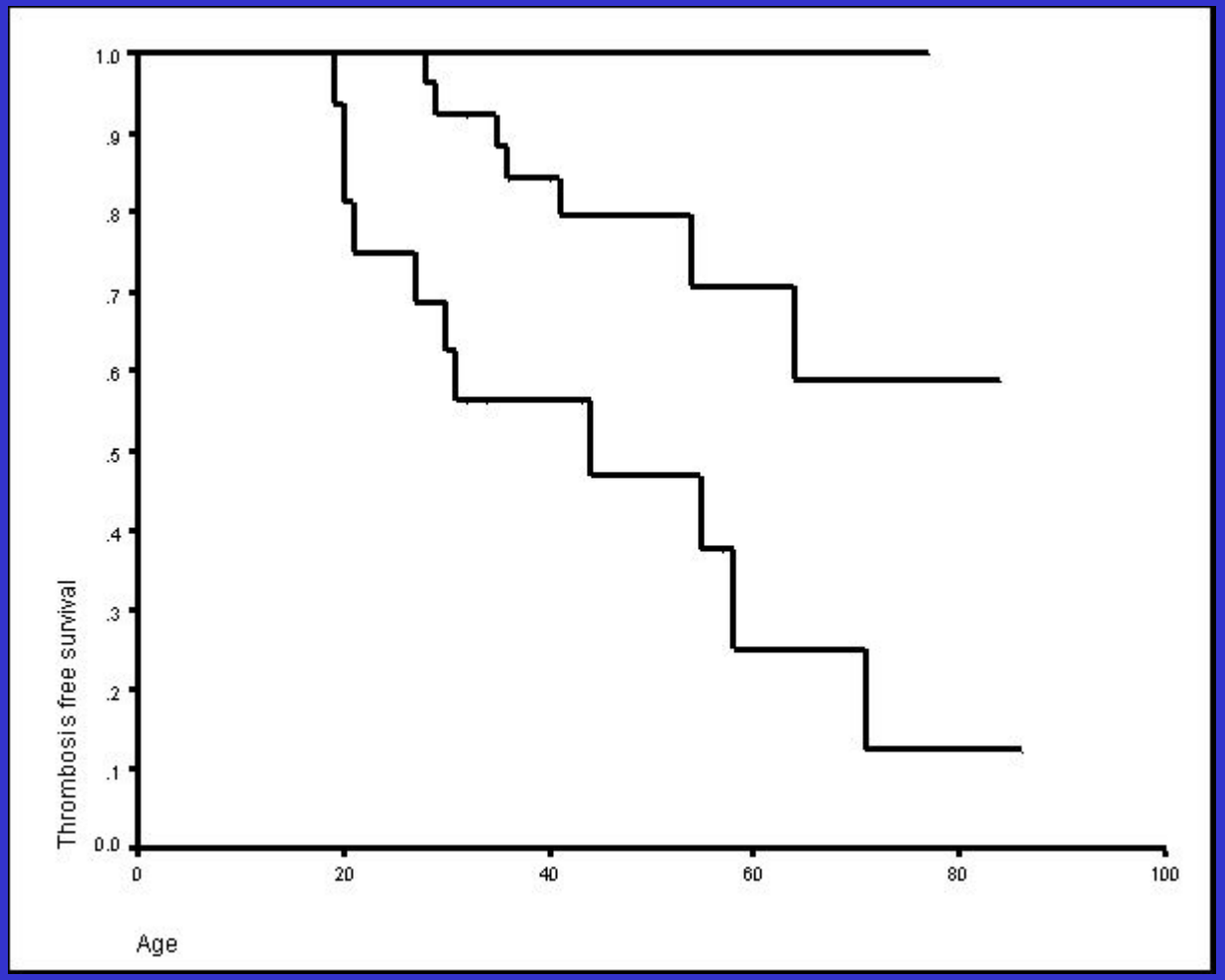
Protein C deficiency: 1994 view



no defect

one defect

(PC or FVL)

two defects

(PC and FVL)

(Koeleman, Blood 1994)

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Weak risk factors

	pop(%)	OR	
FXIII			
val34leu (rec.)	6	0.6	
Protein C			
A2418G	19	1.3	
Fibrinogen			
FGA Thr312Ala	26	1.2	
FGB A8259G (his95arg)		14	1.5
FGB 455G/A	21	1.3	
FGG C10034T	6	2.4	

(van Hylckama Vlieg, BJH 2002; Spek, ATVB 1995; Pomp TH 2009; Carter, Blood 2000; Komanasin, JTH 2005; Uitte de Willige, Blood 2005; Smith, JAMA 2007; den Heijer JTH 2005; Bezemer, Arch Intern Med 2007)

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Are there more genetic causes?

- in families with hereditary thrombophilia, 30% no defect found

- high recurrence risk idiopathic thrombosis, compared to low recurrence rate after surgery

- study of 751 pedigrees in Minnesota
  - 16650 individuals
  - polygenic model
  - heritability 62% (idiopathic thrombosis)

(Heit, J Thromb Haemost 2004)

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How to find them?

- association studies
  - unrelated individuals
  - usually case-control
  - can be large: high power
  - may suffer from admixture

- family studies
  - related individuals
  - usually linkage or case-control
  - relatively small (low power)
  - enriched for heritable factors (high power)
  - information on relations can be used (linkage)
    - no admixture

Recent studies

- Studies looking at a few SNPs in candidate genes
  - Smith, JAMA 2007: 24 candidates
- GWAS on disease
  - Bezemer, JAMA 2008 (coding variants)
  - Trégouet, Blood 2009 (genome wide)
- GWAS on hemostatic markers
  - Smith, CHARGE consortium, Blood 2009

Functional Genome-wide Screen

- gene-centred approach

- SNPs likely to be functional
  - 20 000 SNPs in 10 000 genes
    - missense/nonsense, modifiers of splice sites
    - mostly MAF>5%

- re- and triplication (total 10 000 samples)
  - allele frequencies in pools (n=30-100)
  - individual genotyping
  - fine mapping

(Bezemer, JAMA 2008; Bezemer ,JAMA 2010; Li, J Thromb Haemost 2009)

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Study Populations

- LETS (Leiden Thrombophilia Study)
- MEGA (Multiple Environmental and Genetic Assessment of risk factors for venous thrombosis)

- LETS 474 patients with VT of the leg (DVT)
  474 control subjects
- MEGA-1 1420 patients with VT of the leg (DVT)
  1784 control subjects
- MEGA-2 1356 patients with VT of the leg (DVT)
  2907 control subjects

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Functional Genome-wide Screen

19,682 SNPs

1,206 SNPs

1,206 SNPs

104 SNPs

104 SNPs

18 SNPs

18 SNPs

4 SNPs (p<0.05, FDR<0.20)

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18 SNPs in 18 genes

NR112
GP6
APOH
NAT8B
SERPINC1
MET
EPS8L2
CASP8A2
SELP
ZNF544
RGS7
TACR1
CYP4V2
F5
SMOYKEEBO
C1orf114
F9
ODZ1

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Triplication in MEGA-II

GP6

CYP4V2 (next to PK and FXI)

F5

SERPINC1 (antithrombin)

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Risk estimates (MEGA-II)

gene		frequency (%)	RR
CYP4V2	rs13146272	64	1.24
SERPINC1	rs2227589	10	1.29
GP6	rs1613662	82	1.15
F5 *	rs4524	73	1.33

CYP4V2 explained by two SNPS in FXI (FXI:5U/dl/allele)

		p_0	RR
F11	rs2289252		0.411.35
F11	rs2036914		0.521.20

(* previously described by Smith, JAMA 2007)

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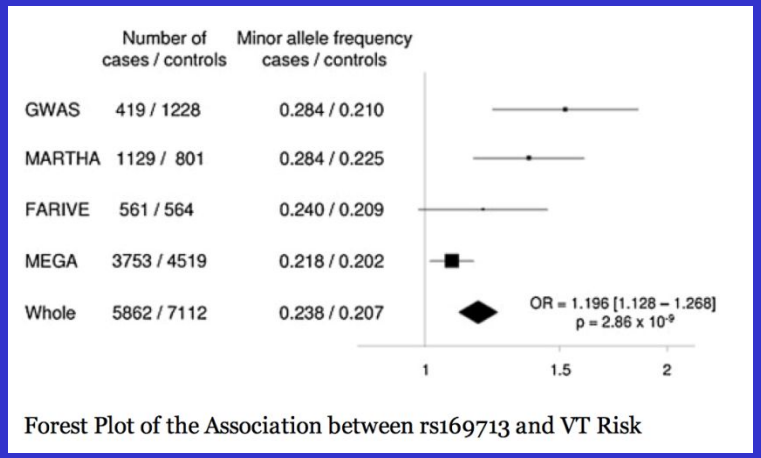
Overall findings

- several new variants
- all common and weak
- all in coagulation genes
  - One exception: HIVEP 1 (Morange, Am J Hum Genet 2010)



Forest Plot of the Association between rs169713 and VT Risk

Unresolved question 5

- how to find new genetic risk factors?

..... And 6

- what's the point?

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Techniques and strategies

- linkage with variable markers
- sequencing candidate genes
- genotyping known SNPs on a few genes
- genotyping many SNPs on many genes (GWAS)
- sequencing all exons (exome)
- sequencing all genes (genome)

approaches over time

- finding the gene and the (null) mutation for known proteins
- finding causative SNPs in known (candidate) genes
- counting number of SNPs in genes (burden test)
- counting number of SNPs in series of genes (burden test)

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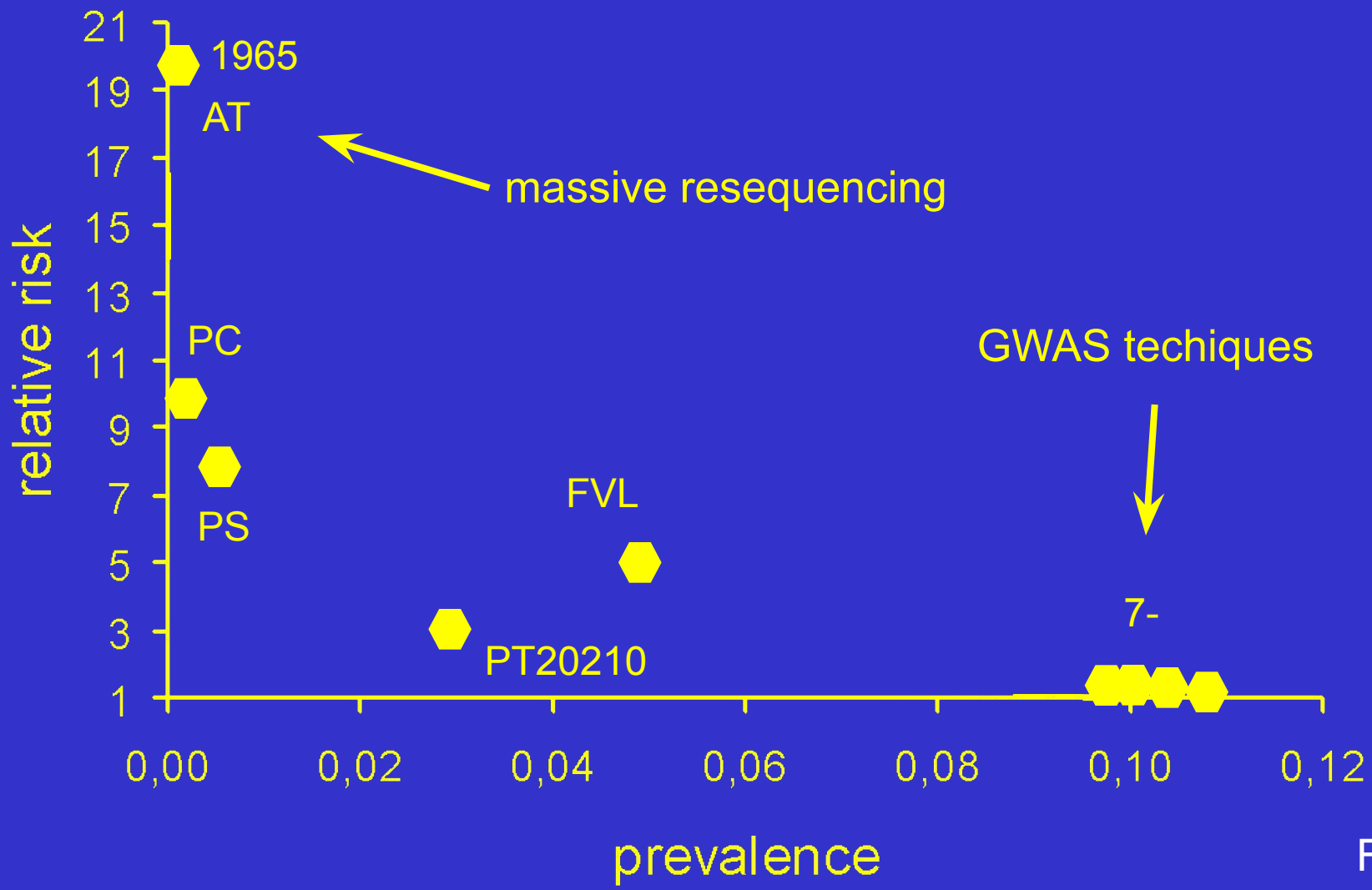
Progress.....



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Clinical relevance weak risk factors

- combined effect of more than one variant SNP
  - risk enhancing allele very frequent
  - few people carry none
  - many people carry several

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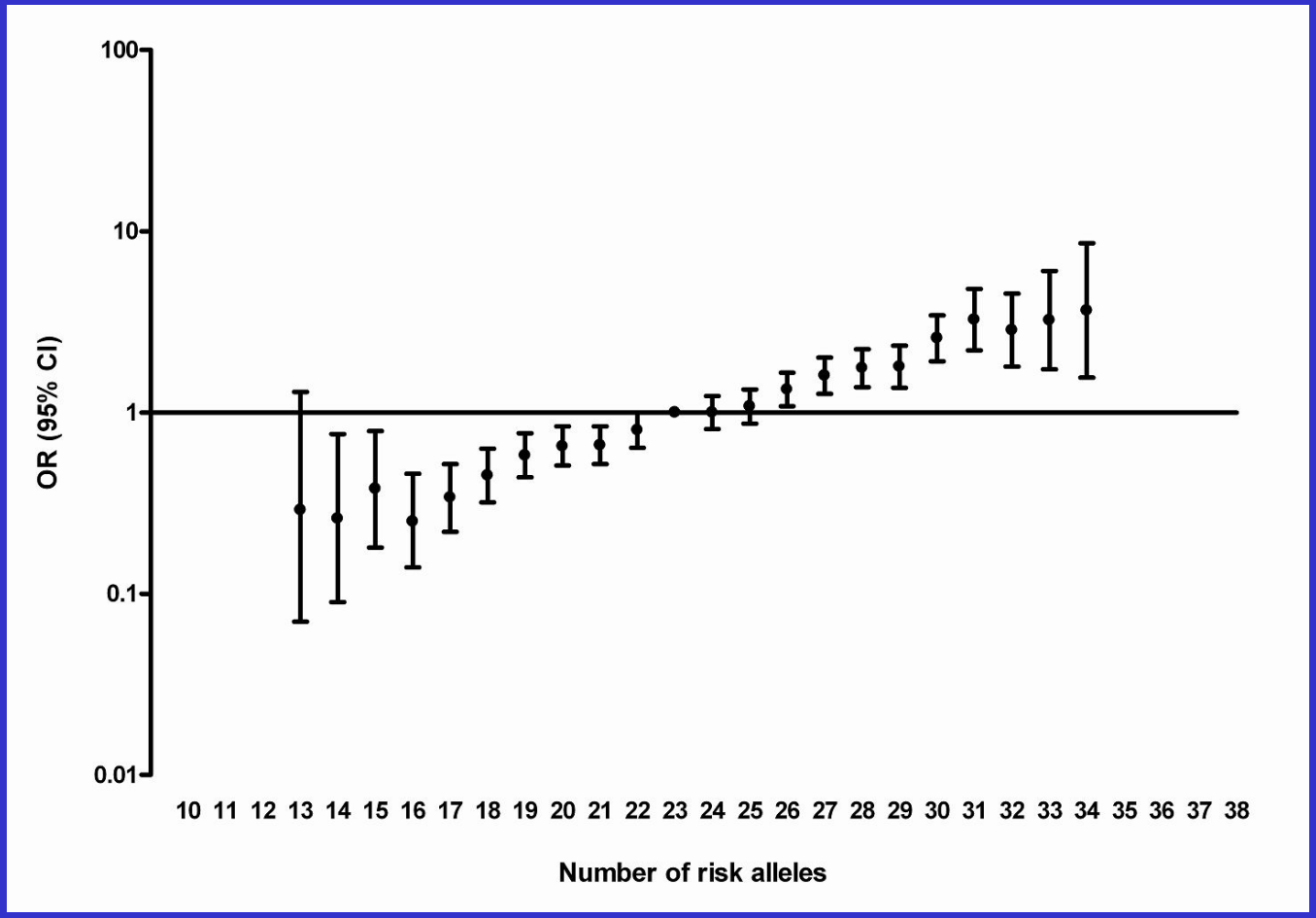
Combinations of frequent variants



(de Haan et al, Blood 2012)

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Predicting venous thrombosis

- parsimonious model
  - limiting to 5 SNPs equal predictive power
  - factor V Leiden, F2, 20210 G>A), ABO blood group, FGG 10034 C>T) and F11 (rs2289252)
  - 100-fold risk gradient
- predictive power (ROC-curve)
  - genetic score: AUC = 0.68
  - environmental factors: AUC = 0.74
  - combination: AUC = 0.80

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Recurrent venous thrombosis

- rates vary between 2.5 - 10% per year
- most studies find no effect of coagulation abnormalities
- some consistency for inhibitor deficiencies
- consistent results for persistent transient factors
  - oral contraceptives
  - cancer
  - lupus

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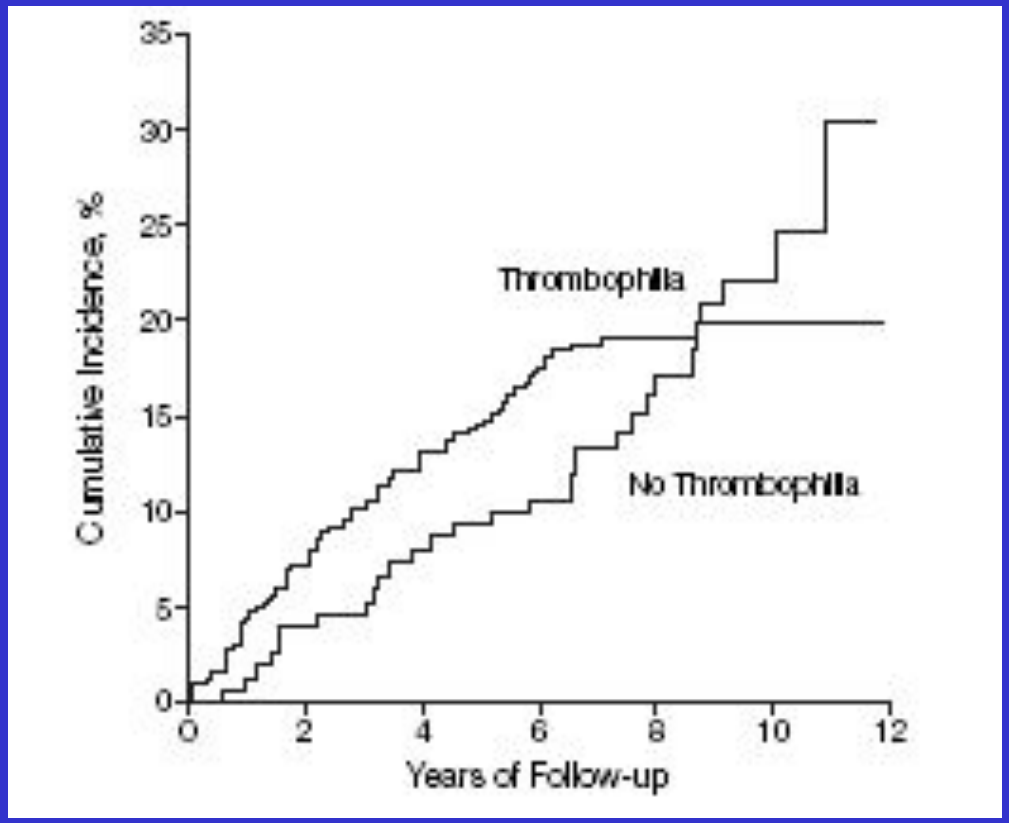
LETS: >7 years follow-up



- all laboratory abnormalities
  - PC, PS, AT
  - FVL, PT20210A
  - FVIII, FIX, FXI
  - homocysteine
- HR: 1.4 (CI95: 0.9-2.2)

(Christiansen, JAMA 2005)

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Recurrence risk by defect

	RR	CI95
factor V Leiden	1.2	0.7 - 1.9
prothrombin 20210A	0.7	0.3 - 2.0
PC/PS/AT deficiency	1.8	0.9 - 3.7
high FVIII	1.1	0.7 - 1.8
high FIX	0.9	0.5 - 1.7
high FXI	0.6	0.3 - 1.1
hyperhomocysteinemia	0.9	0.5 - 1.6

(Christiansen, JAMA 2005)

Non-transient predictors

	Relative risk
sex	
men vs women	3- to 4-fold
type of first event	
idiopathic vs secondary	2- to 3-fold

(Baglin, Lancet 2003; Baglin, JTH 2004; Kyrle, NEJM 2004; Christiansen, JAMA 2005)

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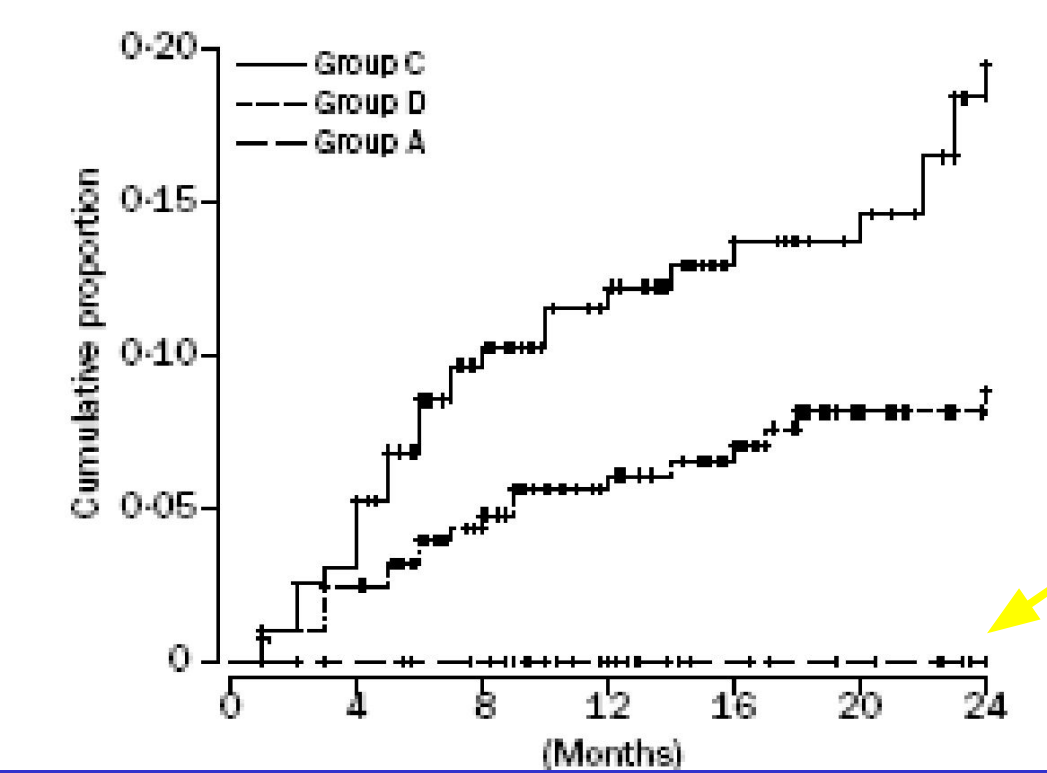
Idiopathic vs provoked



initial event post-surgical

(Baglin, Lancet 2003)

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Unresolved question 7-9

- why do risk factors for first events not predict recurrence?
- what are risk factors for recurrence?
  - genetic
  - acquired
- why is there a sex difference for recurrence?
  - genetic
  - acquired

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Risk factors for first and second VT

- Is it logical that risk factors for a first VT also increase risk of second VT?

- 1. No
- 2. Maybe they do

Possible answer 1: No

- Example
- Suppose there are only two genetic risk factors FVL and FVM. They are identical, but FVL is known, and FVM not yet.
- Both increase risk of first and recurrent risk

- For first VT, we see more FVL in patients than in population: recognised as risk factor
- For recurrent VT, we see people with FVL and we do not see those with FVM - so we see equal recurrence risks for those with and without FVL

Index Event Bias as an Explanation for the Paradoxes of Recurrence Risk Research
JAMA 2011;305:822-823.

Dahabreh, IJ, Kent DM

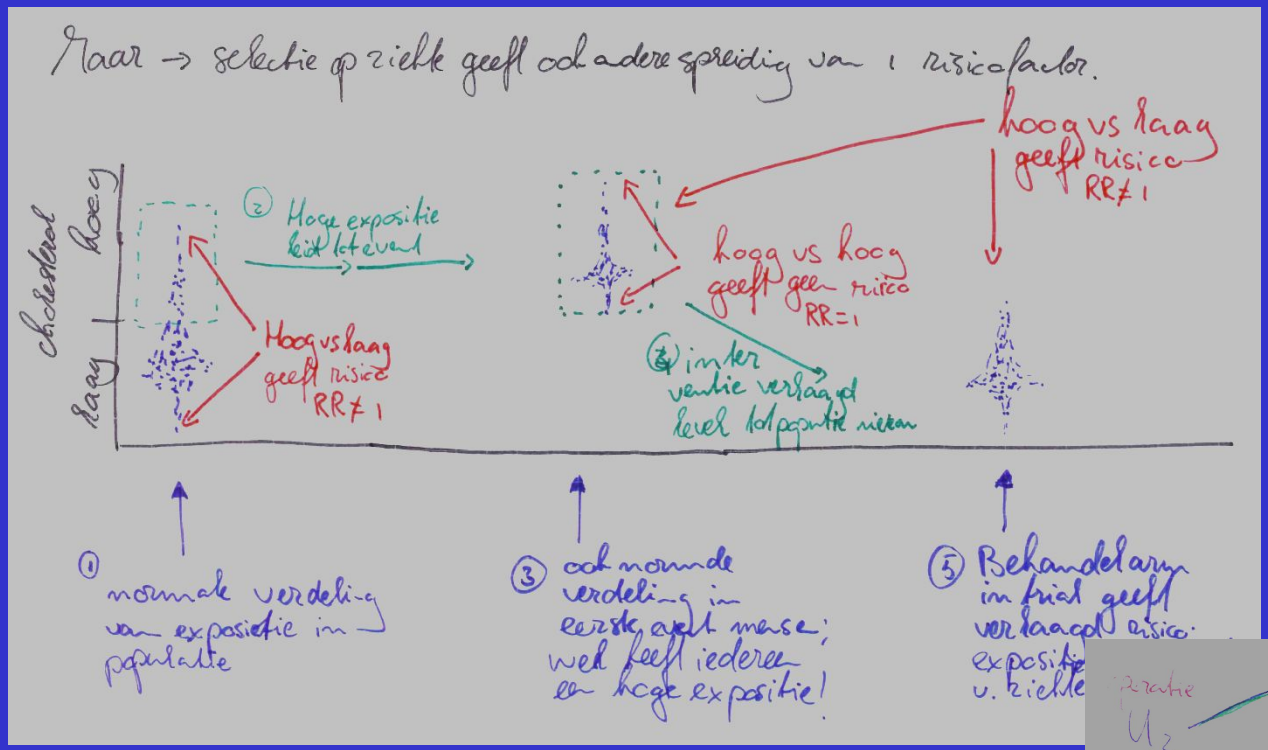
Possible answer 2: maybe they do

- example: look at absolute numbers
- PC deficiency
  - RR first VT: 10
  - RR risk recurrence: 1.8
- incidence of first and second VT very different

- first VT: 1/1000 * 10 = 10/1000: delta = 9 /1000
- second VT: 3/100 * 1.8 = 54/1000: delta = 24/1000

- FVL: 1/1000 * 5 = 5 / 1000: delta = 4/1000
- FVL: 3/100 * 1.2 = 36/1000: delta = 6/1000

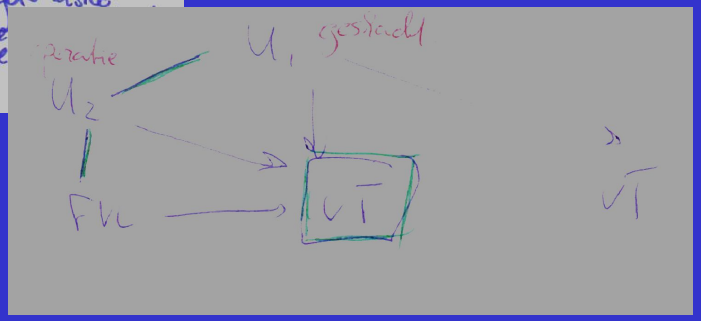
Progress



simulations

- little effect of index event bias unless under extreme circumstances

- mainly scaling problem



(Siegerink, le Cessie, Cannegieter, ms in preparation)

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Genetic or environmental?

Risk factors for thrombosis

- genes
- environment
- behaviour (including life style)
- combinations

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Cancer and thrombosis

	patients	controls	OR	CI95
cancer				
no	2831	2062	1	
yes	389	69	4.1	3.2-5.3
metastatic	93	1	68	9.4-487

(Blom, JAMA 2005)

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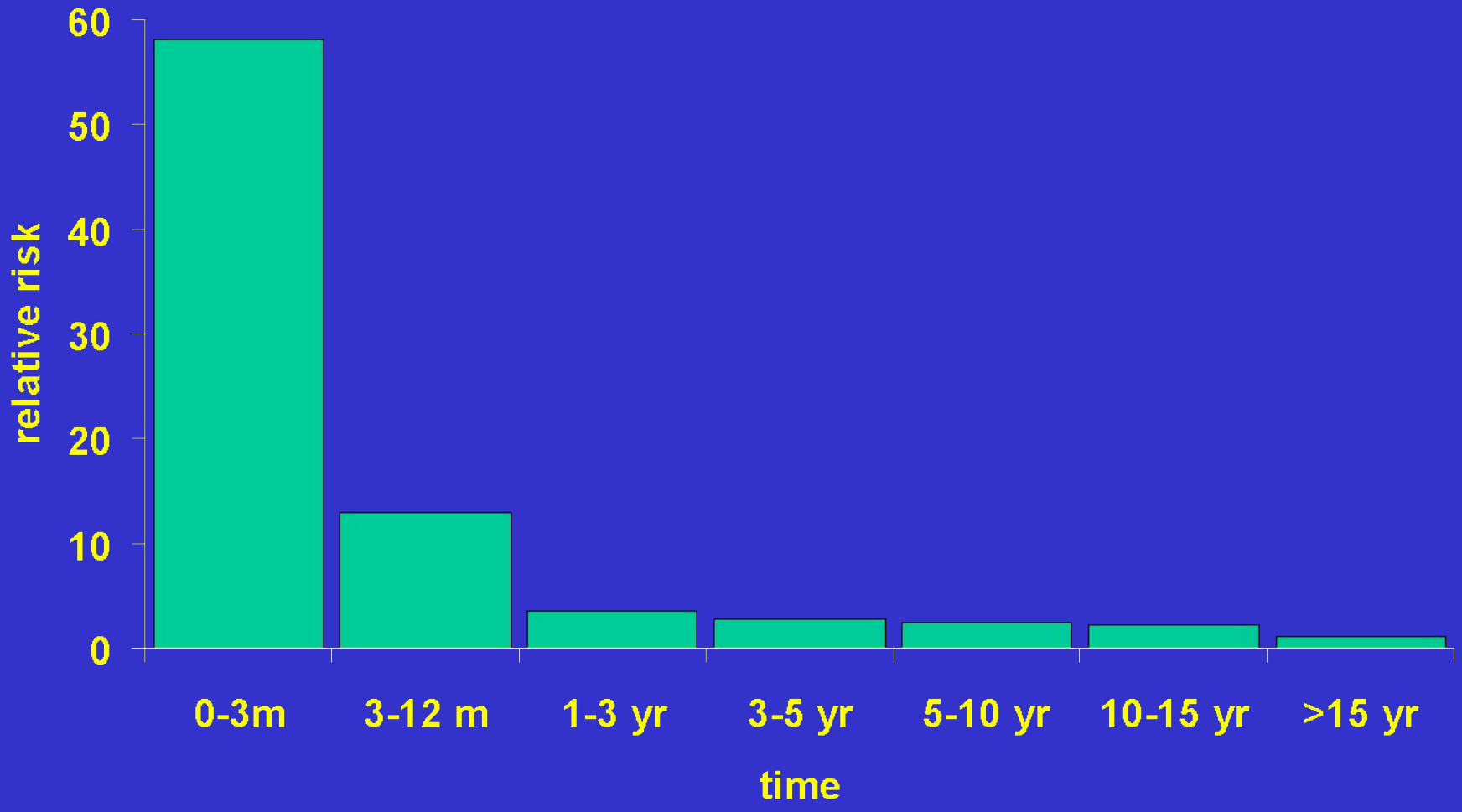
Time between cancer and thrombosis



(Blom, JAMA 2005)

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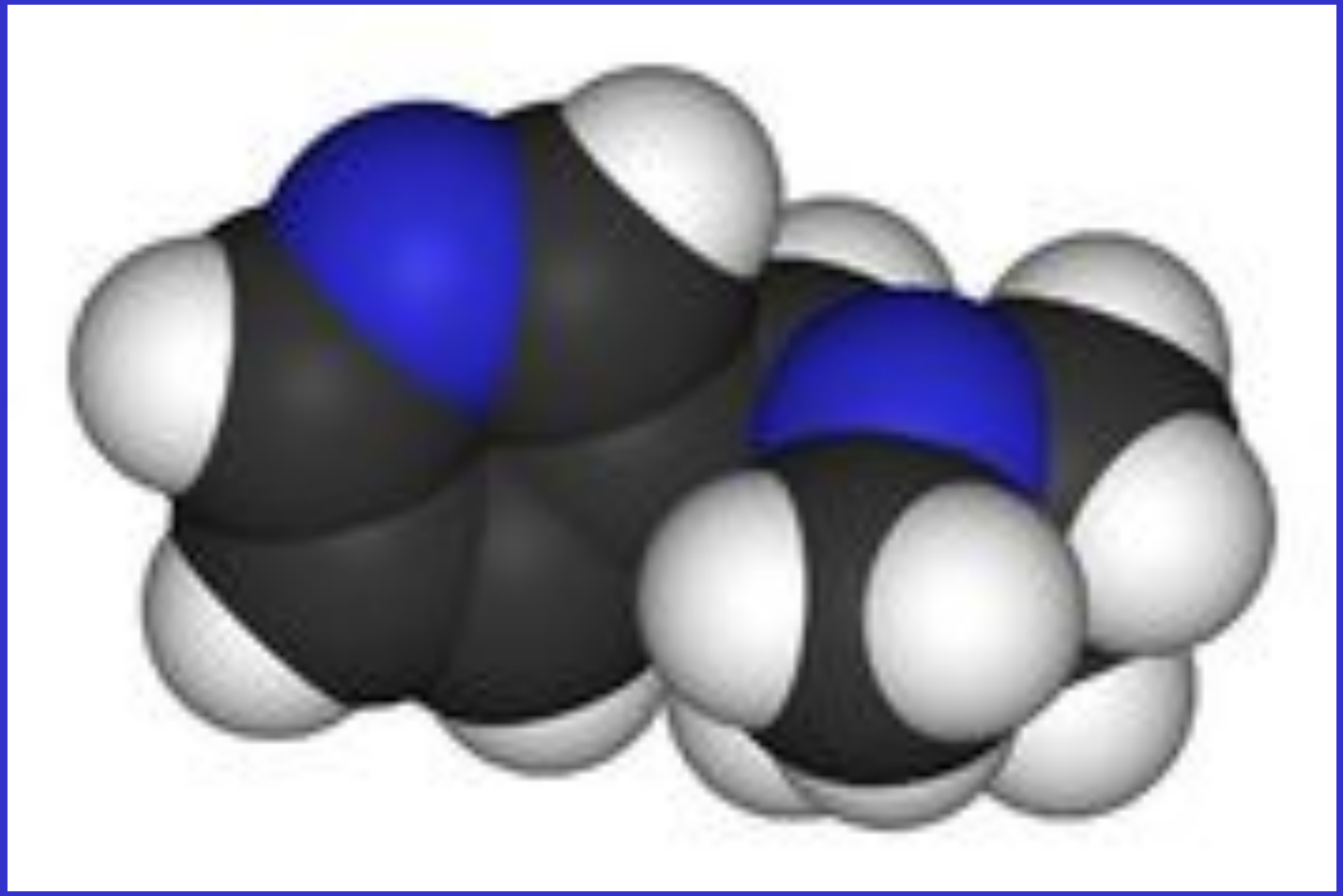
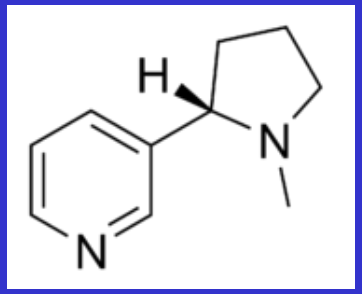
Lifestyle

- smoking
- drinking
- eating
- drugs
- travel
- sex

‘Frau Antje’

(Der Spiegel, 1994)

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113,597 DOCTORS FROM COAST TO COAST WERE ASKED:

According to this recent Nationwide survey:

MORE DOCTORS SMOKE CAMELS THAN ANY OTHER CIGARETTE!

Smoking

- well-established risk factor for all forms of arterial disease

- unclear effect on venous thrombosis
  - ‘Men born in 1913’: OR = 2.8
  - Leiden Thrombophilia Study: no effect
  - Sirius study: protective

(Hansson, Arch Intern Med 1999; Samama, Arch Intern Med 2000)

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MEGA study

- Multiple Environmental and Genetic Assesment of risk factors for venous thrombosis
- large case-control study
- 5000 cases, 5000 controls
- first DVT or PE
- no exclusion criteria, except age <70 yrs
- questionnaire, DNA, plasma

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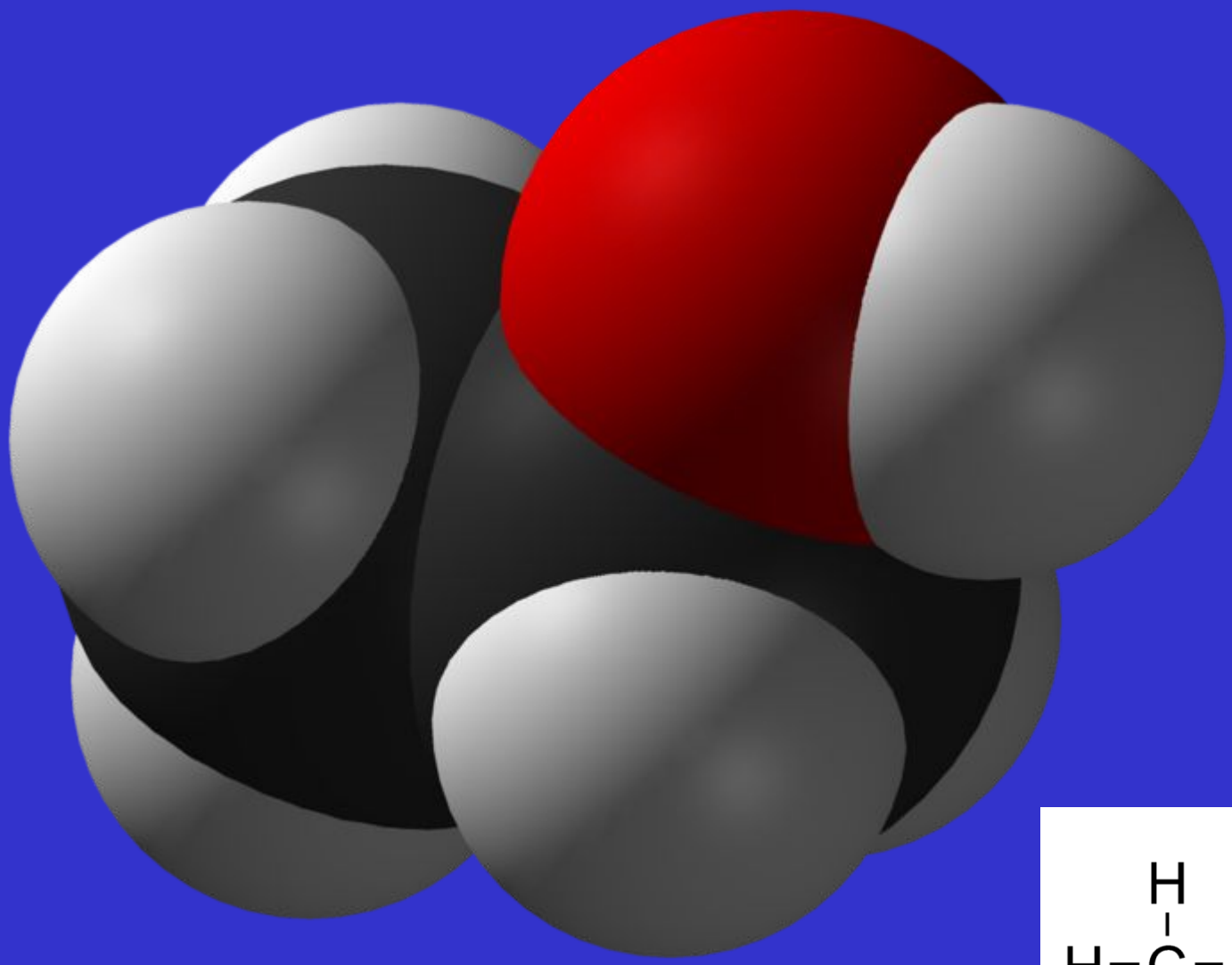
Smoking and venous thrombosis

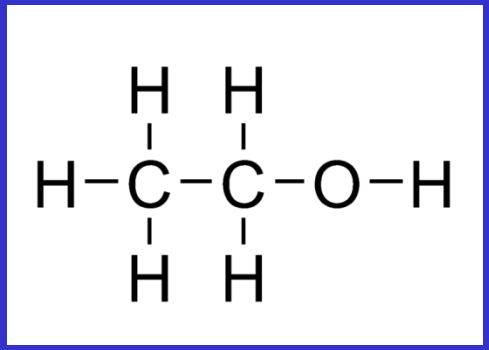
	patients	controls	OR*	CI95
never	1391	1976	1	
former	1136	1357	1.23	1.09-1.38
current	1462	1567	1.43	1.28-1.60

*: pooled controlgroups, adjusted for age an sex

(Pomp, Am J Hematol 2008)

FRR




H H
H C C O H
H H

Drinking alcohol

- established association with arterial disease
  - protective chronic effect
  - deleterious acute effect

- few data on venous thrombosis
  - protective effect in Italian elderly
  - no effect in American cohort (LITE)
  - no effect in Sirius study

(Pahor, JAGS 1996; Tsai, Arch Intern Med 2002; Samama, Arch Intern Med 2000)

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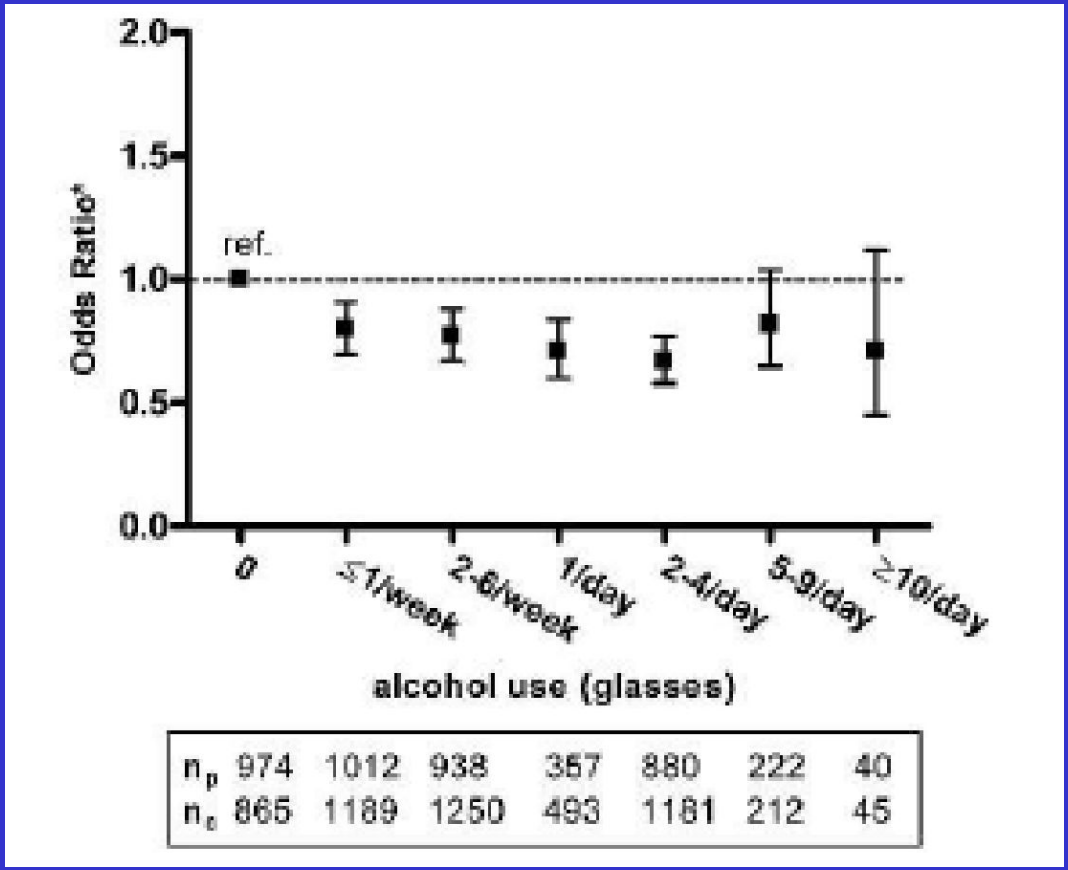
MEGA study



4423 patients

5235 controls

2-4 glasses/day

OR= 0.67 (CI95 0.58-0.77)

(Pomp, Thromb Haemost 2008)

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Eating

- obesity well established risk factor for arterial disease

- related to venous thrombosis in several studies
  - Leiden Thrombophilia Study
  - Copenhagen City Heart Study

(Abdollahi, Thromb Haemost 2003; Juul, Ann Intern Med 2004)

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MEGA study

	patients	controls	OR*	CI95
BMI (kg/m^2)				
<25	1393	2357	1	
25-30	1629	1728	1.70	1.55-1.87
>30	812	598	2.44	2.15-2.78

*: pooled controlgroups, adjusted for age an sex

(Pomp, Br J Haematol 2007)

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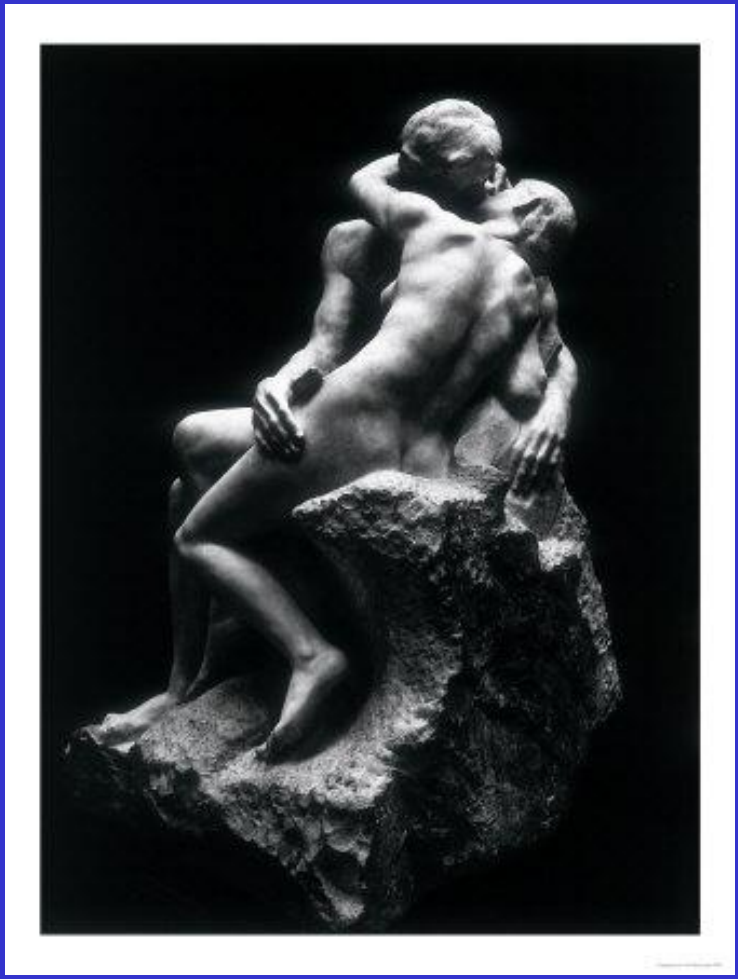
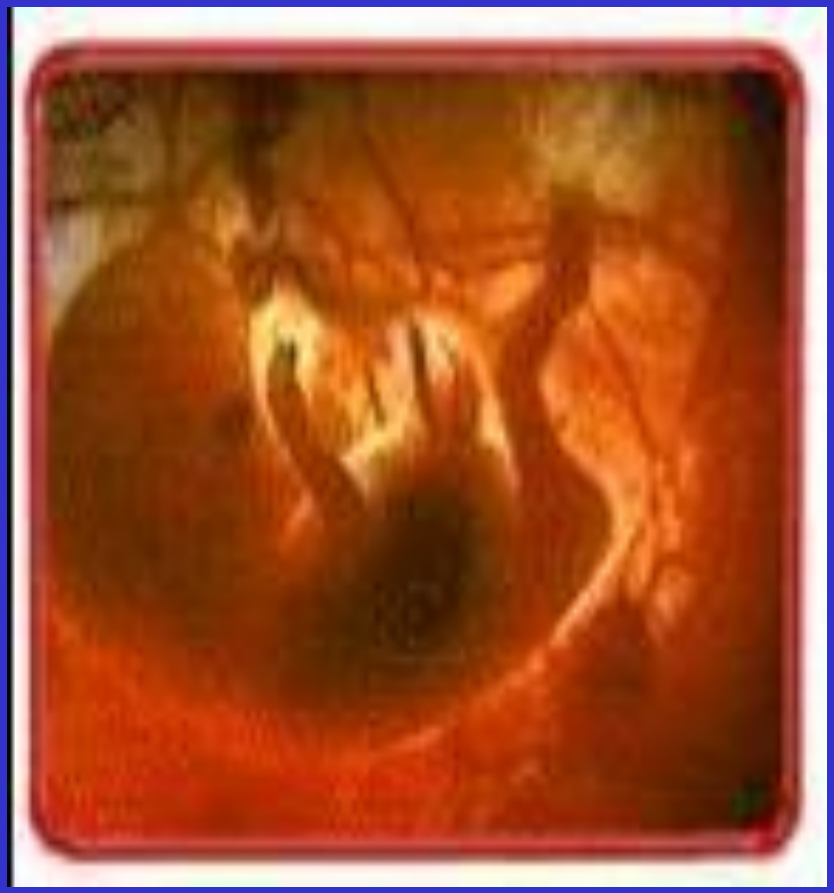
Sex and venous thrombosis



no data

FRR

Reproduction



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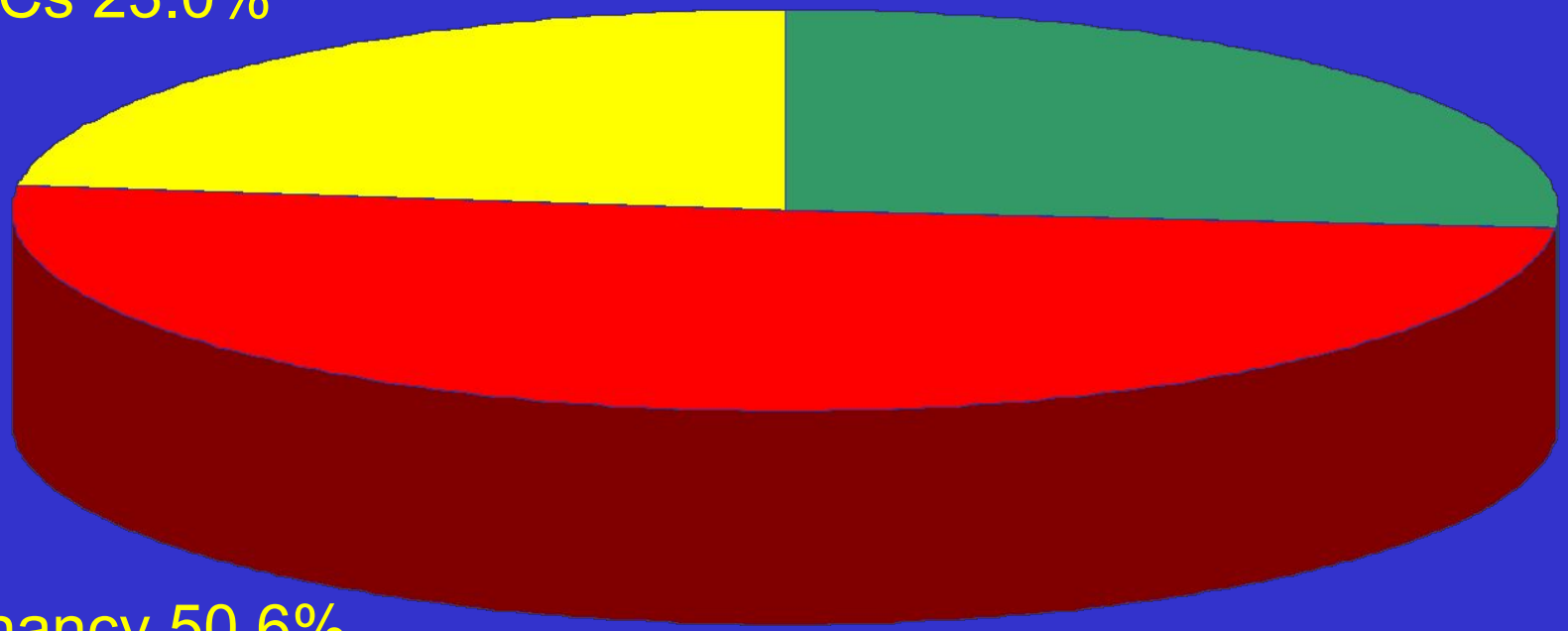
Thrombosis in women (15–39 yr)



risk: 1 per 1000 pregnancies

- current OCs: 4x increased risk
- some OCs have higher risk

(McColl, MD Thesis 1999, van Hylckama Vlieg, BMJ 2009)

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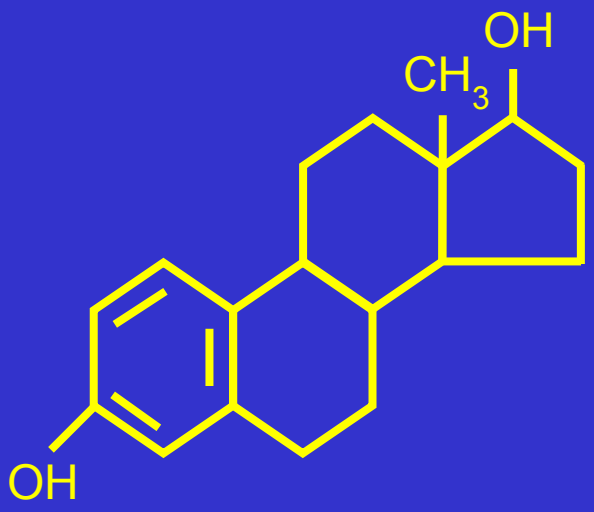
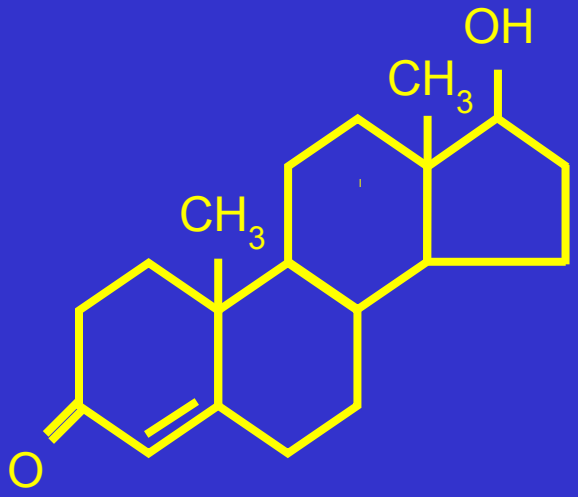
Natural sex-steroids



oestradiol



testosterone

progesterone

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MUSCLEMAG

Oral contraceptives

Type of progestogen	Thrombosis patients (n=1524)	Controls (n=1760)	Odds ratio (95% CI)*
Levonorgestrel†	485 (31.9)	373 (21.2)	3.6 (2.9 to 4.6)
Gestodene†	119 (7.8)	67 (3.8)	5.6 (3.7 to 8.4)
Desogestrel†	289 (19.0)	108 (6.2)	7.3 (5.3 to 10.0)
Lynestrenol†	44 (2.9)	19 (1.1)	5.6 (3.0 to 10.2)
Norethisterone	11 (0.7)	7 (0.4)	3.9 (1.4 to 10.6)
Cyproterone acetate	125 (8.2)	62 (3.5)	6.8 (4.7 to 10.0)
Norgestimate	9 (0.6)	4 (0.2)	5.9 (1.7 to 21.0)
Drospirenone	19 (1.2)	14 (0.8)	6.3 (2.9 to 13.7)
No oral contraceptive (reference)	421 (27.7)	1102 (62.8)	1

(all 30-35 µg ethinyloestradiol)

(van Hylckama Vlieg, BMJ 2009)

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Unresolved question 10

- how do these ‘arterial’ risk factors cause venous thrombosis?
  - one disease causes the other (how?)
  - common risk factors
  - form of index event bias

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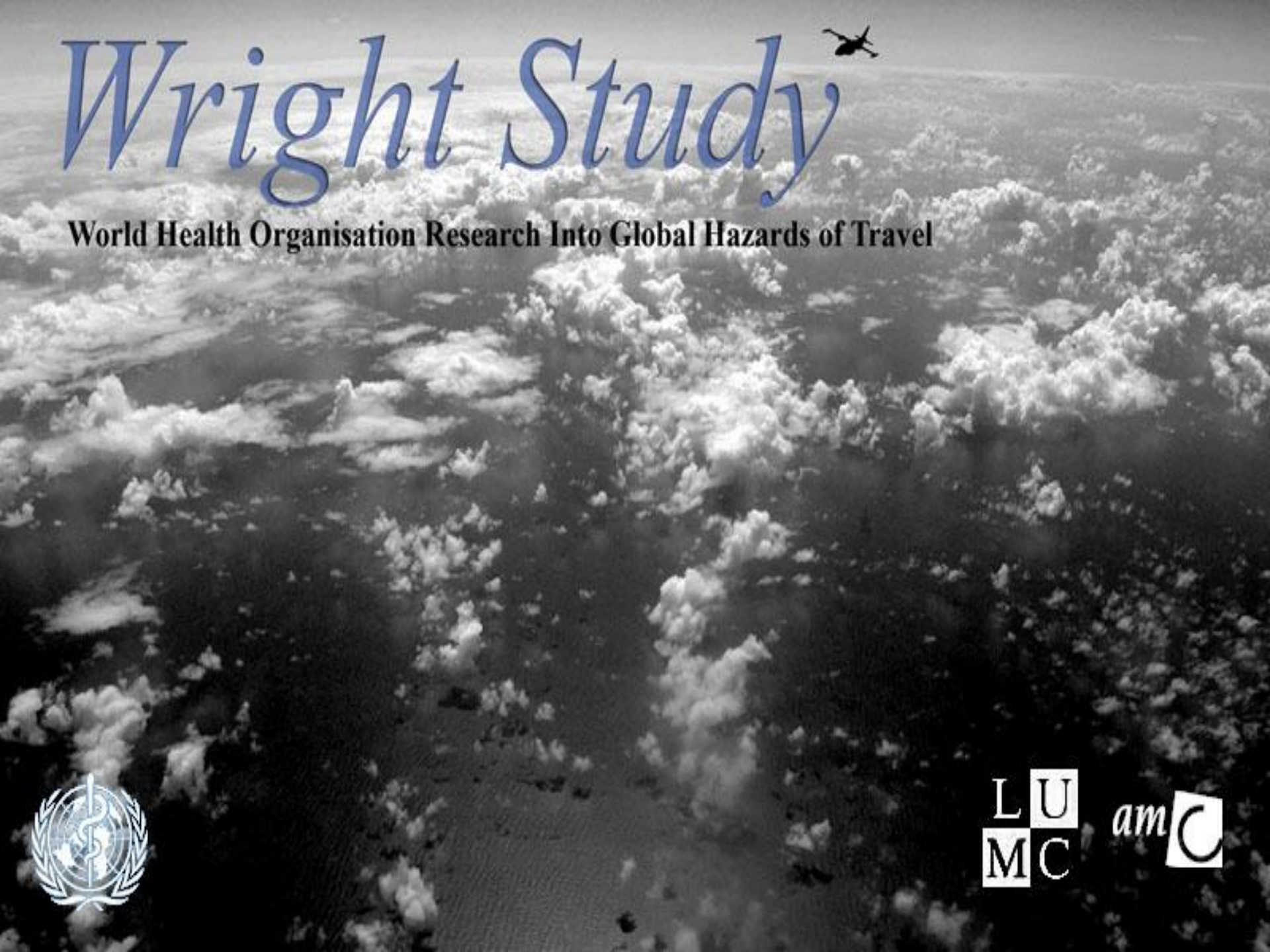
Wright Study
World Health Organisation Research Into Global Hazards of Travel
LUMC
amc

WRIGHT study

- 8755 frequently travelling employees multinationals and international organisations
  - (Nestlé, Royal Dutch, TPG, General Mills, CDC, IMF, Worldbank)

- web-based questionnaire
- cohort study: absolute risk of thrombosis after flying

- 5 yrs: 115 000 flights > 4 hr, 53 thromboses

(Kuipers S, PLoS Med 2007)

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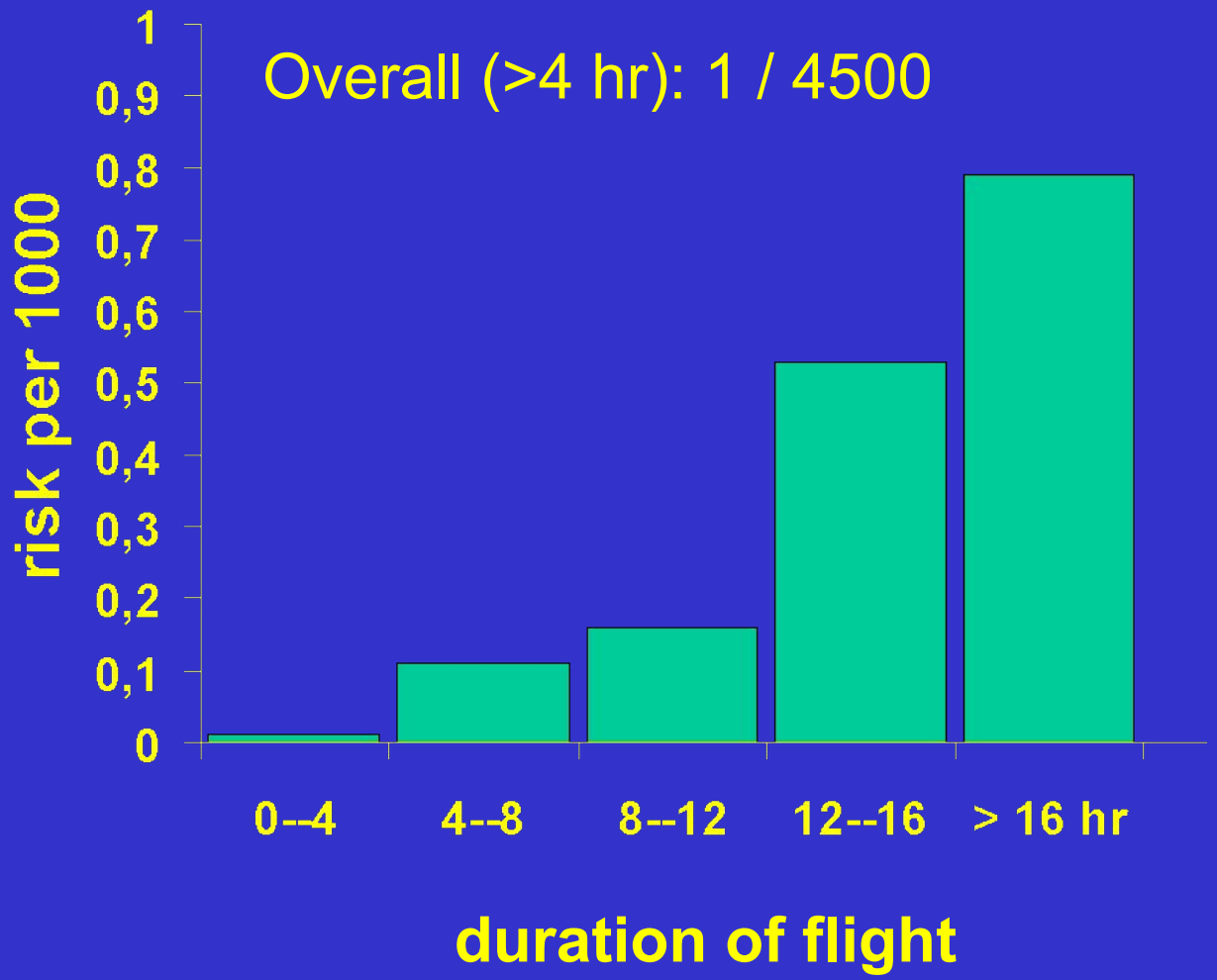
WRIGHT study



(Kuipers, PLoS Med 2007)

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Unresolved question 11

- Why so much more interest for genetic risk factors than acquired ones, while the latter are clearly more important?

Conclusions

- venous thrombosis usually the result of both genetic and environmental factors
  - strong risk factors
    - surgery, trauma, cancer
  - moderate risk factors
    - anticoagulant deficiencies, lifestyle factors, medical conditions
  - weak risk factors
    - all other known genetic variants
- only few causes of recurrence known
  - persistent transient factors, male sex

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My 10 unresolved questions

1. Why the steep age-increase?
2. Too many risk factors
3. What is the use of finding more and more risk factors (with marginal odds ratios)?
4. Are there more genetic causes of thrombosis?
5. How to find new genetic risk factors?
6. What's the point?
7. Why do risk factors for first events not predict recurrence?
8. What are risk factors for recurrence?
9. Why is there a sex difference for recurrence?
10. How do these 'arterial' risk factors cause venous thrombosis?

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