# Genetics of Haemophilia

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Edward
Duke of Kent
(1767-1820)

Victoria
Princess of Saxe-Coburg
(1786-1861)

Albert
(1819-1861)

Victoria
Queen of England
(1819-1901)

Victoria

Frederick

Edward VII
of England

Alexandra

Alice

Louis of Hesse

Alfred

Helena

Louise

Arthur

Leopold

Helen

Beatrice

Henry

Wilhelm II

Sophie of
Greece

George V

Irene

Henry

Fred

Alix

Nikolas II
of Russia

Alice of Athlone

Alfonso XIII
of Spain

Eugenie

Leopold

Maurice

George VI

Waldemar

Prince
Sigmund of
Prussia

Henry

Olga

Tatiana

Marie

Anastasia

Alexis

Lady May
Abel Smith

Rupert

?

Alfonso

Gonzalo

Princess
Margaret

Queen
Elizabeth

Prince Phillip

JC

Juan Carlos
of Spain

Princess
Ann

Prince
Charles

Prince
Andrew

Prince
Edward

Normal Male

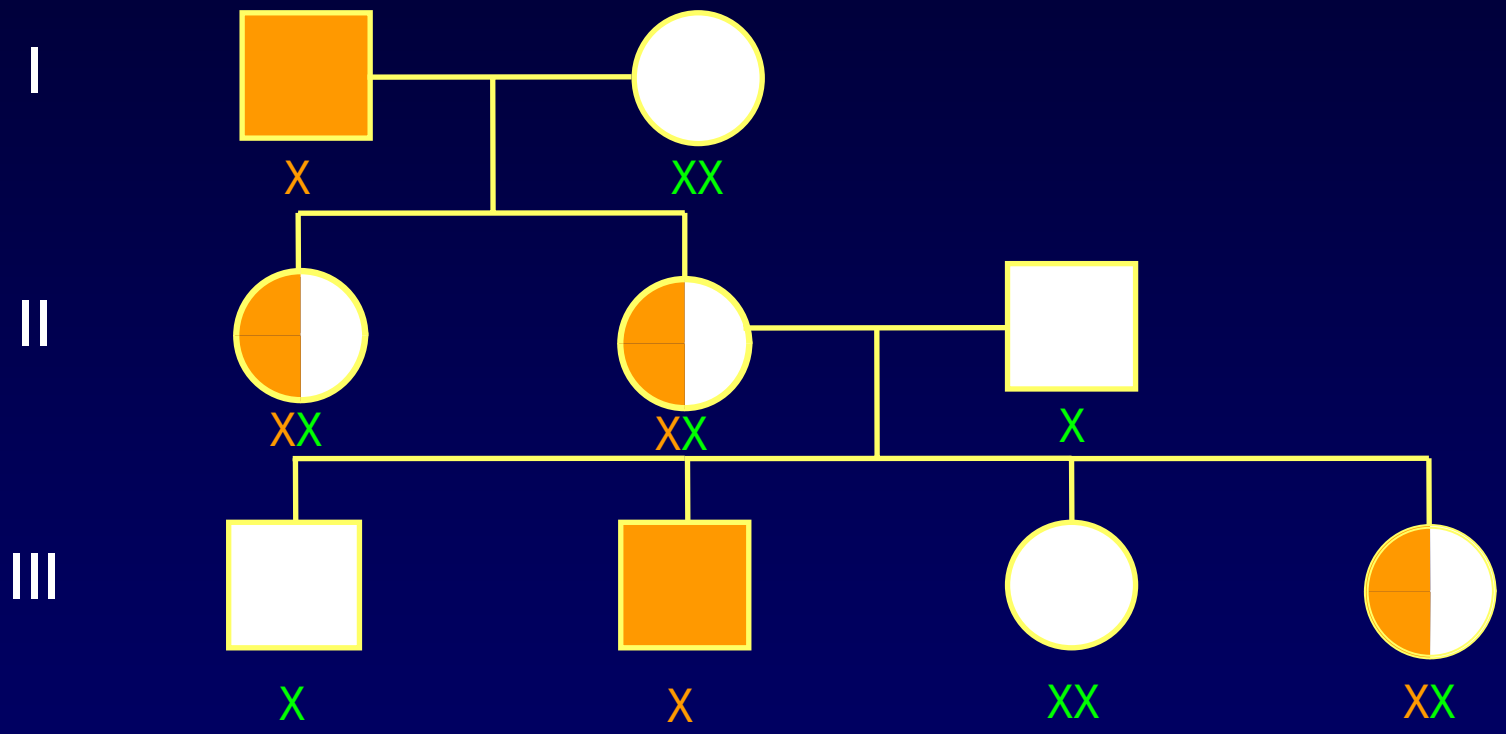
Normal Female

Hemophilic Male

Carrier Female

? Male died in infancy, possible hemophilic

# 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

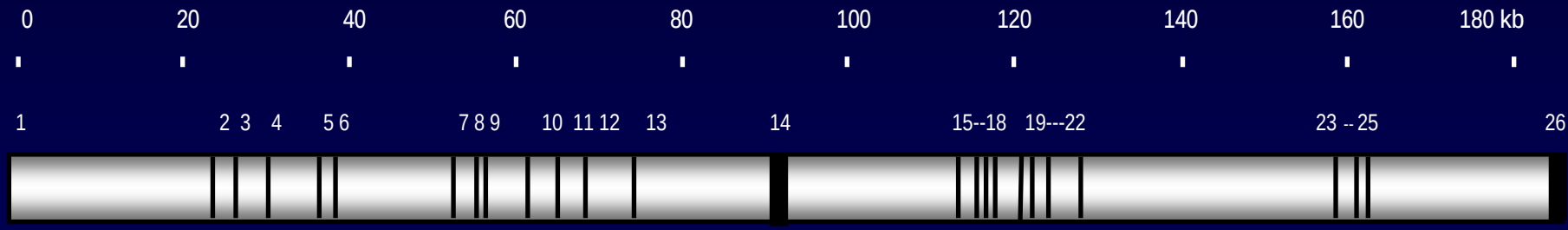
- X-chromosome linked inherited bleeding disorder
- 2 varieties
  - haemophilia A; 1 in 10,000 population
  - coagulation factor VIII deficiency

  - haemophilia B; 1 in 50,000 population
  - coagulation factor IX deficiency

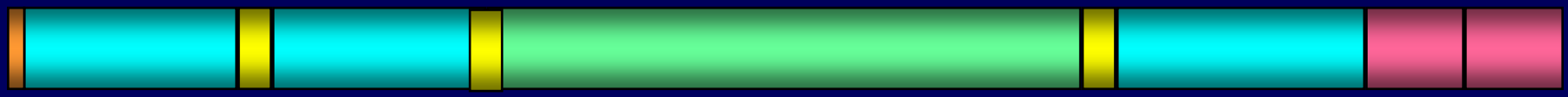
# Mutations Responsible for Haemophilia A and B

# Factor VIII Gene and Protein

*F8* Gene



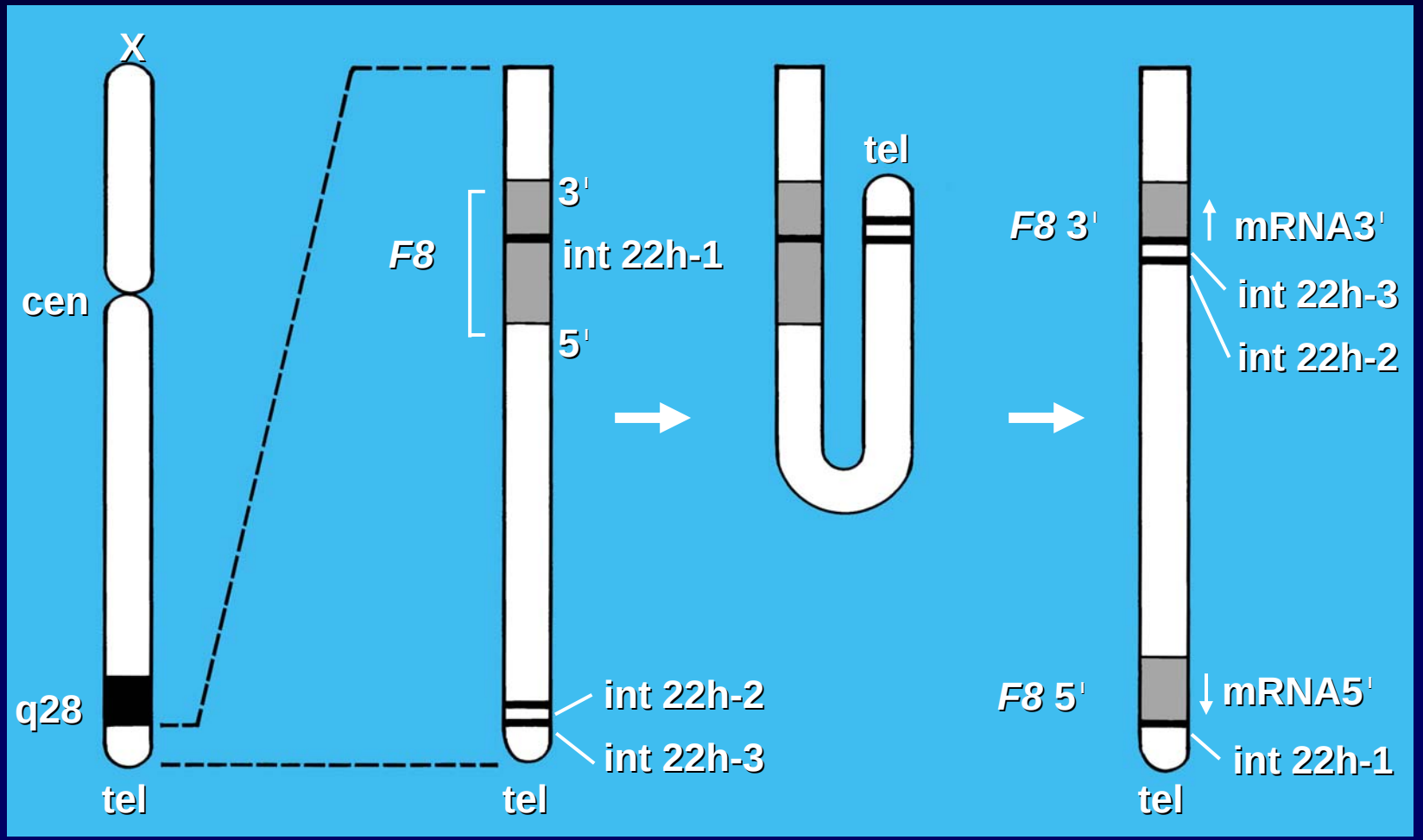

FVIII Protein



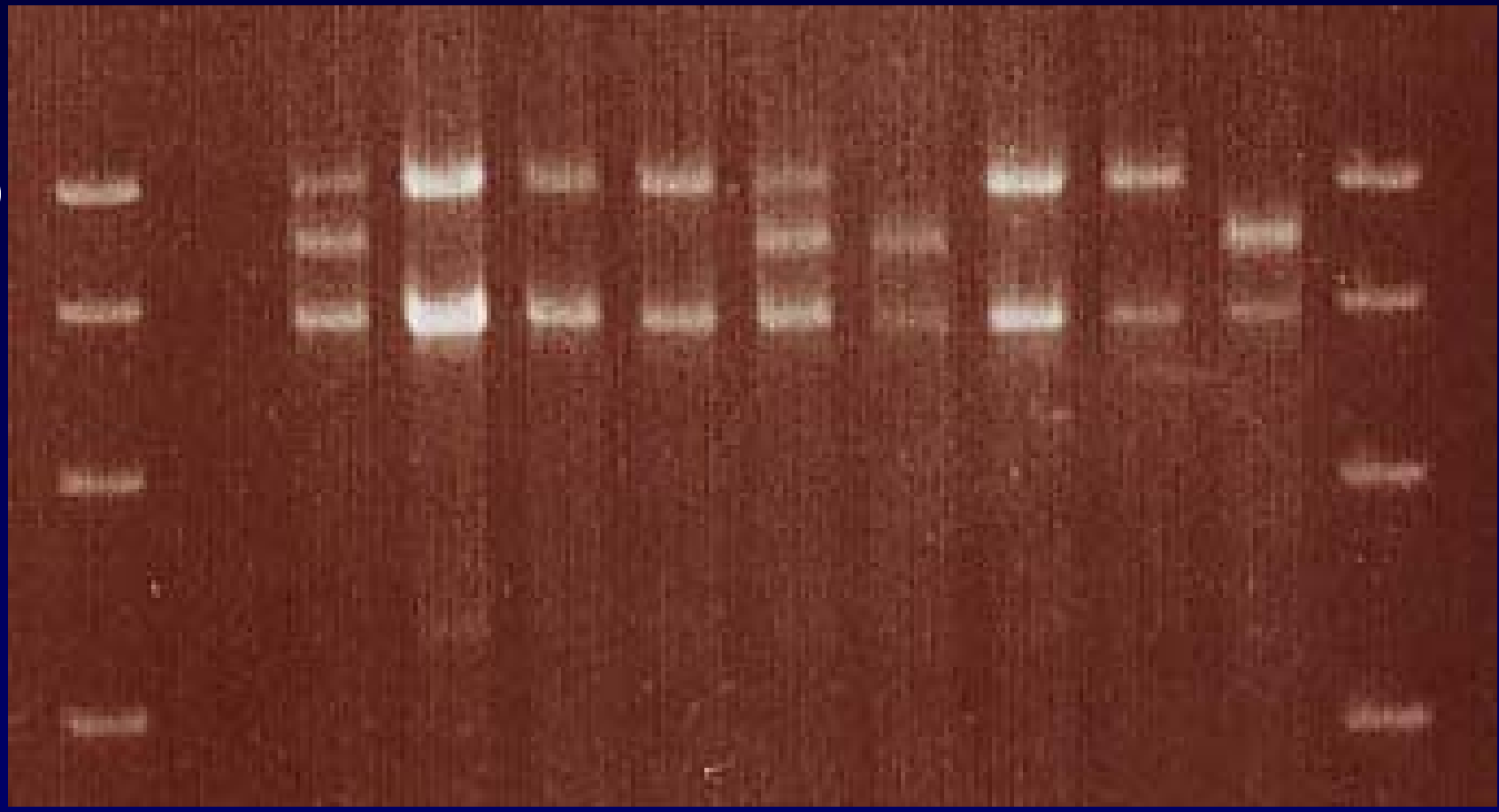
A1 a1 A2 a2 B a3 A3 C1 C2

Heavy chain Connecting region Light chain

# *F8* Intron 22 Inversion



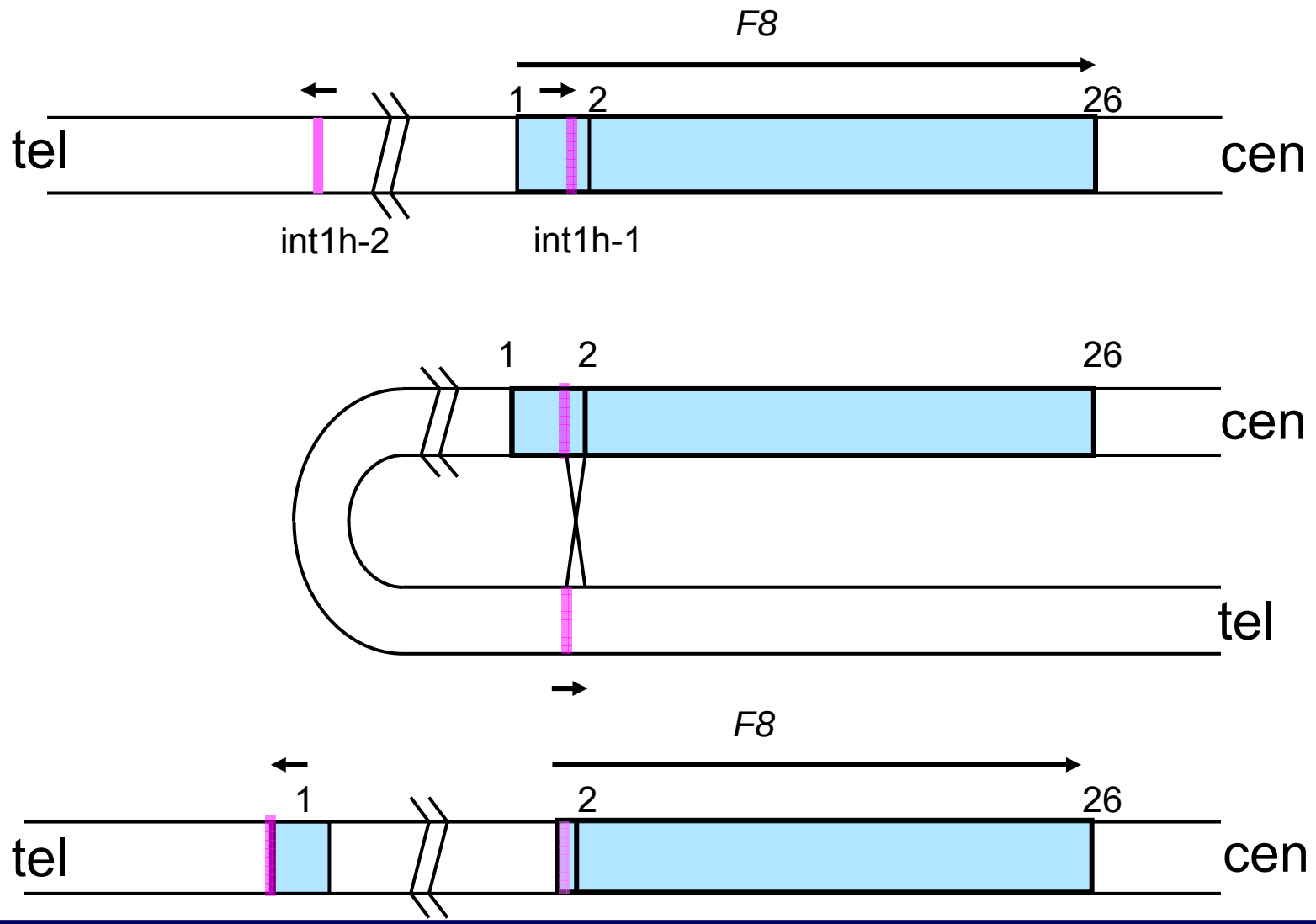
# *F8* Intron 22 Inversion Analysis



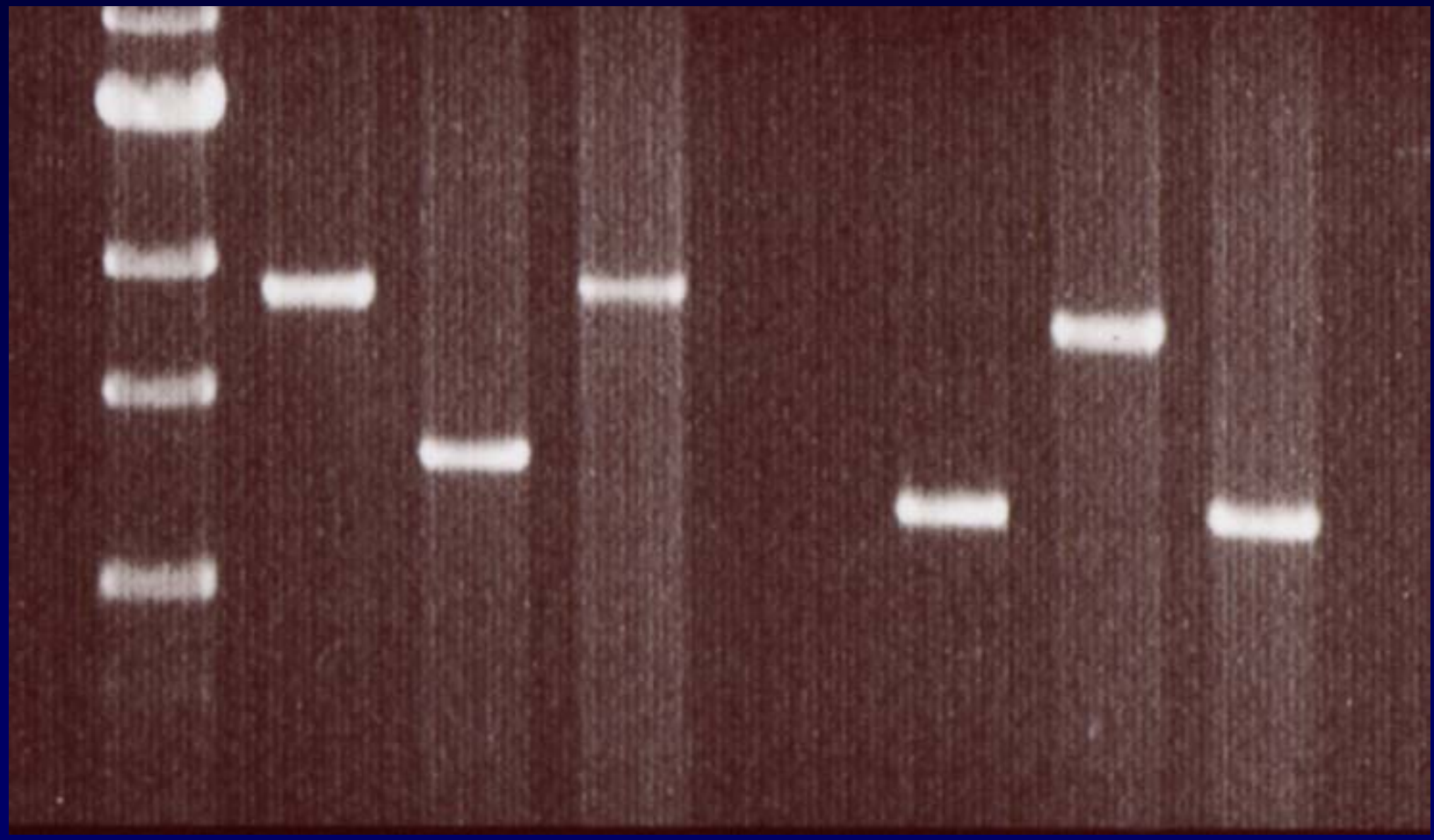
# *F8* Intron 22 Inversion

- 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



# *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 $A_8$>$A_7$) |

# 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)

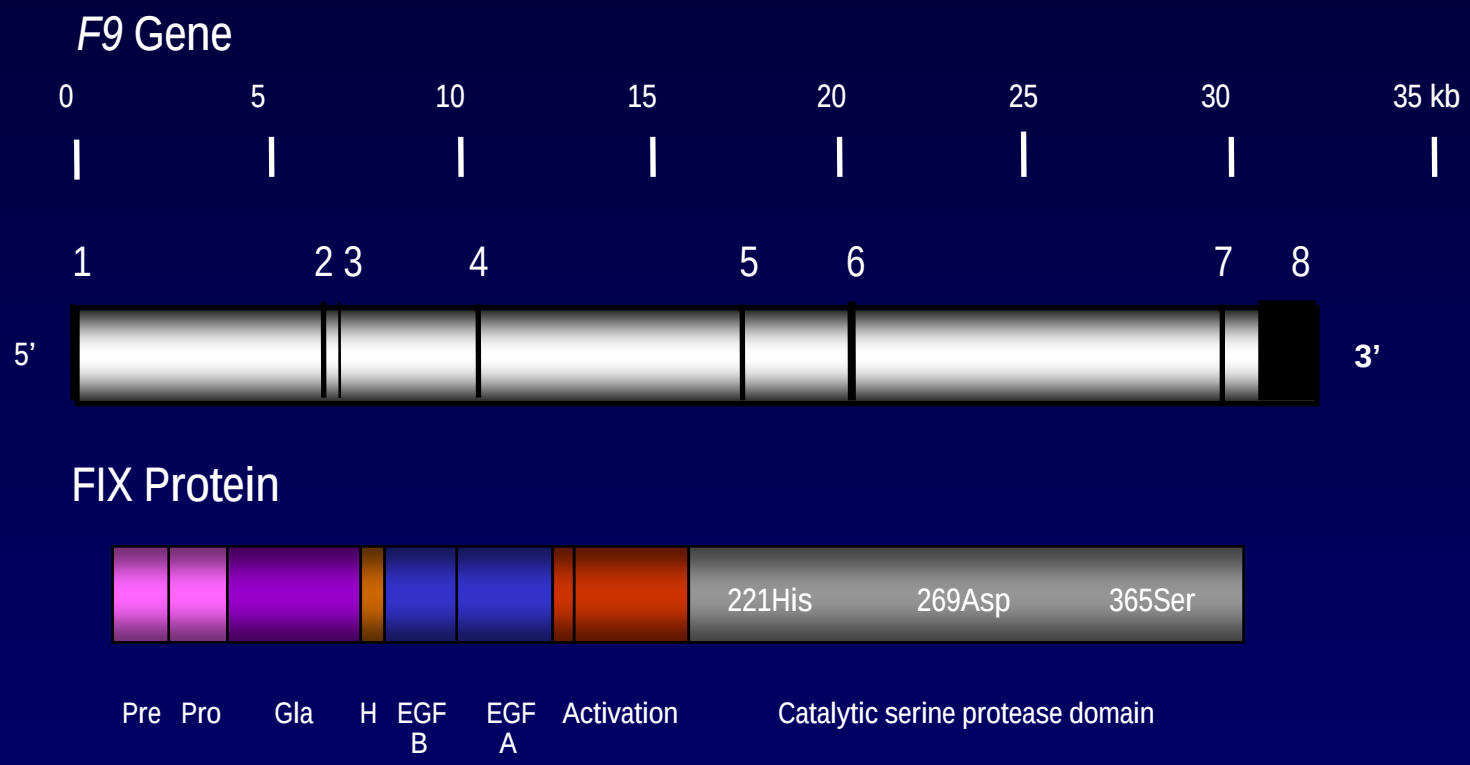
- Intron 1 or 22 inversion
- Delete part of gene
- Insert extra nucleotides
- Nonsense mutation
- Splice site defect
- Missense mutation at strategic amino acid

## 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

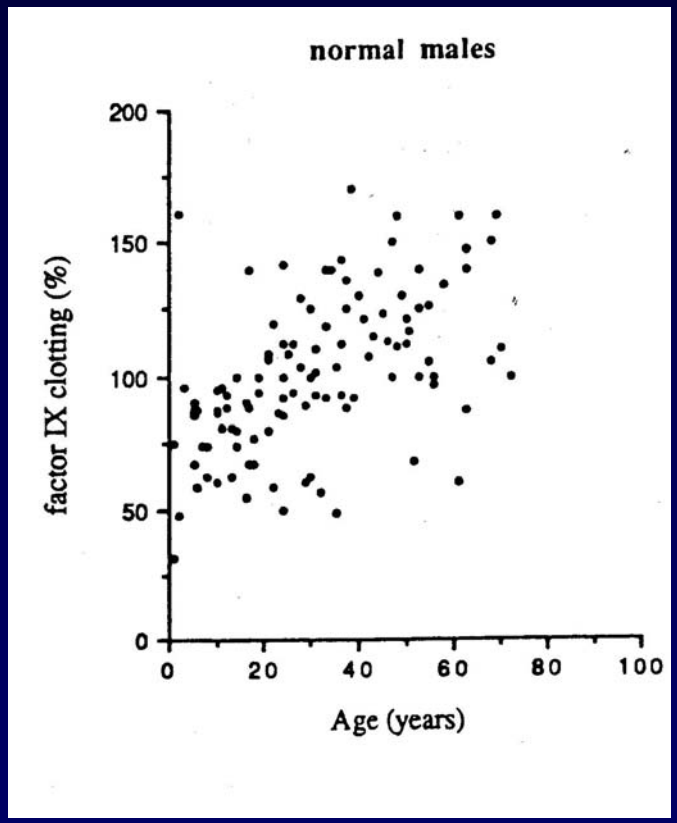


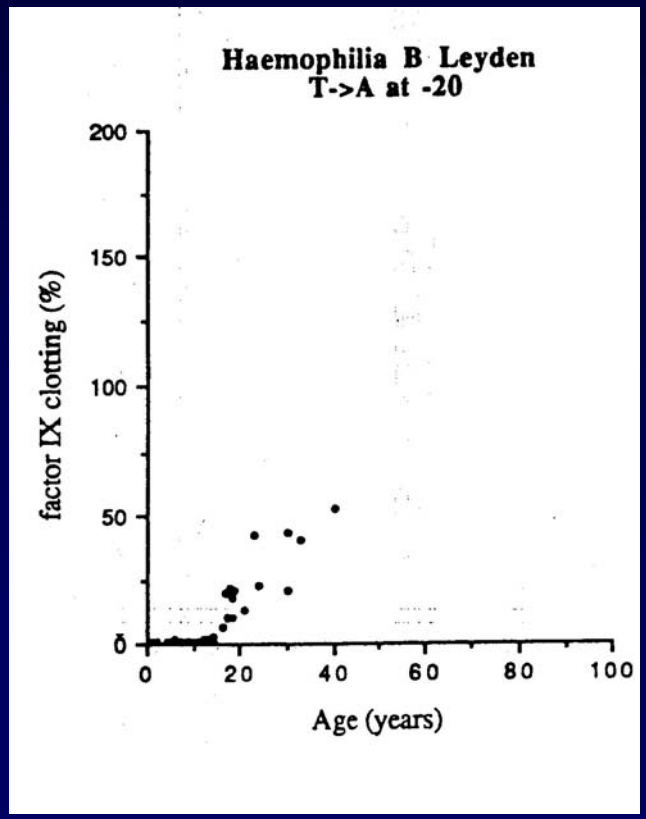
# *F9* Mutations

# 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

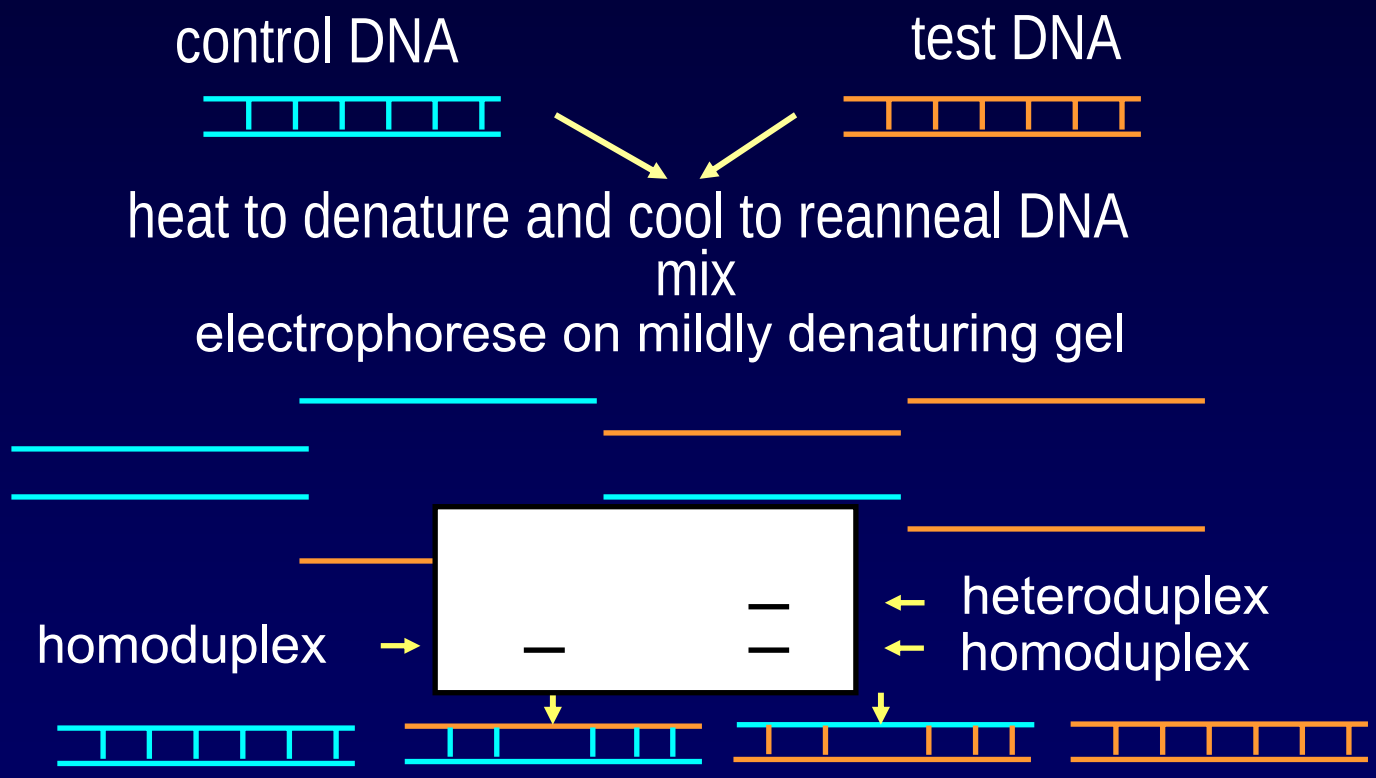
1. Seek mutation in affected male, then use presence/absence of mutation to determine female carrier status and enable PND

2. Use linkage analysis to track affected allele around the family, without knowledge of the causative mutation

# *F8* or *F9* Gene Mutation Screen

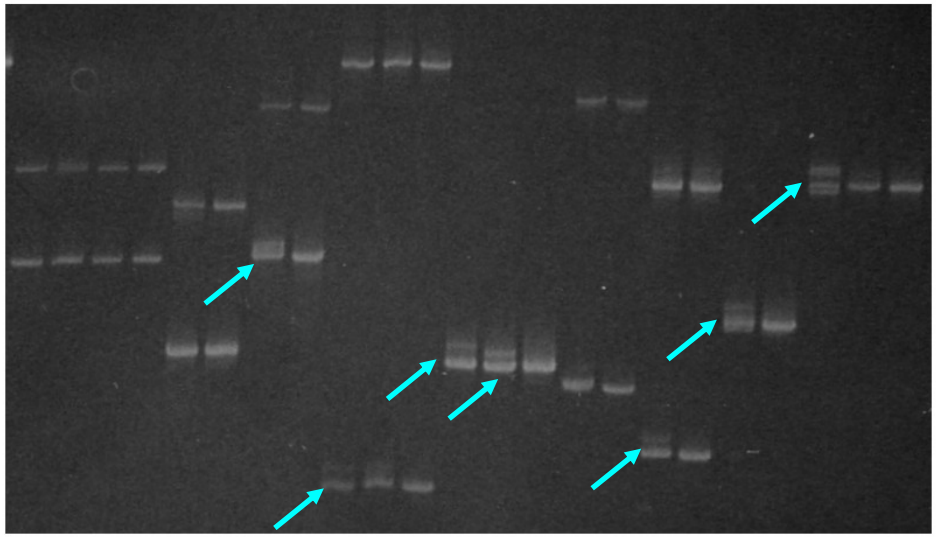
- Extract DNA from blood (white cells)
- PCR amplify exons & promoter
  - 30 PCR amplicons for *F8* (26 exons)
  - 10 PCR amplicons for *F9* (8 exons)
- Use mutation screening technique (CSGE, DHPLC, SSCP etc) or DNA sequence each amplicon to identify mutation
- Polymorphisms (neutral variation) also seen

# DNA Heteroduplex Formation



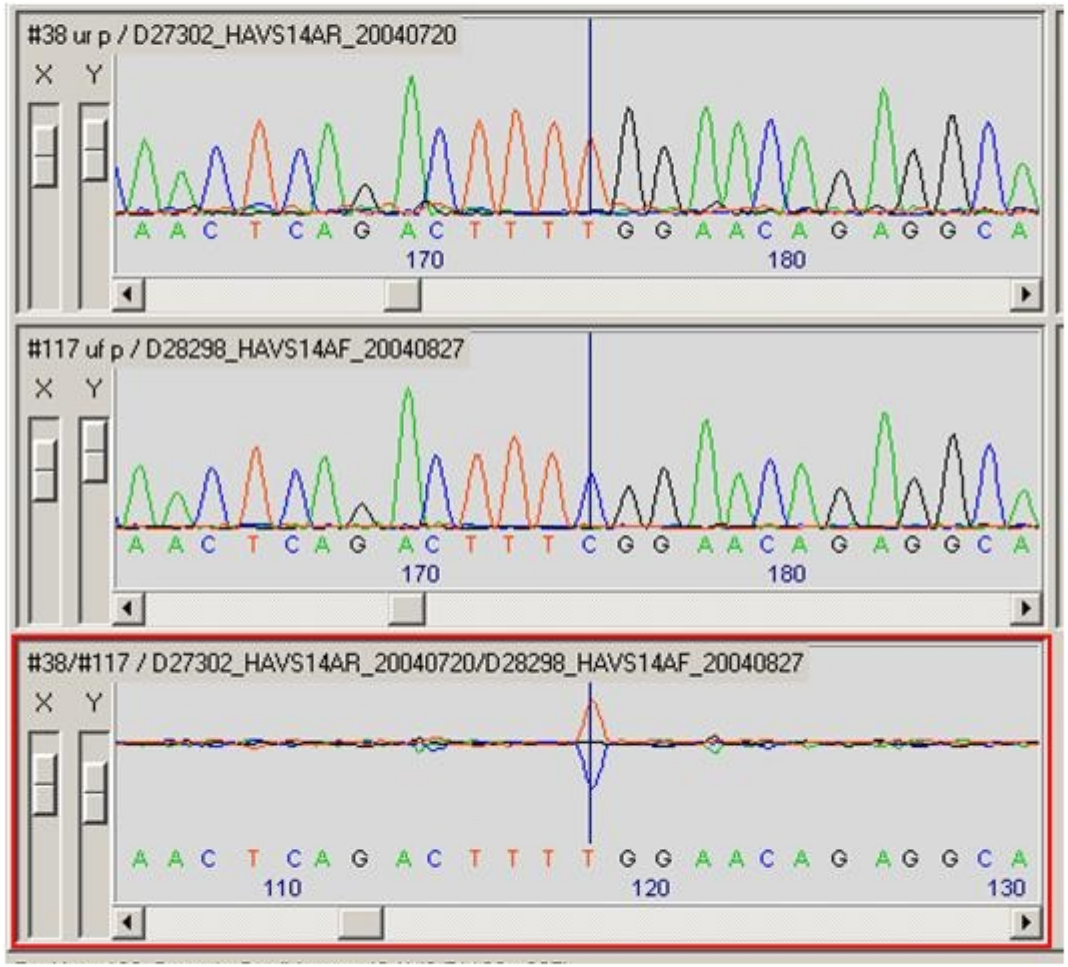

heteroduplex indicates alteration from control sequence

# Conformation Sensitive Gel Electrophoresis



CSGE analysis of *F8* gene amplicons identifies several sequence alterations in heteroduplexed DNA

# DNA Sequence Analysis




DNA sequence comparison of two patients to identify a sequence alteration using Staden sequence analysis software

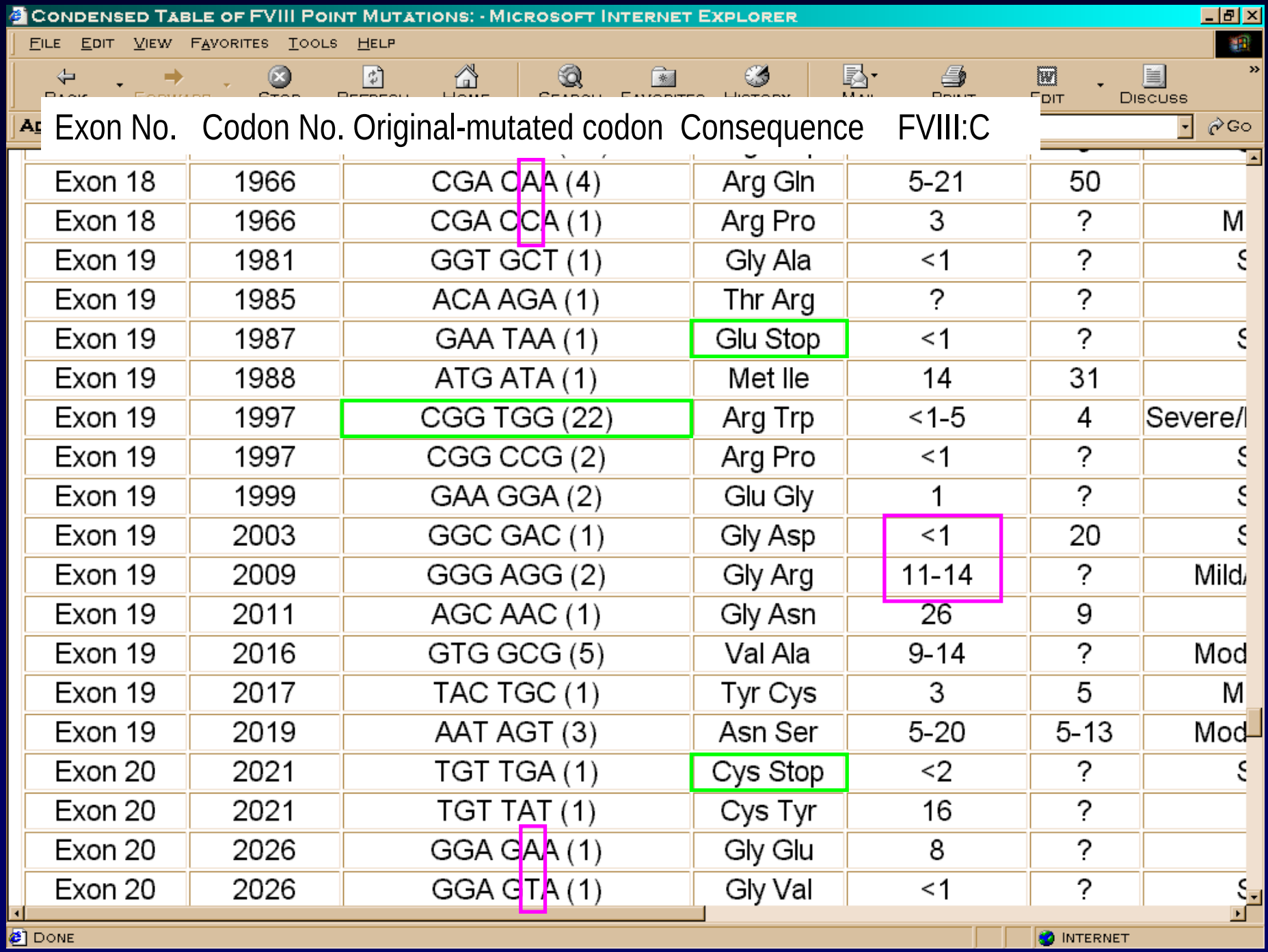
# Mutation Analysis

- Sequence affected male's DNA
- Identify amplicon (1 of 30 for *F8*) with altered sequence
- Use reference (*F8*) sequence to interpret result of nucleotide change (eg 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



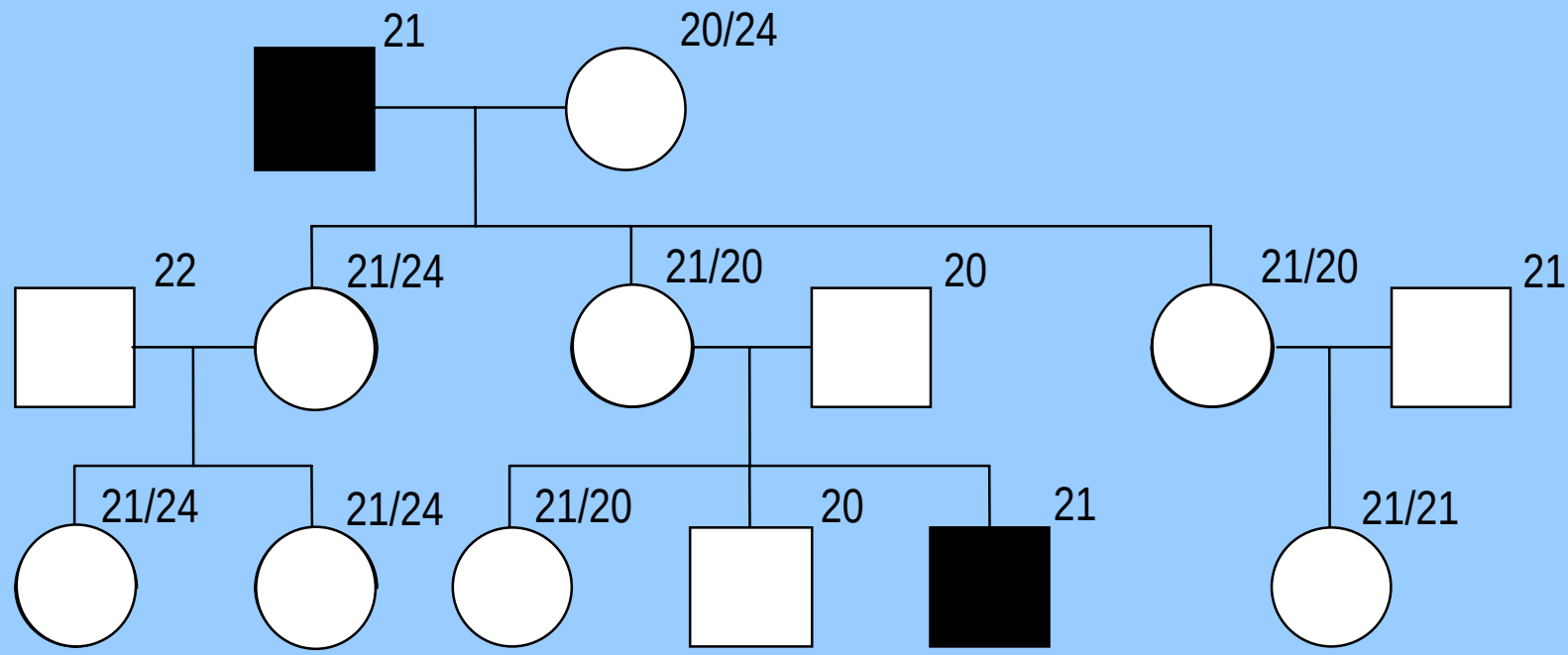

| Exon No. | Codon No. | Original-mutated codon | Consequence | FVIII:C | | |
|---|---|---|---|---|---|---|
| Exon 18 | 1966 | CGA CAA (4) | Arg Gln | 5-21 | 50 | |
| Exon 18 | 1966 | CGA CCA (1) | Arg Pro | 3 | ? | M |
| Exon 19 | 1981 | GGT GCT (1) | Gly Ala | <1 | ? | S |
| Exon 19 | 1985 | ACA AGA (1) | Thr Arg | ? | ? | |
| Exon 19 | 1987 | GAA TAA (1) | Glu Stop | <1 | ? | S |
| Exon 19 | 1988 | ATG ATA (1) | Met Ile | 14 | 31 | |
| Exon 19 | 1997 | CGG TGG (22) | Arg Trp | <1-5 | 4 | Severe/I |
| Exon 19 | 1997 | CGG CCG (2) | Arg Pro | <1 | ? | S |
| Exon 19 | 1999 | GAA GGA (2) | Glu Gly | 1 | ? | S |
| Exon 19 | 2003 | GGC GAC (1) | Gly Asp | <1 | 20 | S |
| Exon 19 | 2009 | GGG AGG (2) | Gly Arg | 11-14 | ? | Mild/ |
| Exon 19 | 2011 | AGC AAC (1) | Gly Asn | 26 | 9 | |
| Exon 19 | 2016 | GTG GCG (5) | Val Ala | 9-14 | ? | Mod |
| Exon 19 | 2017 | TAC TGC (1) | Tyr Cys | 3 | 5 | M |
| Exon 19 | 2019 | AAT AGT (3) | Asn Ser | 5-20 | 5-13 | Mod |
| Exon 20 | 2021 | TGT TGA (1) | Cys Stop | <2 | ? | S |
| Exon 20 | 2021 | TGT TAT (1) | Cys Tyr | 16 | ? | |
| Exon 20 | 2026 | GGA GAA (1) | Gly Glu | 8 | ? | |
| Exon 20 | 2026 | GGA GTA (1) | Gly Val | <1 | ? | S |

# FIX Home Page

http://www.kcl.ac.uk/ip/petergreen/haemBdatabase.html

| | FIX:C | FIX:Ag | Nt No. | Nt chg | CpG? | Consequence | | | |
|---|---|---|---|---|---|---|---|---|---|
| Münster 12 | 5 | | 30970 | C→G | N | 283, S→R | | Eigel & Horst | 1937 |
| GER 9589 | 6 | | 30970 | C→G | N | 283, S→R | | Wulff ,Herrmann et al | 2348 |
| France HB228 | <1 | | 30970 | C→G | N | 283, S→R | double with 291 A→P (nucleotide 30992 G→C) | Goossens et al | 2601 |
| HB270 | | | 30972 | A→G | N | 284, Y→C | Double (see 31,328) | Sommer et al | 1048 |
| HB 270 | <5 | | 30972 | A→G | N | 284, Y→C | | Ketterling, R, 1999 | 2217 |
| HB816, Fr | <1 | 2 | 30973 | C→A | N | 284, Y→Stop | | Tartary et al (1993) | 1049 |
| HB203 | 1 | <1 | 30973 | C→A | N | 284, Y→Stop | | Ketterling et al (1993) | 1050 |
| Besancon 7 | 1 | | 30973 | C→A | N | 284, Y→Stop | | Goossens et al | 1984 |
| HB006 | 14 | | 30980 | C→A | N | 287, P→T | | Vidal et al (2000) | 2563 |
| GER 2234A | | | 30981 | C→A | N | 287, P→H | | Wulff et al (1995) | 735 |
| es82 | 3 | 3 | 30981 | C→A | N | 287, P→H | | Montejo et al (1999) | 1857 |
| Unnamed | <1 | <1 | 30981 | C→T | N | 287, P→L | | Chen et al (1991a) | 251 |
| GER 11606 | | | 30981 | C→T | N | 287, P→L | | Wulff ,Herrmann et al | 2855 |
| 9313 | 6 | 2 | 30984 | T→C | N | 288, I→T | | Thompson (unpublished) | 2750 |
| HB109 | <1 | <1 | 30985 | T→G | N | 288, I→M | | Bottema et al (1991a) | 519 |
| GER 8832 | | | 30986 | T→C | N | 289, C→R | | Wulff & Herrmann (1999) | 1936 |
| PA 648 | <1 | | 30987 | G→A | N | 289, C→Y | | Tagariello | 2590 |
| LY83 | <1 | | 30987 | G→A | N | 289, C→Y | | Negrier et Vinciguerra | 3064 |
| Belem 4 | 4 | | 30987 | G→C | N | 289, C→S | | Pestana et al | 1488 |
| UK 58 | 2 | | 30987 | G→T | N | 289, C→F | | Saad et al (1994) | 736 |
| HB162 | | | 30987 | G→T | N | 289, C→F | Female | Gostout et al (1993) | 1051 |
| Unnamed | <1 | | 30987 | G→T | N | 289, C→F | | Driscoll et al (1996) | 1487 |
| Unnamed | <1 | | 30987 | G→T | N | 289, C→F | | Driscoll et al (1996) | 1646 |
| Toulouse 4 | <1 | | 30987 | G→T | N | 289, C→F | | Goossens et al | 2637 |
| GER 11623 (Ru) | | | 30989 | AT→CA | N | 290, I→H | | Wulff ,Herrmann et al | 2856 |
| UK 13 | 10 | | 30992 | G→A | N | 291, A→T | | Montandon et al (1989) | 253 |
| UK 33 | 7 | 19 | 30992 | G→A | N | 291, A→T | | Green et al (1992a) | 254 |
| UK 41 | 9 | 11 | 30992 | G→A | N | 291, A→T | | Green et al (1992a) | 255 |
| UK 71 | 7 | 10 | 30992 | G→A | N | 291, A→T | | Saad et al (1994) | 520 |
| UK 239 | 10 | | 30992 | G→A | N | 291, A→T | | Saad et al (1994) | 737 |
| UK 249 | 6 | | 30992 | G→A | N | 291, A→T | | Saad et al (1994) | 1052 |
| UK 416 | | | 30992 | G→A | N | 291, A→T | | Rowley et al | 1489 |
| HB 689 | <1 | | 30992 | G→A | N | 291, A→T | | Li, X, 2000 | 2219 |
| HB 690 | 1 | | 30992 | G→A | N | 291, A→T | | Li, X, 2000 | 2220 |
| Oxford h2 | 2 | 3 | 30992 | G→C | N | 291, A→P | | Winship & Dragon (1991) | 252 |
| France HB228 | <1 | | 30992 | G→C | N | 291, A→P | double with 283 S→R (nucleotide 30970 G→C) | Goossens et al | 2601 |

# Linkage Analysis in a Haemophilia A Family



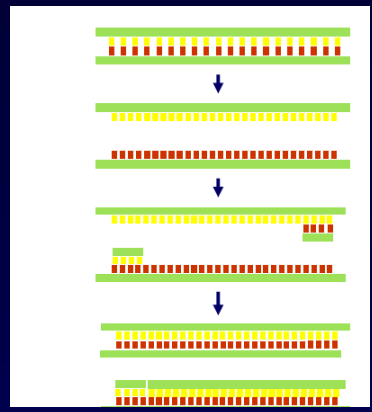

*F8* intron 13 ($CA_n$) repeat; 16-25 repeats

# 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

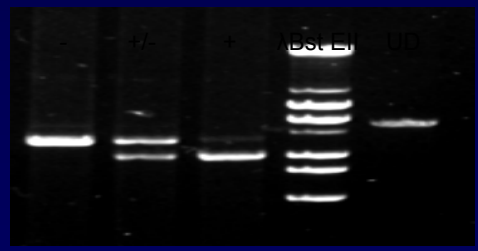


- Single nucleotide polymorphism recognised by restriction enzyme digestion (restriction fragment length polymorphism (RFLP))

G

A

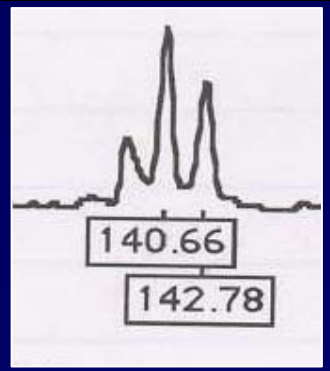
**XbaI**



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

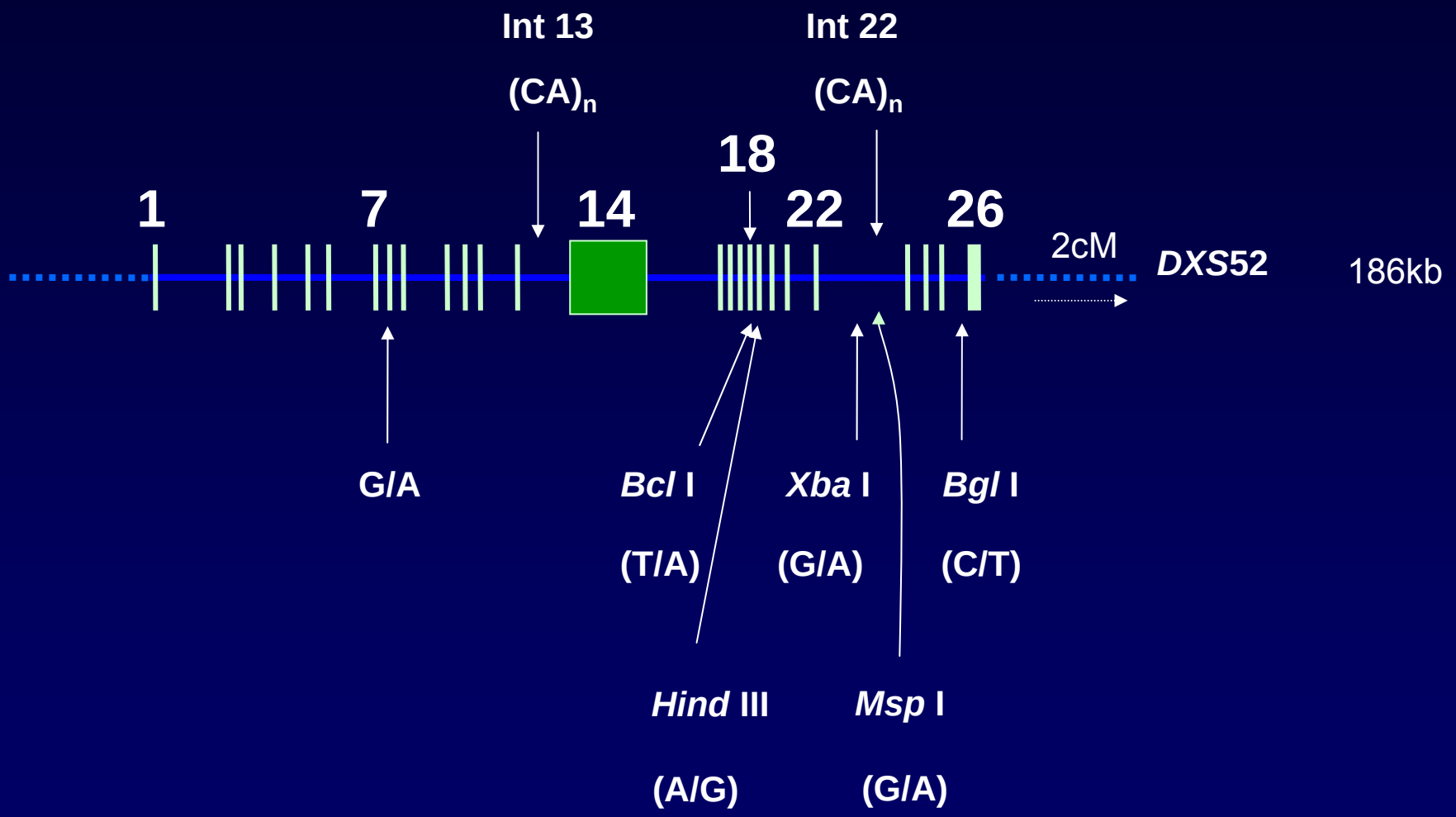
CACACA

CACACACA CA



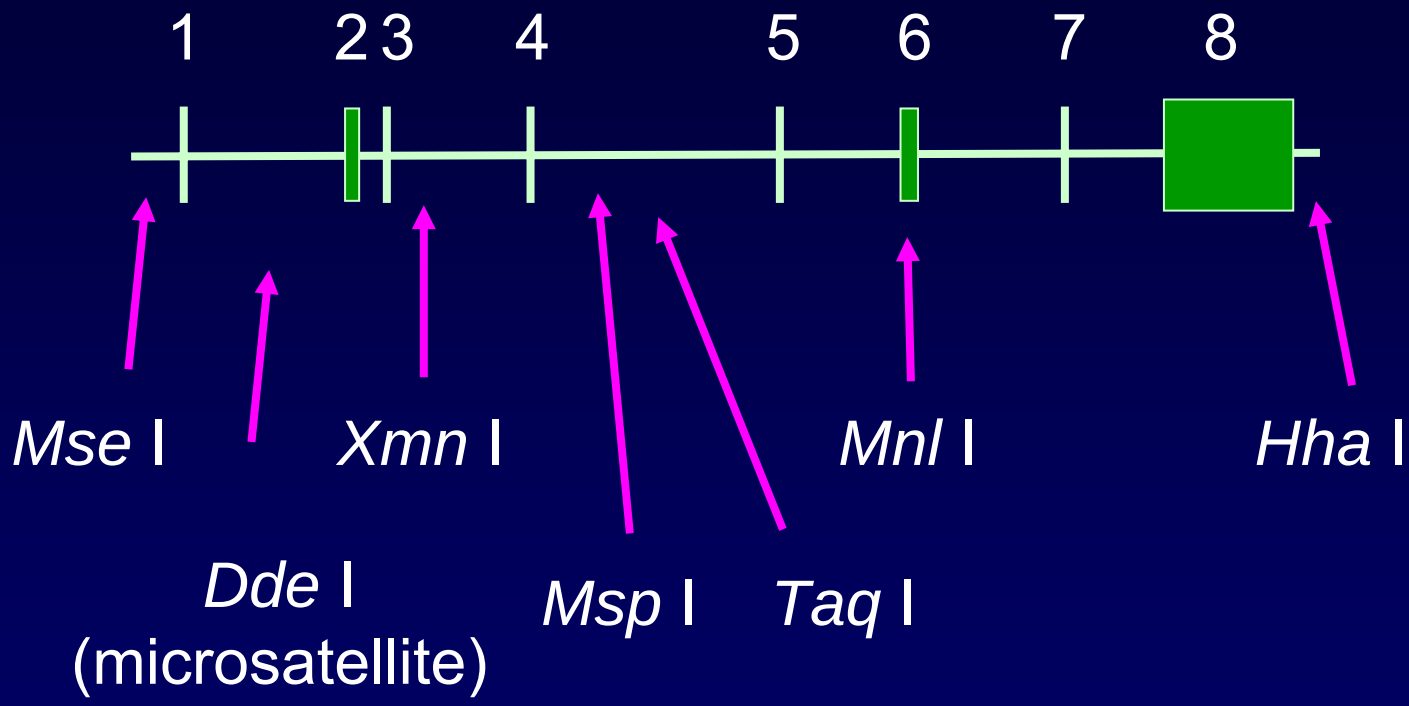

**Intron13 (CA)n**

# Commonly Used Polymorphic Markers in the *F8* Gene




Informativity 80-90%

# Commonly Used Polymorphic Markers in the *F9* Gene




Combined informativity 80-90%

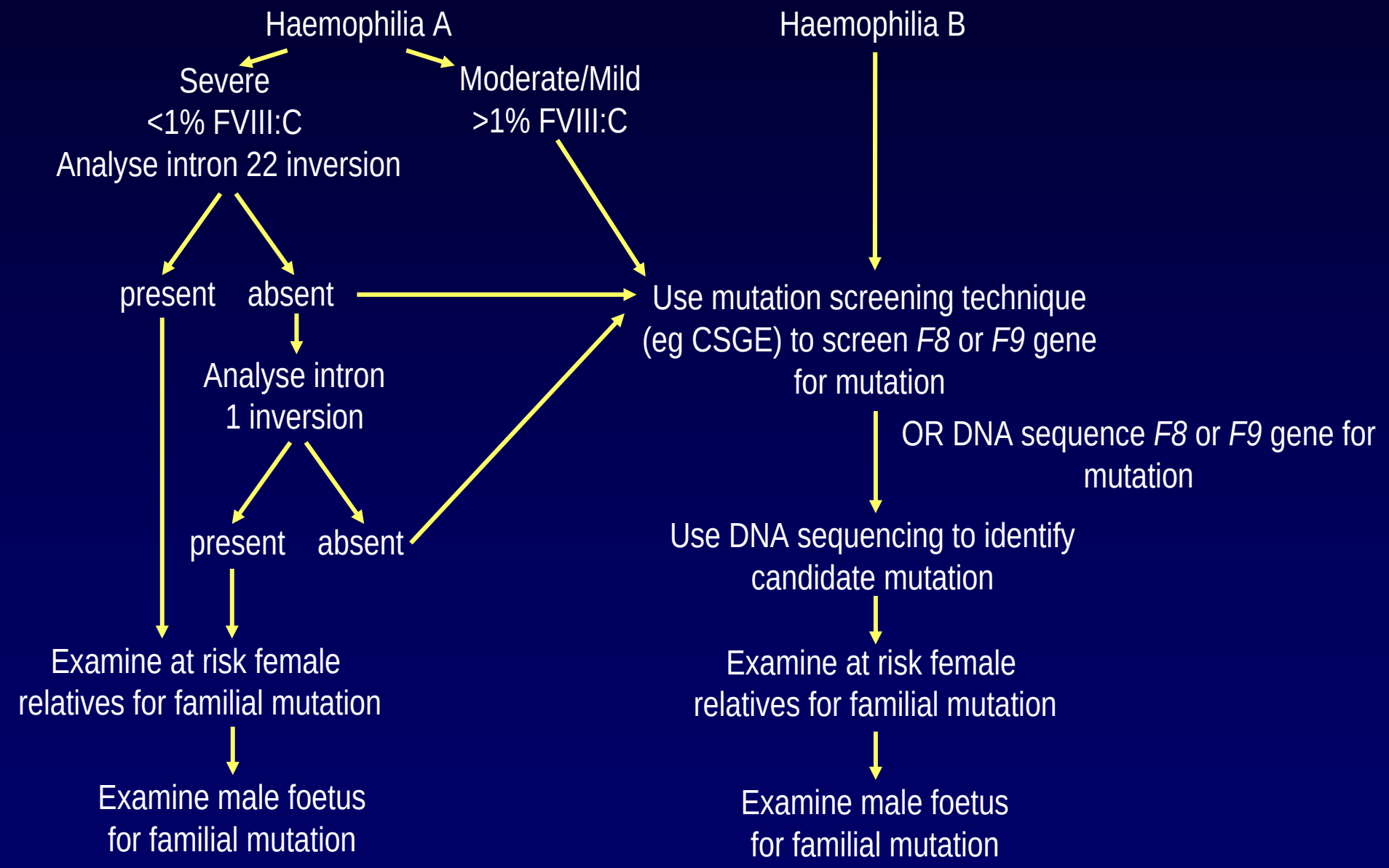
# Genetic Tests for Haemophilia

- *F8* intron 22 inversion
- *F8* intron 1 inversion
- *F8* screen in affected male
- *F9* screen in affected male
- Confirm / exclude mutation in ? carrier female (amplify single exon only)
- Linkage analysis
- PND

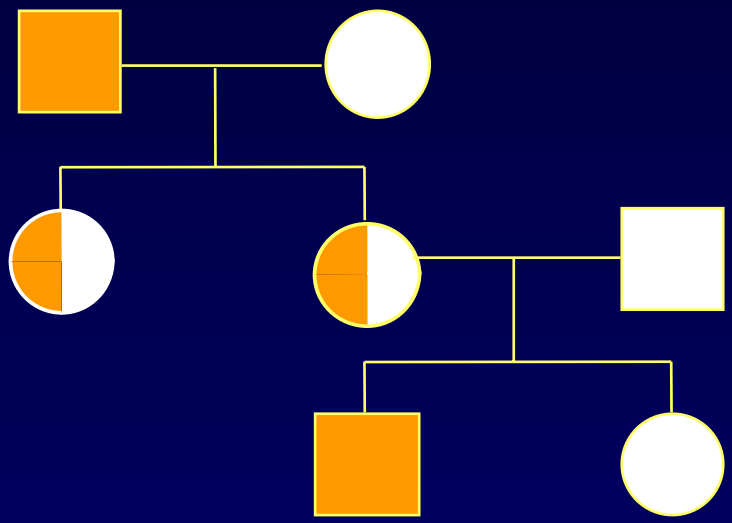
# Prenatal Diagnosis (PND)

- 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

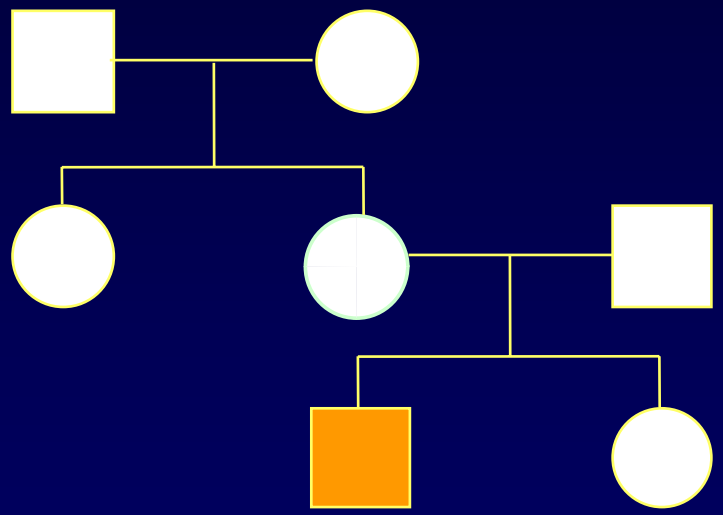


# New Mutations in Haemophilia



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 chromososme 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
  http://www.cmgs.org/BPG/Guidelines/2004/HaemophiliaA.htm
  http://www.cmgs.org/BPG/Guidelines/2004/HaemophiliaB.htm
- About haemophilia
  http://www.haemophilia.org.uk/
  http://www.zlbbehring.co.uk/zb/n26942/PFFAQs.htm


Thank You