Haemophilia

from man

to dog

to man

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Haemophilia

- Lack of a clotting protein
- Bleeding diathesis
- Severe to mild forms
  - Depends on level of clotting protein
  - Depends on which protein is deficient
- Commonest are FVIII and FIX

History of Haemophilia

- References in ancient texts
  - Egyptian Papyri
  - Talmud 2nd century
    - Exemption from circumcision
  - 11th Century Arabian reference
- Symptoms described again in 19th century
  - Origin in one family traced back to 1720
  - Inheritance from mother to son recognised
- 1828 Zurich University
  - Bleeding disorder: first use of term ‘Haemorrhaphilia’

Haemophilia – a Royal Disease

Queen Victoria (1819-1901)

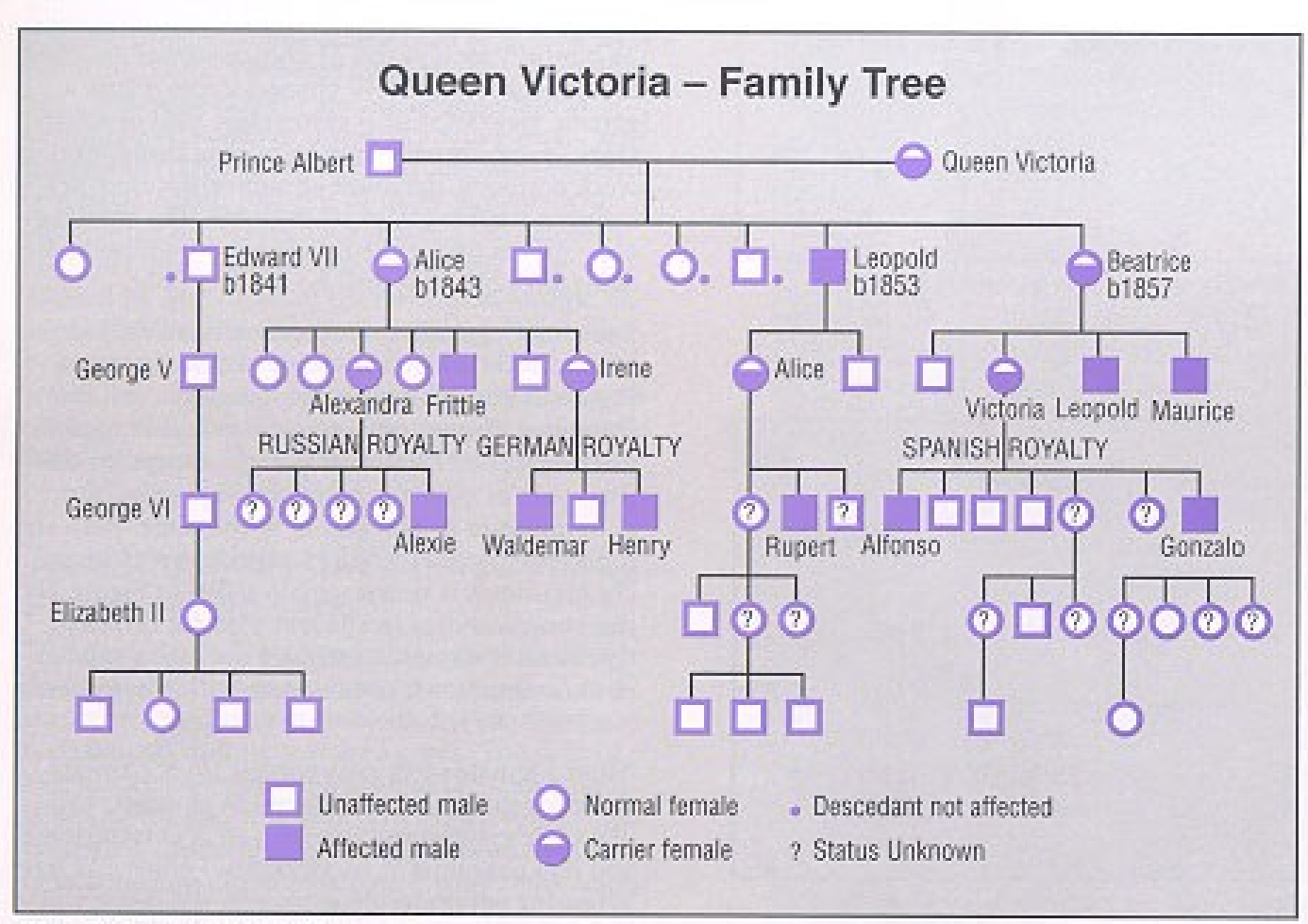
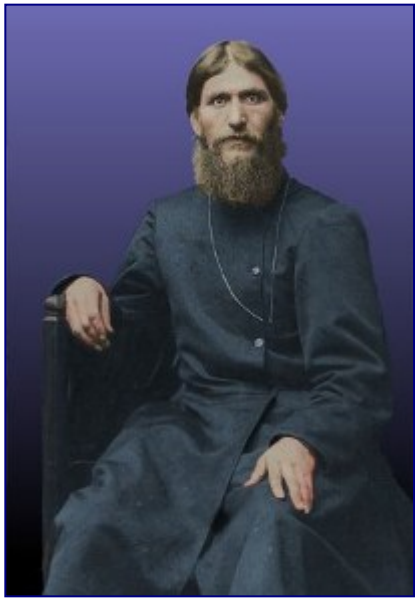


Figure 1. Queen Victoria's family tree.

Haemophilia – a Royal Disease

Rasputin and the Russian Imperial Family



Alexis

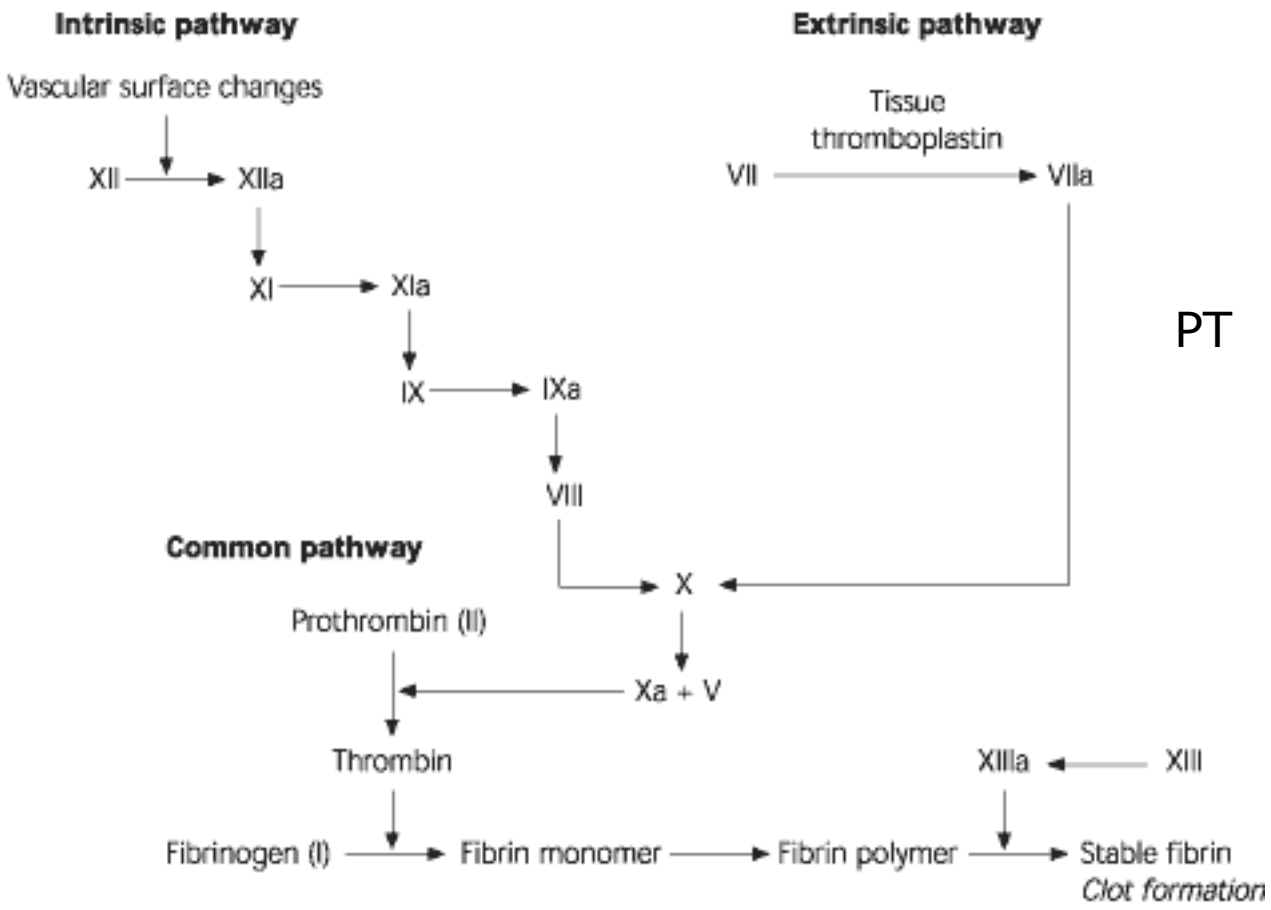
1940s

- 1944 – Buenos Aires
  - Mixing blood from two haemophiliacs
  - Correction of each coagulation defect
- That is:
  - Plasma from 1st corrected defect of 2nd
  - Plasma from 2nd corrected defect of 1st
- Eventual recognition of 2 diseases
  - Haemophilia A and B

Coagulation Cascade

aPTT

PT



Partial Thromboplastin Time

- Reflects the integrity of the intrinsic pathway
  - Factors XII, XI, IX, VIII and X
- Prolonged by
  - Deficiencies of these factors
  - Inhibitors of these factors
- Degree of prolongation dependent on
  - Reagent
  - Position in pathway
- Does not necessarily correlate with bleeding

From bedside to lab…

- If aPTT prolonged
  - 50:50 mix
  - deficiency versus inhibitor
- Deficiency: >50% correction
  - Check individual factors
- Inhibitor: < 50% correction
  - Lupus anticoagulant
  - Heparin
  - Acquired e.g. with malignancy

From lab to kennels…

French Bulldogs in the Netherlands

1971

Utrecht Small Animal Clinic

10 male dogs referred with a bleeding diathesis

8 tested:

- 5 FVIII deficiency and 3 FIX deficiency

All 10 had the same female ancestor

French Bulldogs in the Netherlands

210 male descendants

At least a further 10 with

a bleeding diathesis

170 registered female offspring

Potential high number of carriers of either haem A or B

No further papers found on searching

Classification of Haemophilia

- Haemophilia A
- Haemophilia B
- Haemophilia C

- FVIII deficiency
- FIX deficiency
- Factor XI deficiency

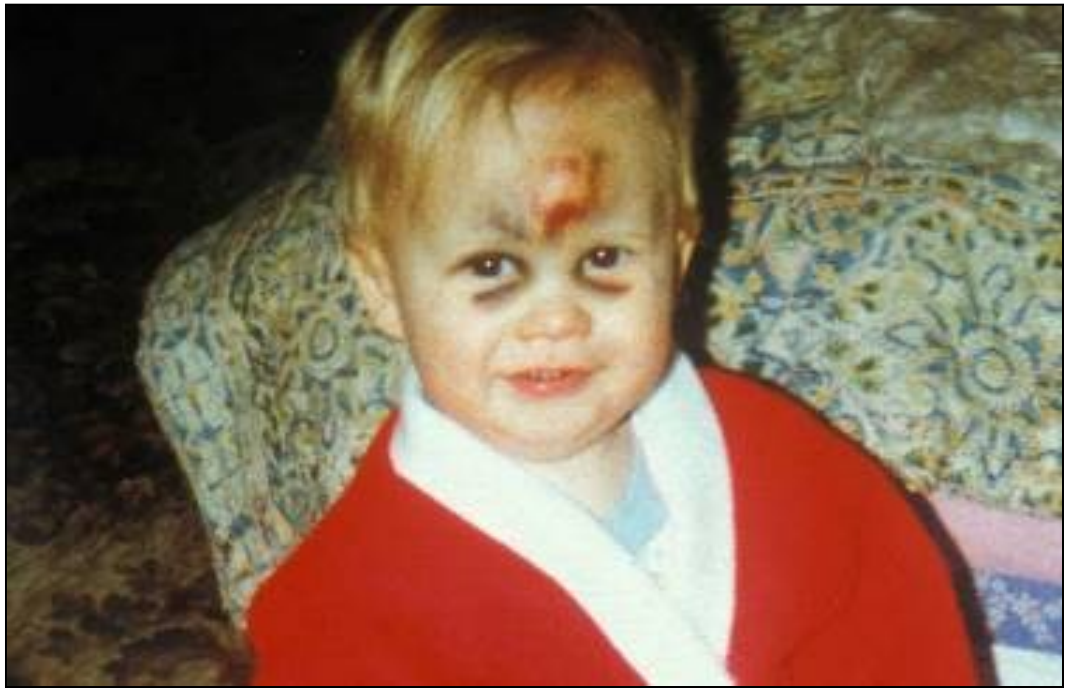
- Haemophilia A
  - 1:5,000*
  - 40% severe
  - X-linked

- Haemophilia B
  - 1:20,000*
  - X-linked
- Haemophilia C
  - Autosomal inheritance

*live male births

Classification of Haemophilia

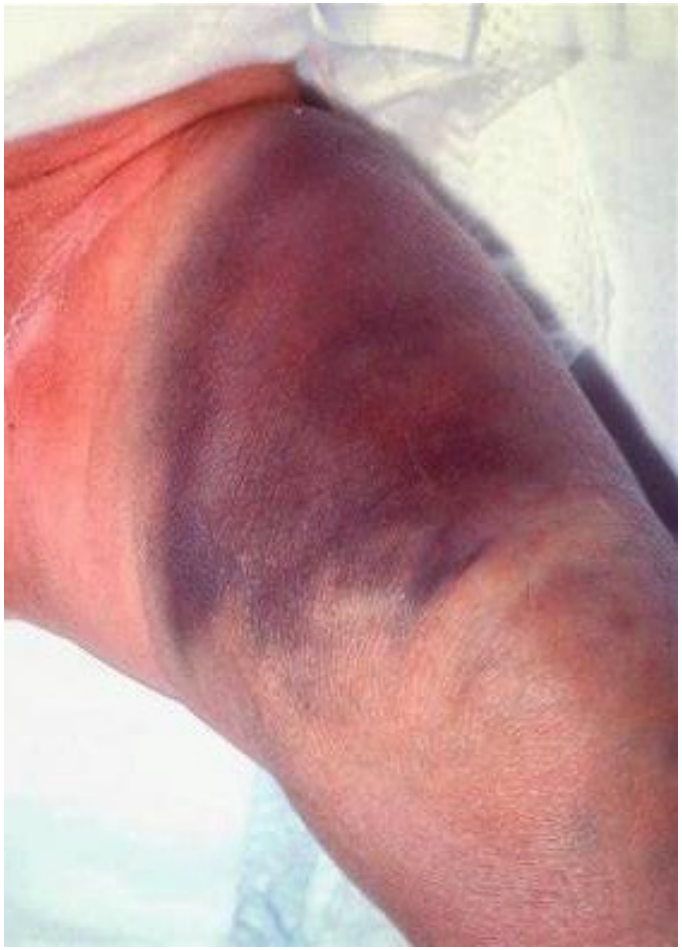
- Severe <1% spontaneous
- Moderate 1-5% minor trauma
- Mild >5% trauma, surgery



Presentations of Haemophilia

- Haematoma after i/m injections
- Bruising especially when toddling
- Bleeding from minor trauma
- Intermittent bleeding from wound
- Not moving a limb
- Swollen painful joint
- Through family studies
- Incidentally

Bleeding post i/m vitamin K



Neonate

2 hours old

Isolated, prolonged aPTT

Factor assays:

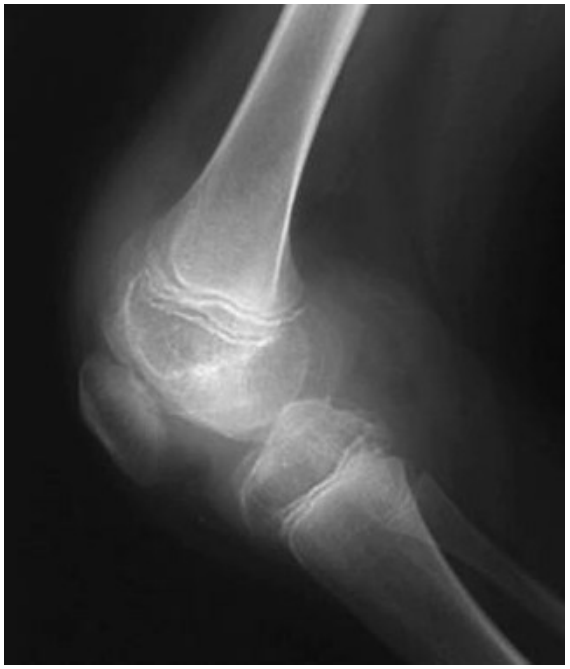
FVIII <0.01iu/mL

FIX 15iu/mL

Severe Haemophilia A

Haemophilia in man

- Initial challenge
  - To find effective treatment
- Subsequent challenge
  - To find safe & effective treatment
    - Cost; convenience
- Curative therapy
  - Gene therapy
  - Gene editing
- Final challenge
  - Treatment to bypass inhibitors



Haemophilia in man

- 11th Century
  - Cautery at the bleeding place
    - Suggested by Albucasis (936-1013)
- Treatment in 1940s
  - Ice on joints
  - Whole blood transfusions
- Recognition that plasma corrected the defect
- Life expectancy <30 years

Haemophilia in man

- Treatment in the 1950s
  - Fresh frozen plasma
  - Fraction I-O
- Treatment in the 1960s
  - Cryoprecipitate (1965)
    - FVIII and von Willebrand factor

Birger Blombäck holds a bottle of fraction, I-0, the first concentrate produced to be used for hemophiliacs, 1956.

Haemophilia in man

- Factor concentrates 1970s
  - Plasma concentrates
  - Recombinant factor concentrates 1990s

- Complications
  - Transfusion transmitted infections
  - Inhibitor (antibody) development

Haemophilia in animals

Haemophilia and animals

- 1950s
  - Haemophilia A and B recognised
- Anti-haemophilic factor
  - Bovine plasma (MacFarlane, Biggs and Bidwell)
- 1964
  - Lab detection of female carriers of canine haem A
- 1970s
  - Liver transplantation in dogs
  - Canine plasma used in human assays
- 1980
  - Porcine factor VIII used in patients with inhibitors

Haemophilia dog model

- 1981 gingival bleeding time
  - Factor VIII bypassing activity
- 1982 cuticle bleeding time
  - Factor VIII replacement
- 1987 thrombogenicity
  - Factor IX products
- 1990s
  - Gene therapy model

First use of FIX concentrate in UK

- 1960: 4 year old from East of Scotland
- Flown from Scotland to Oxford with
  - Orthopaedic surgeon
  - Haematologist
  - Paediatrician
- Allowed amputation of arm
- Learnt to play golf

Aims of treatment

- Reduce bleeding episodes
- Preserve joint function
- Reduce disruption to life
- Minimise complications of treatment
  - Inhibitor development
  - Transfusion transmitted infections
    - HIV; Hepatitis B, Hepatitis C
    - nvCJD?

Current treatments available

- Factor concentrates
  - Recombinant or virally inactivated plasma derived
  - FVIII + vWF
  - DDAVP (mild haemophilia A)
- Patients with inhibitors
  - Antibody development to the deficient factor
  - Factor concentrates essentially ineffective
    - Factor VIII bypassing agents (FEIBA) for haemophilia A
    - rVIIa for haemophilia A and B

New treatments needed

- Cost
- Safety
- Psychological burden
  - Unpredictable risk of bleeding
  - Need for repeated venepuncture
    - Time taken to deliver treatment
  - Being different…
- Will gene therapy be the answer?

Haemophilia and gene therapy

- Prime target for gene therapy
- Gene expression
  - Tight control not essential
  - Wide range of levels
    - Beneficial
    - Non-toxic
- Animal models available
  - FIX and FVIII knock-out mice
  - Dogs with haemophilia A and B

Haemophilia and gene therapy

- Restore gene function
  - Replacement
  - Repair
- Limitation of
  - Cell toxicity
  - Genome alterations
  - Harmful immune responses
    - Gene therapy system used
    - Product of the transgene

Haemophilia and gene therapy

- FIX relatively small gene
- Incorporated into viral vectors
  - Site-specific delivery (liver)
  - Gene-specific delivery (gene editing)
- Potential mutagenesis
- Problems with host immunity
  - In trial animals
  - In man

Gene therapy: site-specific delivery

- Proof of principal in animal models
  - Success seen in dogs with both haem A and B
- Proof of principal now in man for Factor IX
- 10 patients with severe Haemophilia B
  - Adeno-associated virus (AAV8)
  - FIX transgene
- Dose dependent response
  - $2x10^{12}$ vector genome/kg
  - Sustained and clinically useful response (5% level)
    - Management of AAV immune response important

Gene therapy: gene-specific delivery

- Encouraging results in mouse models
- Vector delivers
  - Gene specific nuclease
    - Cleaves DNA at targeted sequence
  - Repair DNA
    - Inserted into cleaved DNA
- Site-specific rather than random
  - Reduces risk of insertional mutagenesis
  - Reduces risk of silencing of transgene expression

What about inhibitor patients?

- Factor VIIIa is the co-factor for IXa and X
- Bi-specific antibody
  - Binds FIXa and FX
  - Brings into spatially appropriate positions
  - Mimics co-factor functions of FVIIIa
- Given subcutaneously
  - Half-life 17 days
- Haemostatic activity shown in NHP model
- Trials now in man

PUPS… Previously Untreated Patients