Genetics of Haemophilia

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Haemophilia Inheritance – X Linked Recessive

- Haemophilia A
  - F8 gene Xq28

- Haemophilia B
  - F9 gene Xq27
Haemophilia Severity

- Haemophilia A <50% normal activity FVIII:C
- Haemophilia B <50% normal activity FIX:C
  - Severe <1% 40% of patients
  - Moderate 1-5% 10% of patients
  - Mild >5% 50% of patients
Haemophilia Prevalence

• X-chromosome linked inherited bleeding disorder

• 2 varieties
  - haemophilia A; 1 in 10,000 population
  - coagulation factor VIII deficiency
  - haemophilia B; 1 in 50,000 population
  - coagulation factor IX deficiency
Mutations Responsible for Haemophilia
A and B
Factor VIII Gene and Protein

F8 Gene

FVIII Protein

A1  a1  A2  a2  B  a3  A3  C1  C2

Heavy chain  Connecting region  Light chain
F8 Intron 22 Inversion Analysis
**F8 Intron 22 Inversion**

- Results from homologous intrachromosomal recombination
- Inversion mutation occurs *de novo* once per 10,000 male meioses
- Every ejaculate contains at least one sperm with a *F8* intron 22 inversion mutation
- Responsible for 45% of severe haemophilia A
F8 intron 1 inversion
**F8 Intron 1 Inversion Analysis**

![Image of F8 Intron 1 Inversion Analysis](image-url)

- **1.5Kb**
- **1.0Kb**

Legend:
- **M**: Reference sample
- **N**: Sample under analysis
- **I**: Inversion present
- **N**: Inversion absent
**F8 Intron 1 Inversion**

- Similar to intron 22 inversion

- 900 bp region 5’ to F8 gene crosses over with homologous region in intron 1

- Results in F8 gene lacking a promoter and first exon

- Responsible for approx 2% of severe haemophilia A
Intrachromosomal inversions cause 50% of cases of severe haemophilia A
Examples of Point Mutation

-Cys Arg Lys Lys Thr Gln- Normal
-TGC CGA AAA AAA ACG CAG - sequence

-Tyr Arg Lys Lys Thr Gln- Missense

-TAC CGA AAA AAA ACG CAG-

-Val Stop Nonsense

-GTC TGA AAA AAA ACG CAG-

-Val Arg Lys Lys Arg Met- Frameshift

-GTC CGA AAA AAA CGC AGT- (eg A₈>A₇)
Other Mutation Types

- Deletion of part or all of gene (200bp to >200kb)
- Insertion into gene (repetitive sequence)
- Splicing error affecting production of mRNA
Ways to Eliminate FVIII Activity (severe disease)

- Intron 1 or 22 inversion
- Delete part of gene
- Insert extra nucleotides
- Nonsense mutation
- Splice site defect
- Missense mutation at strategic amino acid

Ways to Reduce FVIII Production (moderate/mild disease)

- Missense mutation, less important amino acid
- Splice site defect

- Most families have a “private” mutation
- Mutation not identified in ~2% of patients
Factor IX Gene and Protein

**F9 Gene**

- **0** 5 10 15 20 25 30 35 kb
- **1 2 3 4 5 6 7 8**

**FIX Protein**

- Pre  Pro  Gla  H  EGF  B  EGF  A  Activation  Catalytic serine protease domain
- 221His  269Asp  365Ser
F9 Mutations
Haemophilia B Leiden

- Most haemophilia is lifelong disorder of same severity

- Small proportion of haemophilia B patients have FIX levels which increase at puberty

- “Haemophilia B Leiden”
Factor IX Levels in Normal Males and in Haemophilia B Leiden

Haemophilia B Leiden results from specific $F9$ promoter mutations
Ways to Eliminate FIX Activity (severe disease)

• Delete part of gene
• Insert extra nucleotides
• Nonsense mutation
• Splice site defect
• Missense mutation at strategic amino acid
• Promoter mutation

Ways to Reduce FIX Production (moderate/mild disease)

• Missense mutation, less important amino acid
• Splice site defect
• Promoter mutation

• Most families have a “private” mutation
• Mutations detected in 99% of patients
Genetic Analysis Options in Haemophilia

1. Seek mutation in affected male, then use presence/absence of mutation to determine female carrier status and enable PND

2. Use linkage analysis to track affected allele around the family, without knowledge of the causative mutation
**F8 or F9 Gene Mutation Screen**

- Extract DNA from blood (white cells)

- PCR amplify exons & promoter
  - 30 PCR amplicons for F8 (26 exons)
  - 10 PCR amplicons for F9 (8 exons)

- Use mutation screening technique (CSGE, DHPLC, SSCP etc) or DNA sequence each amplicon to identify mutation

- Polymorphisms (neutral variation) also seen
DNA Heteroduplex Formation

control DNA
  →
  heat to denature and cool to reanneal DNA
  mix
  electrophorese on mildly denaturing gel

  ___________
  _______
  ___________

homoduplex
  →
  _______
  heteroduplex
  →
  homoduplex

homo- and hetero-duplexes
heteroduplex indicates alteration from control sequence
Conformation Sensitive Gel Electrophoresis

CSGE analysis of F8 gene amplicons identifies several sequence alterations in heteroduplexed DNA
DNA sequence comparison of two patients to identify a sequence alteration using Staden sequence analysis software.
Mutation Analysis

- Sequence affected male’s DNA
- Identify amplicon (1 of 30 for $F_8$) with altered sequence
- Use reference ($F_8$) sequence to interpret result of nucleotide change (e.g., missense mutation)
- Make judgement as to whether it is causative mutation in that patient (using mutation database, amino acid conservation etc)
- Seek mutation in ? carrier females to determine carrier status
HAMSTeRS
The Haemophilia A Mutation, Structure, Test, Resource Site.

http://europium.csc.mrc.ac.uk
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<th>Codon No.</th>
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Linkage Analysis in a Haemophilia A Family

F8 intron 13 (CA₈) repeat; 16-25 repeats
Linkage Analysis

• Technically simple

• Multiple members, including an affected member from the same family required

• Dependant on heterozygosity of key female relative(s)

• Ethnic variation in informativity

• In families with no prior haemophilia history, can only be used to exclude females as carriers
Approach for Linkage Analysis

- PCR

- Single nucleotide polymorphism recognised by restriction enzyme digestion (restriction fragment length polymorphism (RFLP))

- Repeat sequence polymorphisms (STRs (microsatellites) and variable number tandem repeats (VNTRs))

- Intron13 (CA)n

- XbaI

- BstEII

- UD

- CA

- G

- A

- CACACA

- CACACACA

- 140.66

- 142.78
Commonly Used Polymorphic Markers in the F8 Gene

Informativity 80-90%
Commonly Used Polymorphic Markers in the F9 Gene

Combined informativity 80-90%
Genetic Tests for Haemophilia

- $F_8$ intron 22 inversion
- $F_8$ intron 1 inversion
- $F_8$ screen in affected male
- $F_9$ screen in affected male
- Confirm / exclude mutation in ? carrier female (amplify single exon only)
- Linkage analysis
- PND
Prenatal Diagnosis (PND)

- 10-13 weeks gestation
- CVS biopsy
  - Karyotype
  - Check no chromosomal abnormalities
  - Determine sex
- If male, seek familial mutation. If female, report sex only
- Terminate if affected???
Haemophilia Genetic Analysis Summary

Haemophilia A

Severe
<1% FVIII:C

- Analyse intron 22 inversion
  - present
  - absent
    - Analyse intron 1 inversion
      - present
      - absent
        - Examine at risk female relatives for familial mutation
          - Examine male foetus for familial mutation

Moderate/Mild
>1% FVIII:C

- Use mutation screening technique (eg CSGE) to screen F8 or F9 gene for mutation
  - present
  - absent
  - OR DNA sequence F8 or F9 gene for mutation

Haemophilia B

- Examine at risk female relatives for familial mutation
- Examine male foetus for familial mutation

Use DNA sequencing to identify candidate mutation
New Mutations in Haemophilia

Family history of haemophilia: ~60% families

Sporadic haemophilia: ~40% families
Females with Haemophilia

• Some female haemophilia carriers experience bleeding problems

• Early in embryogenesis, one X chromosome is inactivated in all female cells; “Lyonisation”

• Process is random

• May result in unequal inactivation of X chromosomes
  – Carriers with haemophilia
  – Carriers with normal FVIII/IX levels
Haemophilia Web Resources

• Haemophilia A web page “HAMSTeRS”
  http://europium.csc.mrc.ac.uk/WebPages/Main/main.htm

• Haemophilia B web page
  http://www.kcl.ac.uk/ip/petergreen/haemBdatabase.html

• Best Practice guidelines

• About haemophilia
  http://www.haemophilia.org.uk/
  http://www.zlbbehring.co.uk/zb/n26942/PFFAQs.htm
Thank You