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 2\textsuperscript{nd} century
    • Exemption from circumcision
  – 11\textsuperscript{th} Century Arabian reference

• Symptoms described again in 19\textsuperscript{th} 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)
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
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
- 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-O, 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
  – $2 \times 10^{12}$ 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