Genetic approach to thrombophilia

Island 10 August 2002

Torben Bjerregaard Larsen, MD, PhD
Talk summary

- What is thrombophilia?
- Coagulation factor defects in thrombophilia (brief!)
- How to study a genetic disease?
- Epidemiology and genes
- Clinical and ethical considerations
Superficial phlebitis
Deep venous thrombosis
Fatal venous thrombosis
Flow

Endothelia

Blood

Genes!
Disorders of hemostatic mechanisms

Thrombophilia: Tendency towards thrombosis

Haemophilia: Tendency towards extensive bleeding
Clinical definition of thrombophilia

- Patients with spontaneous thrombosis
- Severe thrombosis without any recognized stimulus
- Recurrent VTE
- VTE in an early age
Genetic definition of thrombophilia

- Disorders of *inherited* hemostatic mechanisms

With NO family history of VTE

WITH a family history of VTE
Genes

The basics!
The Human genome

- Only 3% are coding sequences
- 30,000-40,000 genes
- 1344 disease causing loci found (LocusLink at NCBI - August 2002)
- All individuals carries a library of different polymorphisms
- Variations or polymorphisms underlie the diversity of life
Genetic disease classification

- Single-gene
- Polygenic
- Multifactorial (~ 50%)
- Chromosomal
- Somatic cell
Definition for single gene disorders

- Autosomal recessive (67%)
- Autosomal dominant (26%)
- X-linked (6%)
The central dogma
Classification of mutations

- Type I (decreased antigen)
- Type II (decreased function)
- Others:
  - "Type III" (e.g. Protein binding defect in PS)
  - SNP (single nucleotide polymorphisms)
Single nucleotide polymorphisms

- SNP are one letter variations in the DNA sequence that occur with a population frequency of > 1%
- Commonest type of genetic variation and account for individual characteristics, including risk of disease
Coagulation factor defects in thrombophilia

• In Brief!
Factor V Leiden 1q23
Antithrombin 1q23-q25
Protein C 2q13-q14
Protein S 2p11.1-q11
Factor II 20210 11p1-q12
Factor VIII Xq28
Genetic risk factors affect:

- Probability of disease
- Age of disease outbreak
-Severity of disease
Hereditary risk factors

Relative risk (RR) for VTE

- Antithrombin deficiency = 25-50 (type I)
- Protein C deficiency = 10-15 (type I and II)
- Protein S deficiency = 2 (FV Leiden?)
- Factor II 20210 = 3
- Factor V Leiden = 3-8 (80 for homozygous!)
How to study genetic disease?

• Epidemiological studies
• Clinical observations
• Laboratory analysis
  – Proteins and functional defects
  – RNA
  – DNA
Pedegrees

Observational studies on VTE

Egeberg 1963

Dahlbäck 1993
Single observations

Syndromes

> autoimmune disorders
> type I diabetes
> autoimmune thyroiditis
> lupus erythematosus
> VTE
Klinefelter's syndrome

Normal male

Klinefelter's syndrome

Venous and arterial leg ulcers (RR=50)
Deep venous thrombosis (RR=6)
Pulmonary embolism (RR=5-21)
Deficiency of:
Antithrombin
Protein C+S
FV Leiden mutation
FII 20210 mutation
High FVIII conc.
High Homocystein conc.
Low Fibrinolytic activity
Antiphospholipid ab.
Unknown

Thrombophilia → VTE

Surgery
Immobilisation
Obesity
Cancer
Other

Interaction

Superficial thrombophlebitis
Recurrent miscarriage
Stroke
Preeclampsia?
ALL GENES

Surgery  Immobilisation  Obesity

Cancer  Other

Thrombophilia  VTE

Superficial thrombophlebitis  Recurrent miscarriage  Stroke  Preeclampsia?
A twin study

Contributors:
Torben Bjerregaard Larsen, M.D, Ph.D
Henrik Toft Sørensen, Dr.Med.Sci
Axel Skytthe, M.Sc, Ph.D
Søren Paaske Johnsen, M.D
James W. Vaupel, Ph.D
Kaare Christensen, Dr.Med.Sci
Population Twins 20 YEARS

VTE in monozygotic

TB Larsen et al 2002

Concordance rates (Odds Ratios)
Overall genetic susceptibility to venous thrombosis?

RESULTS: probandwise concordance rates (OR)

- Monozygotic: 0.22 (13.5)
- Dizygotic: 0.08 (3.8)

Conclusion:

Heritability is > 55 %
Molecular genetic analysis
Molecular technology

- Chromosome analysis
- DNA mapping
- Cloning DNA
- Sequencing DNA
- Positional cloning
- DNA amplification
- Mutation analysis
- Expression of recombinant DNA
- CHIP technology
Epidemiological methods
Basic study designs available for genetic analytic studies

- Observational studies
  - Follow up studies
  - Case-control studies
  - Cross-sectional studies

- Experimental studies
The case-control design

1. Select a sample from a population of people with the disease (cases)
2. Select a sample from a population at risk that is free of the disease
3. Measure predictor variable (e.g., genetic determinant) and Odds Ratios
Case-control Strengths

♦ Efficiency for rare outcomes

♦ Often cheaper

♦ Multiple exposures can be studied

♦ Ability to address important questions rapidly

♦ Ability to address important genetic associations
Case-control Weaknesses

- Only one outcome
- No direct estimate of incidence rate or excess risk
- More complicated to design
- Increased susceptibility to bias
INTERNATIONAL GUIDELINE ON ETHICAL ISSUES IN MEDICAL GENETICS AND GENETIC SERVICES

Report of a WHO Meeting on Ethical Issues in Medical Genetics

Geneva, 15-16 December 1997
Genetic counselling

Aspects of genetic thrombophilia and testing - the codex

- Pedigree of the family
- Genetic counselling
  - non directive approach
  - time to consider
  - no susceptibility testing in children
- Result of the test - personally!!
Summary

• VTE is highly genetic in nature
• General guidelines are difficult to establish regarding screening
• General guidelines are difficult to establish regarding treatment
• Genetic counselling is mandatory in genetic screening