CARRIER TESTING – A HISTORY

DR JOHN ROWELL

DIRECTOR OF HAEMOPHILIA CENTRE, ROYAL BRISBANE AND WOMENS HOSPITAL

- Haemophilia is an X linked disorder characterised by deficiency of plasma protein – factor VIII or IX
- Gene for production of FVIII (FIX) is on X chromosome (sex chromosome)
- Variable symptoms related to level of Factor VIII in plasma

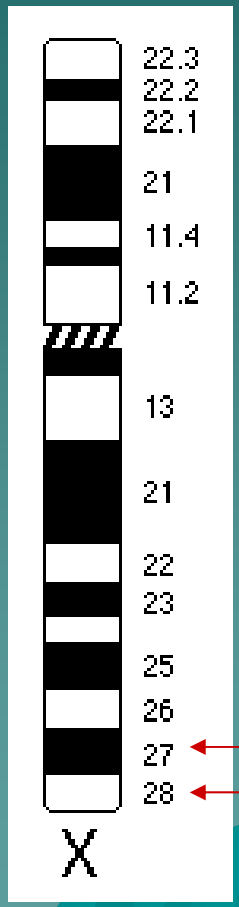
– Severe	Spontaneous haemorrhages	<2%
– Moderate	Traumatic / spontaneous	2-5%
– Mild	Traumatic / post surgery	5-40%

- Treated with replacement Factor VIII (FIX)

Genetics of Haemophilia

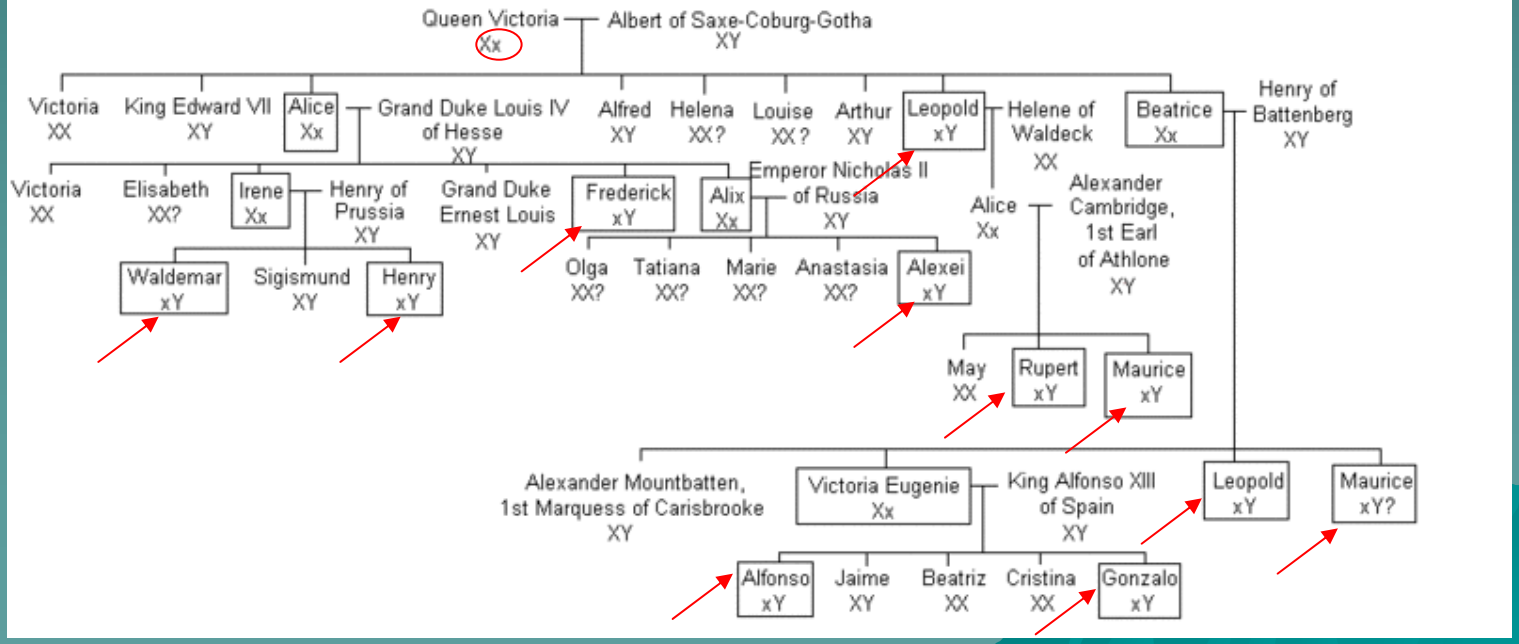
X-linked Inheritance

- Haemophilia A – Factor VIII (F8) gene (Xq28)
- Haemophilia B – Factor XI (F9) gene (Xq27.1-q27.2)



The British Haemophilia Line

Key
X: Unaffected X-chromosome
Y: Y-chromosome
x: Affected X-chromosome



1966

TABLE XI

COMPARATIVE DISTRIBUTION OF FACTOR-VIII LEVELS IN HETEROZYGOUS FEMALES*, POSSIBLY HETEROZYGOUS FEMALES†, HEMIZYGOUS AFFECTED MALES, NORMAL MALES AND FEMALES IN HAEMOPHILIC FAMILIES, AND FEMALES FROM NORMAL FAMILIES

Genotype class	*No. of individuals*	*Factor-VIII levels (%)*			
		Mean	*Range*	*Standard deviation*	*Coefficient of variation*
Homozygous normal females (normal families)	99	93	32–200	27	29
Hemizygous normal males (normal families)	118	96	44–157	24	25
Homozygous normal males (haemophilic families)	58	87	48–209	34	39
Possibly heterozygous females	43	71	26–150	29	41
Heterozygous females	29	48	26–126	22	45
Hemizygous affected males	37	3	0–20	5	—

* Daughters of hemizygous affected males or females with at least two Factor-VIII-deficient male relatives in appropriate lineage.

† Mothers of a single haemophiliac and no other known affected male relative or females with half or quarter chance of being heterozygous.

Kerr C, Preston A, Barr A, Biggs R. Further studies on the inheritance of Factor VIII. Br J Haem 1966, 12,212

1971

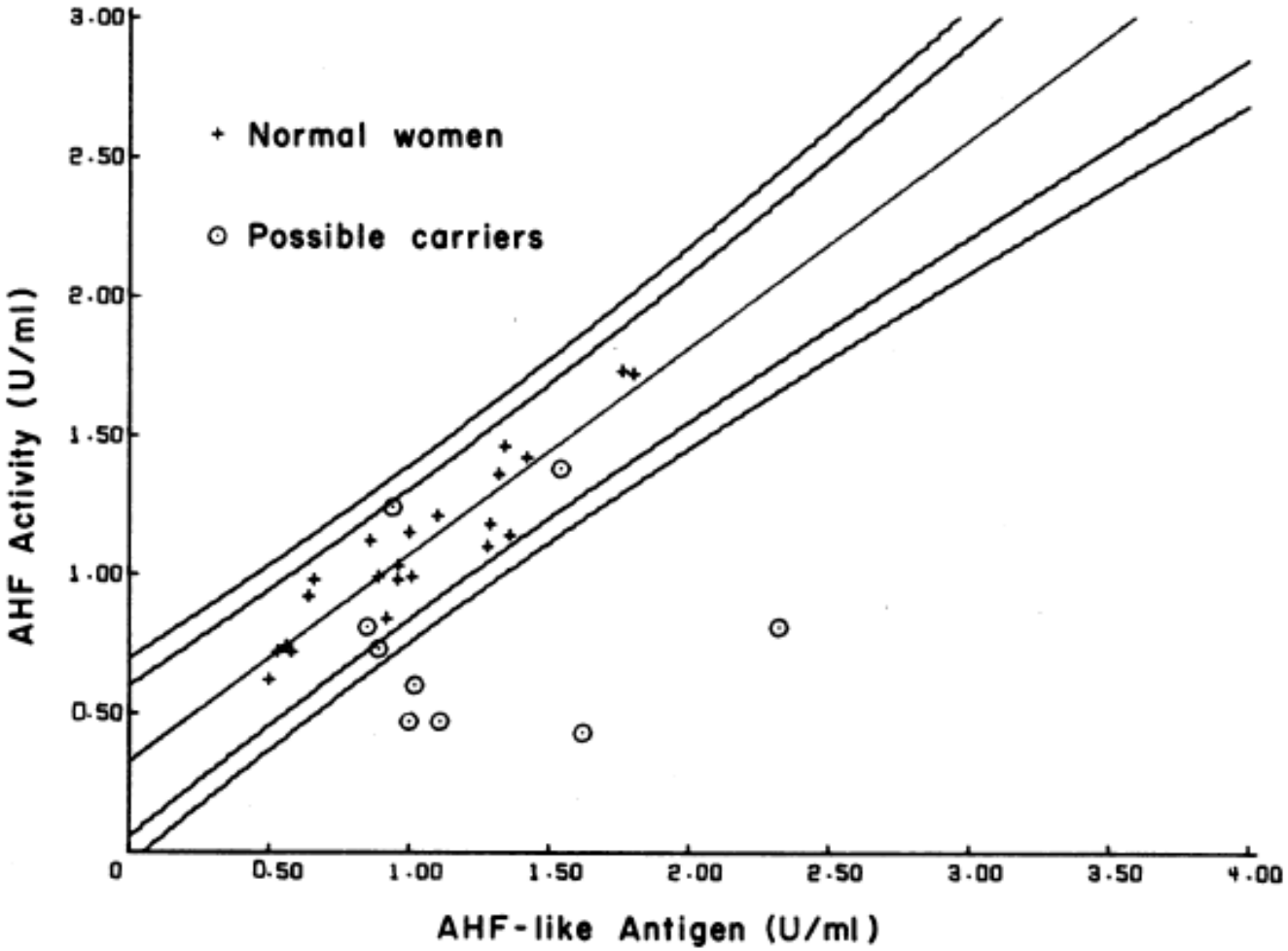


FIGURE 2 The relationship of AHF activity to AHF-like antigen in possible carriers and normal women. The center line is the regression line for the data obtained in normal women, the outermost lines represent the 99% confidence belt, and the other two lines represent the 95% confidence belt.

Zimmerman TS, Ratnoff O, Littell A. Detection of carriers of classic Haemophilia using an immunologic assay for Antihaemophilic Factor (factor VIII) JCI 1971, 50, 255

DNA – (deoxyribonucleic acid)

G - guanine

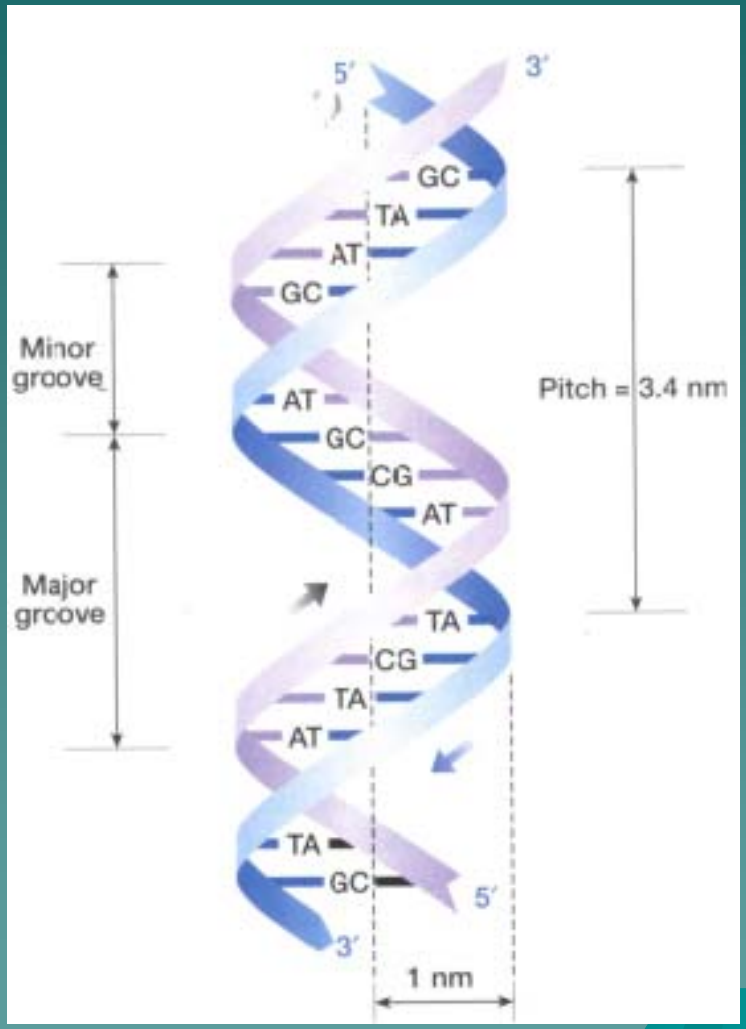
C - Cytosine

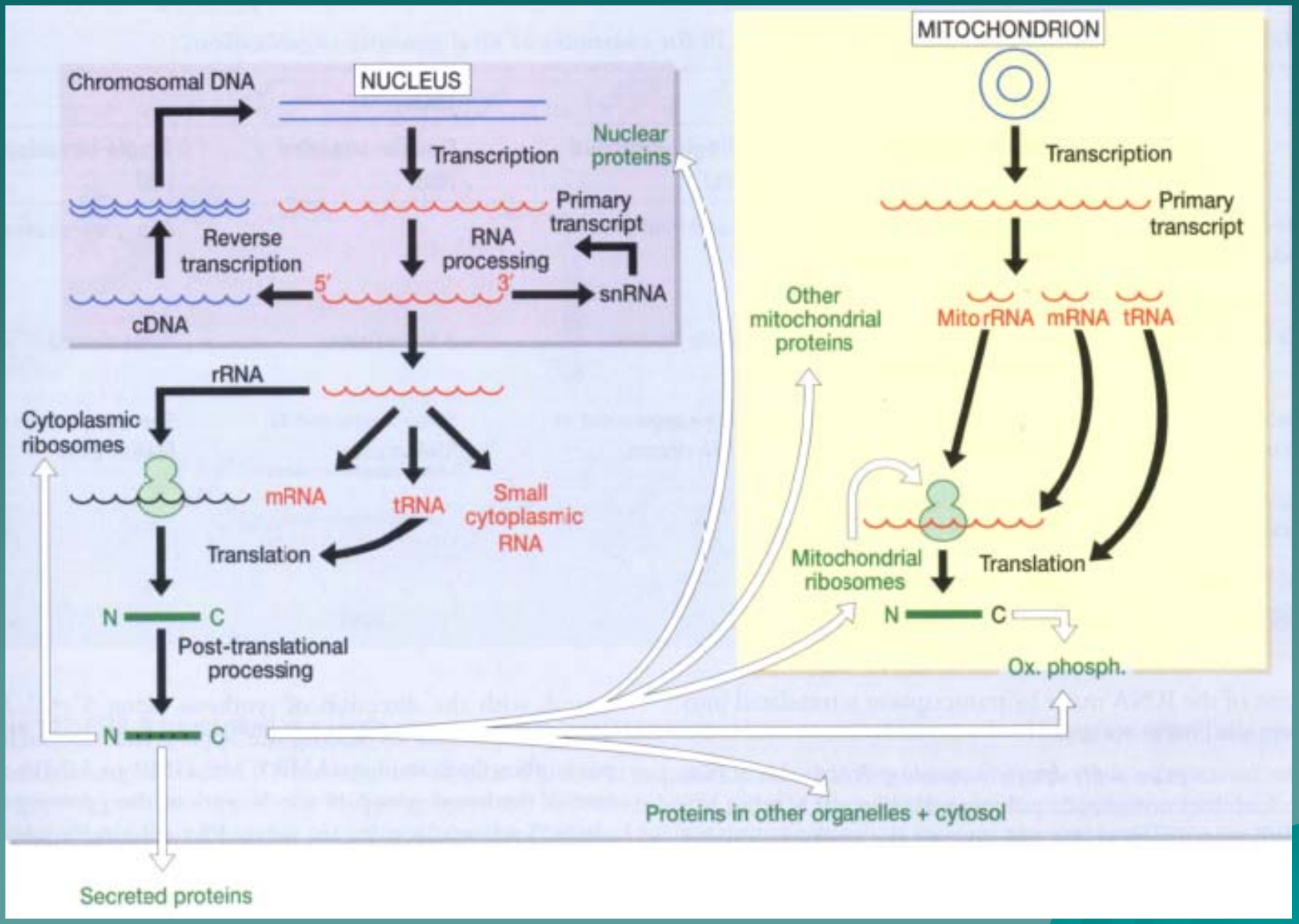
T - Thymine

A - Adenine

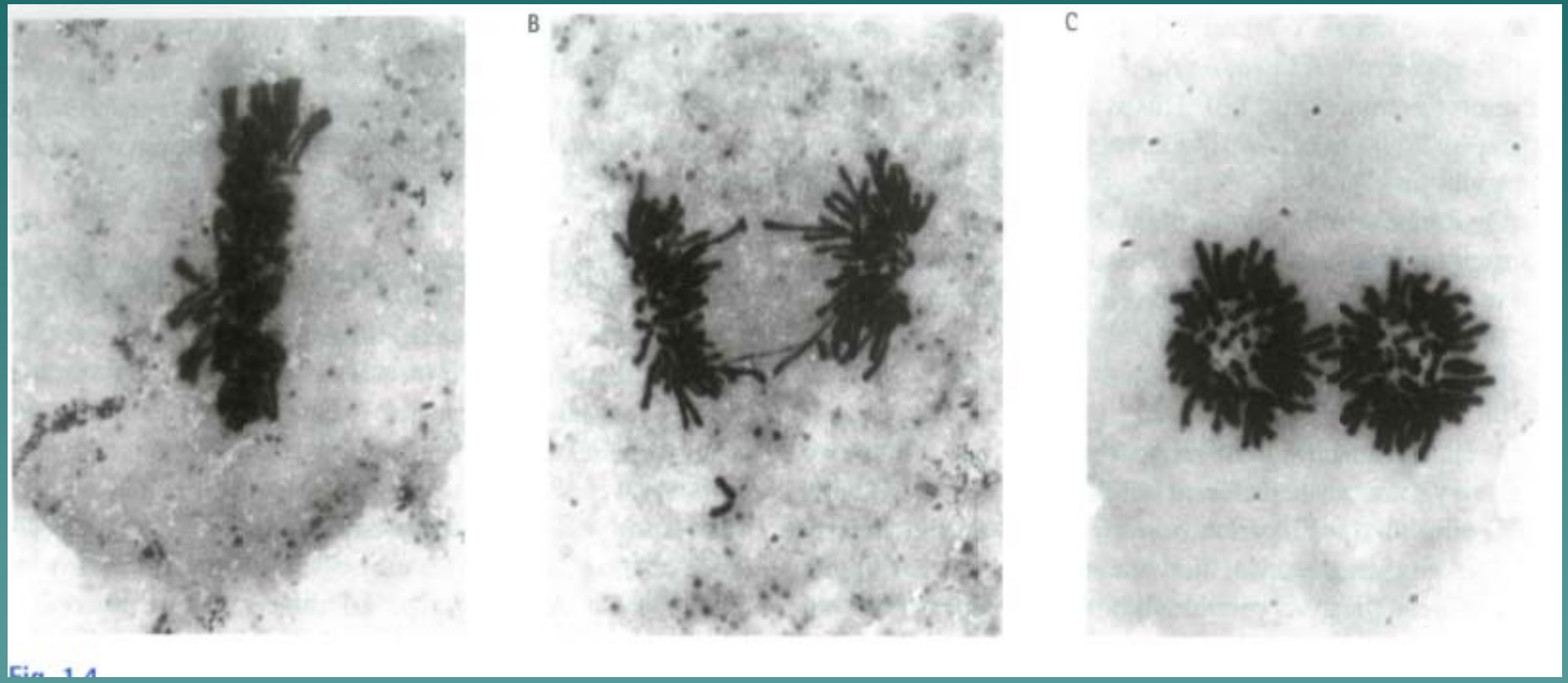
Sequence of bases – codons (3 bases) code for aminoacids

Proteins consist of aminoacids

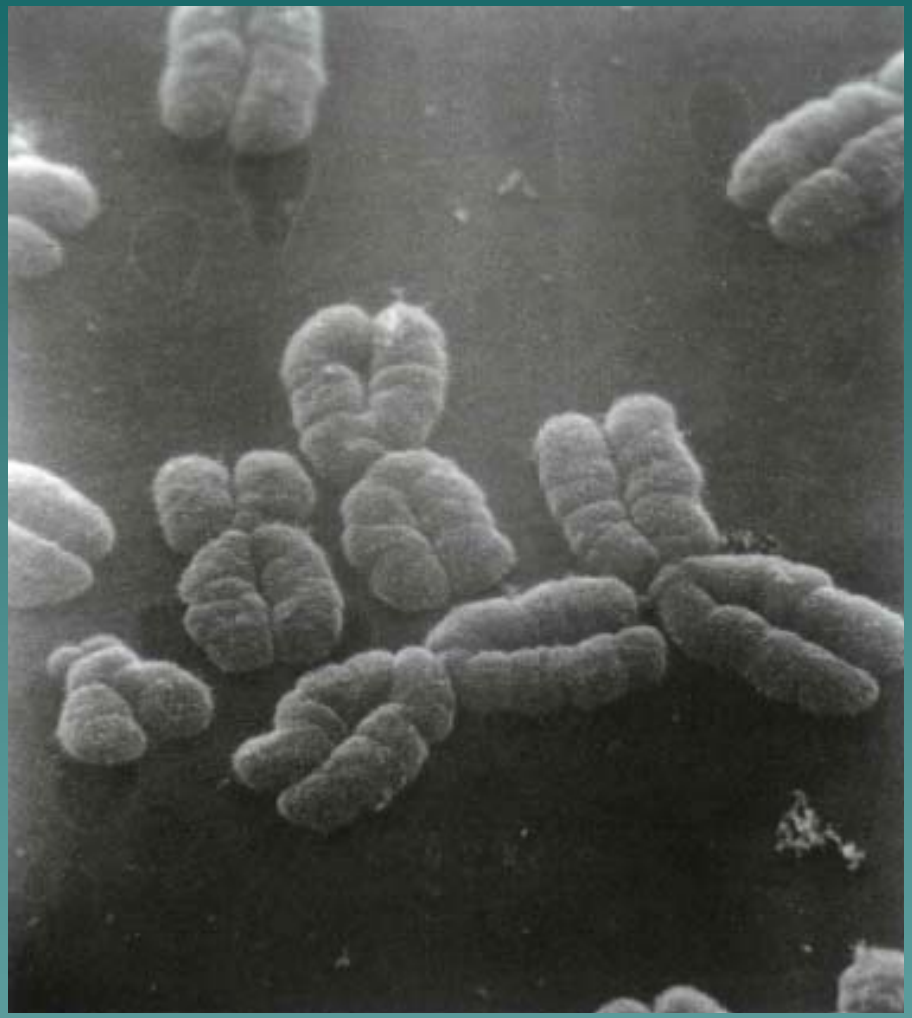




Strachan T, Read AP. Human Molecular Genetics 3rd ed. 2004 Garland science, London



Turpenny PD, Ellard S. Emerys Elements of Medical Genetics 2005 Elsevier, London



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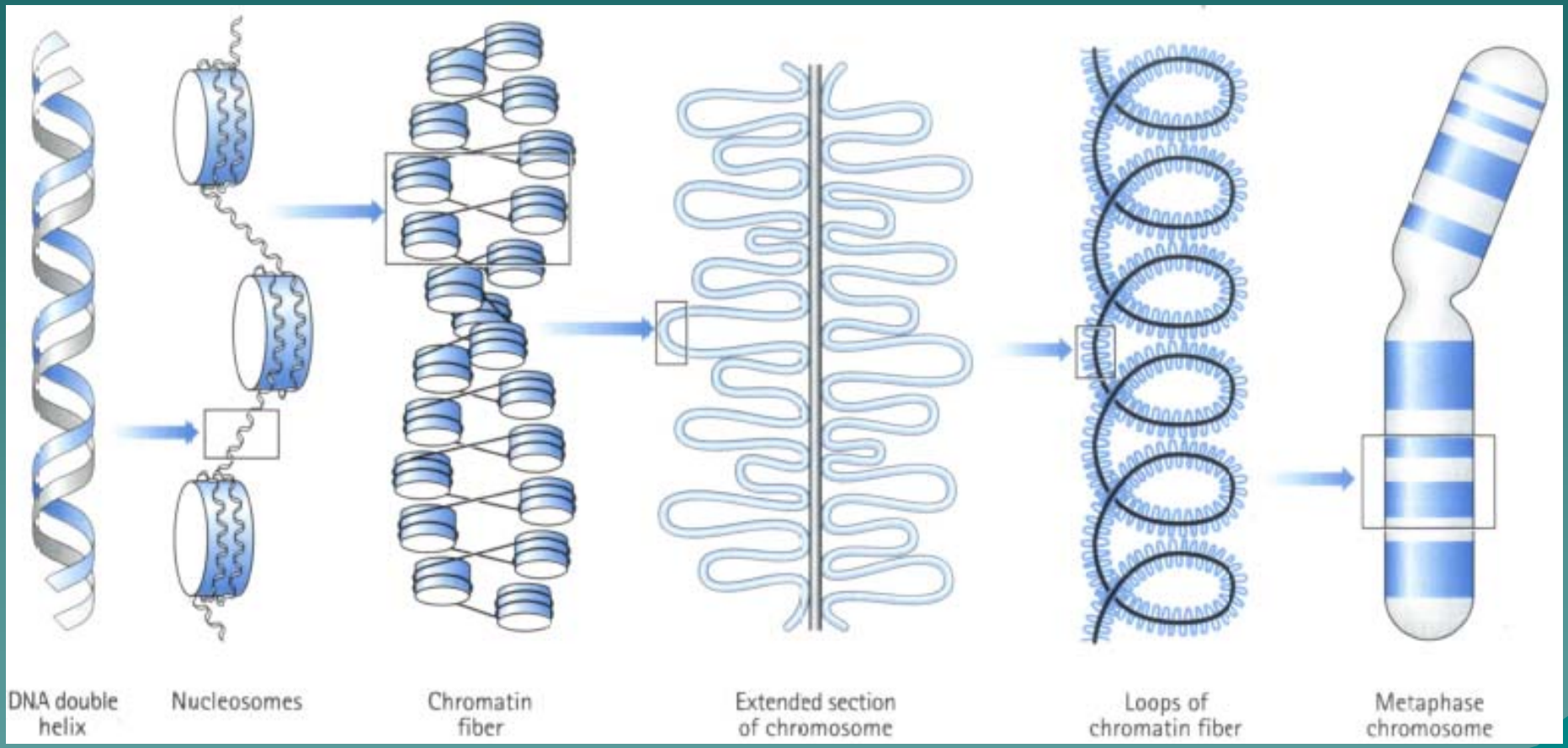

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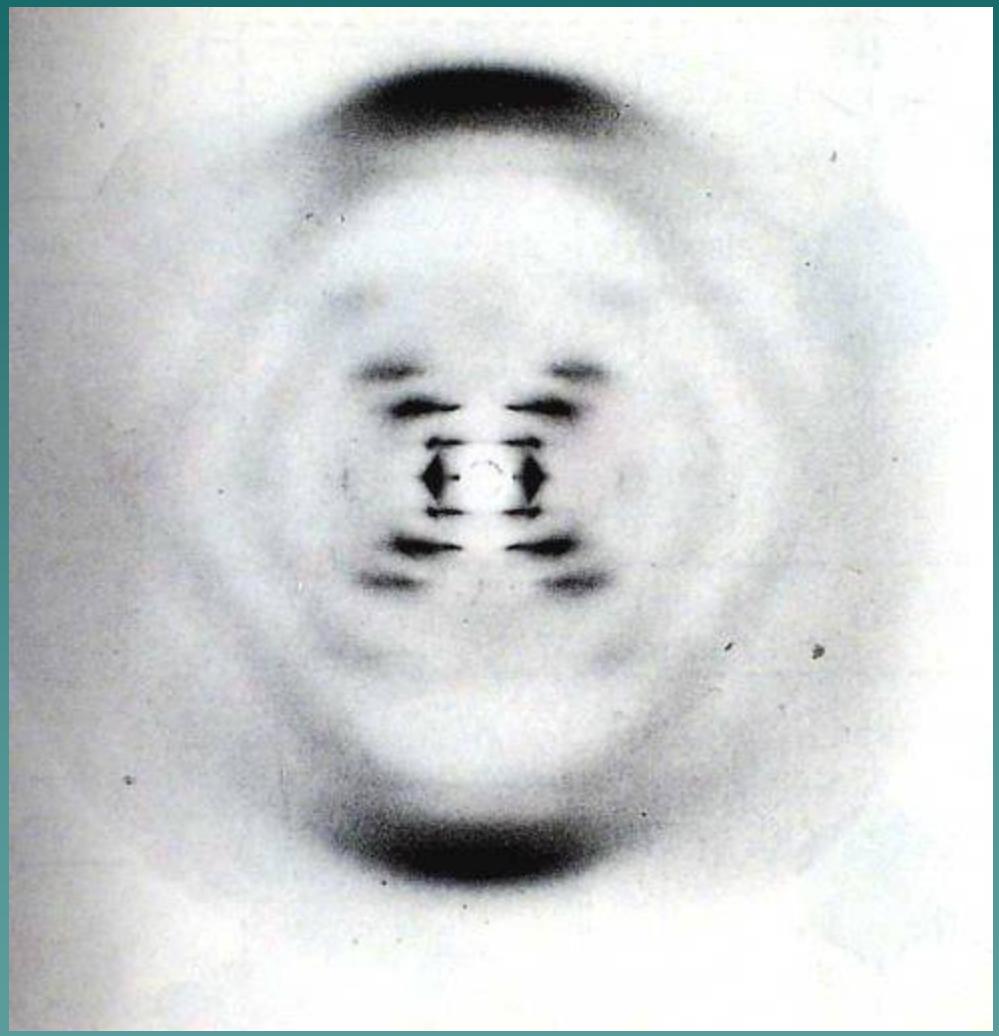


1984

Characterization of the human factor VIII gene

Jane Gitschier, William I. Wood, Therese M. Goralka, Karen L. Wion, Ellson Y. Chen, Dennis H. Eaton, Gordon A. Vehar*, Daniel J. Capon & Richard M. Lawn

Departments of Molecular Biology and * Protein Biochemistry, Genentech, Inc., 460 Point San Bruno Boulevard, South San Francisco, California 94080, USA

The complete 186,000 base-pair (bp) human factor VIII gene has been isolated and consists of 26 exons ranging in size from 69 to 3,106 bp and introns as large as 32.4 kilobases (kb). Nine kb of mRNA and protein-coding DNA has been sequenced and the mRNA termini have been mapped. The relationship between internal duplications in factor VIII and evolution of the gene is discussed.

Expression of active human factor VIII from recombinant DNA clones

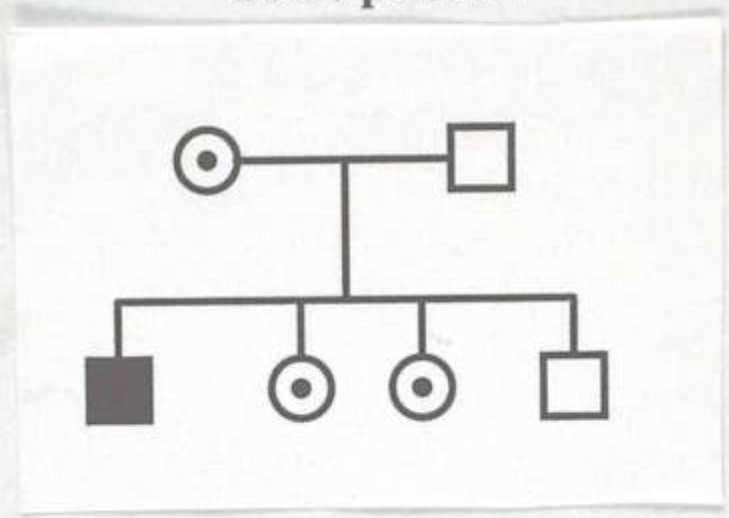
William I. Wood, Daniel J. Capon, Christian C. Simonsen, Dan L. Eaton*, Jane Gitschier, Bruce Keyt*, Peter H. Seeburg, Douglas H. Smith, Philip Hollingshead, Karen L. Wion, Eric Delwart, Edward G. D. Tuddenham†, Gordon A. Vehar* & Richard M. Lawn

Departments of Molecular Biology and *Protein Biochemistry, Genentech, Inc., 460 Point San Bruno Boulevard, South San Francisco, California 94080, USA
† Haemophilia Centre, Royal Free Hospital School of Medicine, London WC1N 1BP, UK

DNA clones encoding the complete 2,351 amino acid sequence for human factor VIII have been isolated and used to produce biologically active factor VIII in cultured mammalian cells. The recombinant protein corrects the clotting time of plasma from haemophiliacs and has many of the biochemical and immunological characteristics of serum-derived factor VIII.

Haemophilia A

Bcl1 / p114.12



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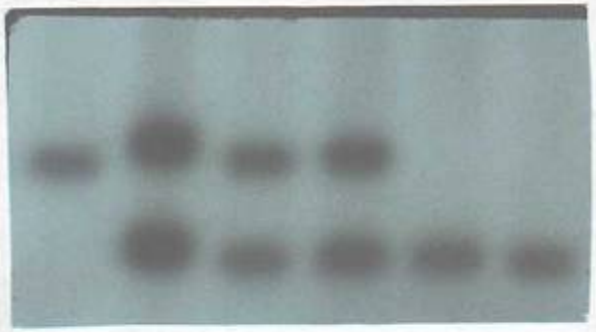
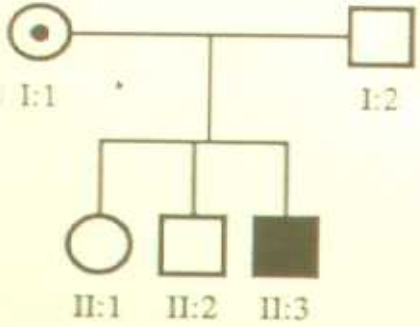
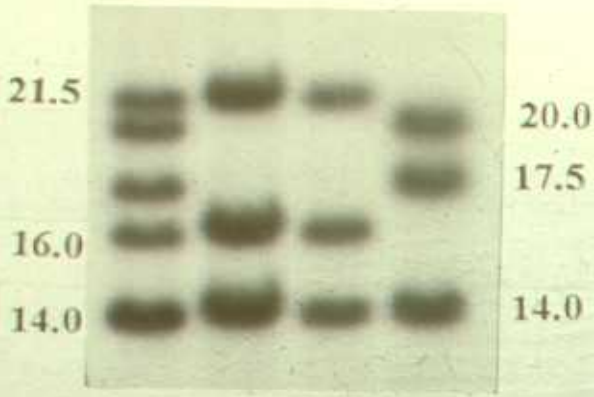


HAEMOPHILIA A

Inversion Mutations





p482.6 / BclI

- Carrier testing moved from probability testing with clotting tests – to definitive testing with genetic testing
- Prenatal testing
- PGD / IVF (Prenatal genetic determination)
- New therapies

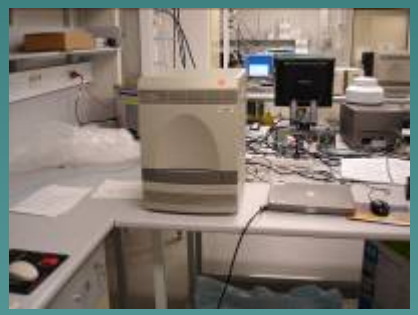
WORKFLOW

Sample reception

Mastermix preparation

DNA Extraction

PCR setup



DHPLC

Detection / Sequencing

Data analysis / report generation

1982

Molecular cloning of the gene for human anti-haemophilic factor IX

K. H. Choo, K. G. Gould, D. J. G. Rees & G. G. Brownlee

Sir William Dunn School of Pathology, University of Oxford, South Parks Road, Oxford OX1 3RE, UK

1983

Gene deletions in patients with haemophilia B and anti-factor IX antibodies

F. Giannelli*†, K. H. Choo†, D. J. G. Rees†, Y. Boyd‡, C. R. Rizza§ & G. G. Brownlee†

† Sir William Dunn School of Pathology, University of Oxford, Oxford OX1 3RE, UK
‡ Genetics Laboratory, Department of Biochemistry, University of Oxford, Oxford OX1 3QU, UK
§ Haemophilia Centre, Churchill Hospital, Oxford OX3 7LJ, UK

1985

Expression of active human clotting factor IX from recombinant DNA clones in mammalian cells

D. S. Anson*, D. E. G. Austen† & G. G. Brownlee*

* Sir William Dunn School of Pathology, University of Oxford, South Parks Road, Oxford OX1 3RE, UK
† Haemophilia Centre, Churchill Hospital, Oxford OX3 7LJ, UK

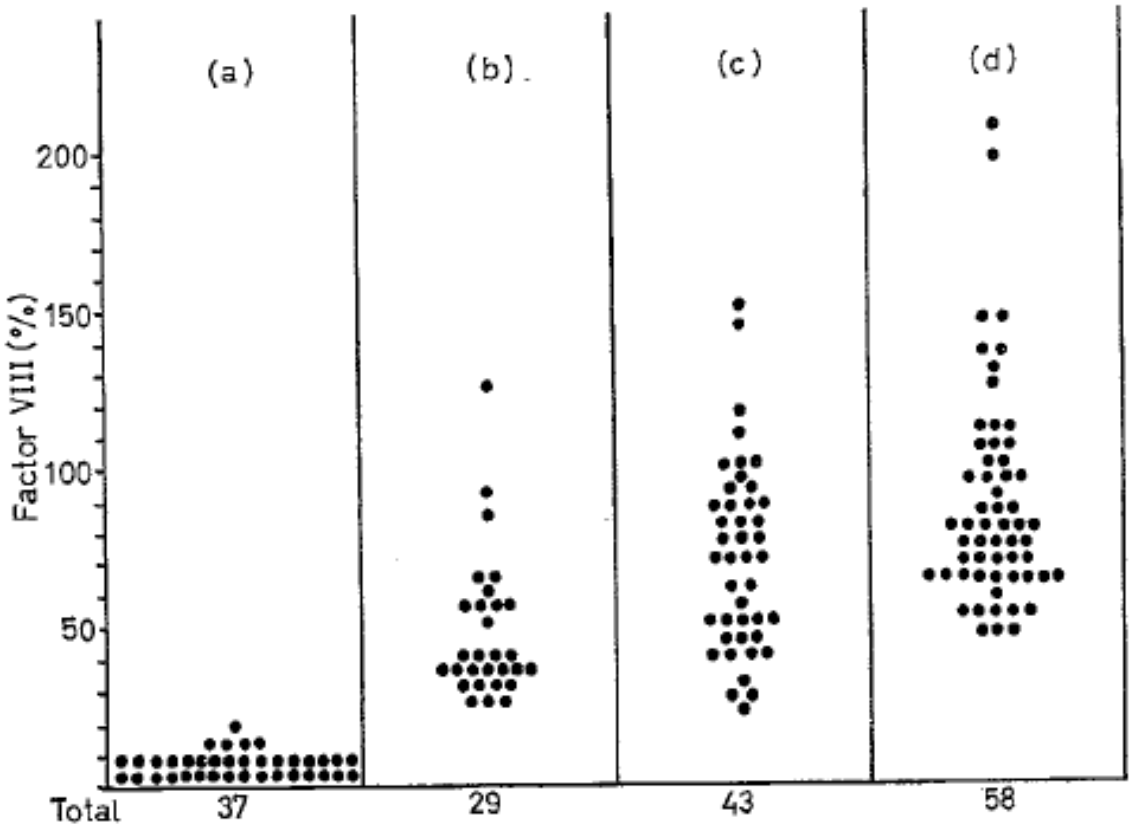
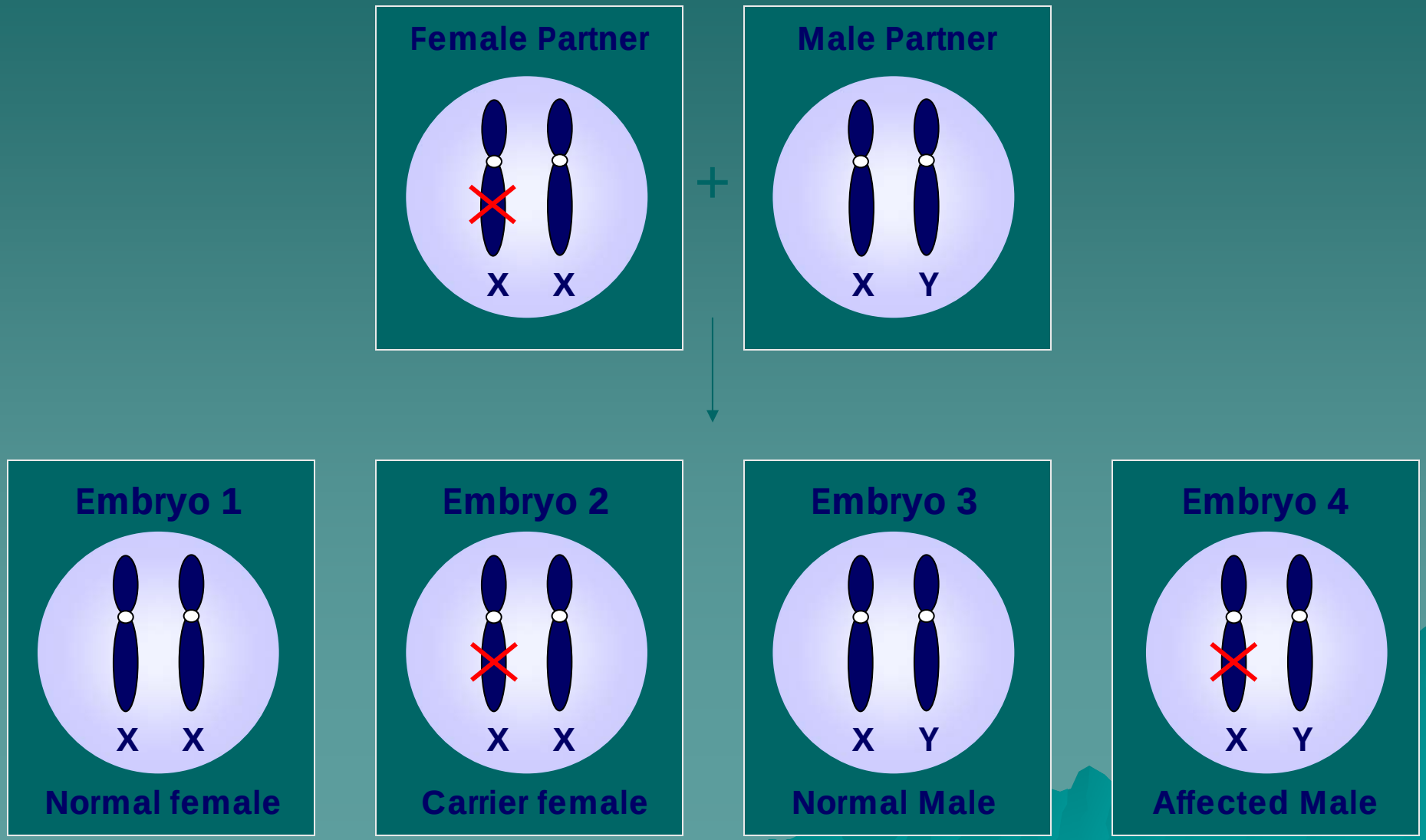


FIG. 3. Distribution of Factor-VIII levels in members of haemophilic families grouped according to genotype. (a) Factor-VIII-deficient males, (b) Proven heterozygous females, (c) Possible heterozygous females, and (d) Normal males and females.

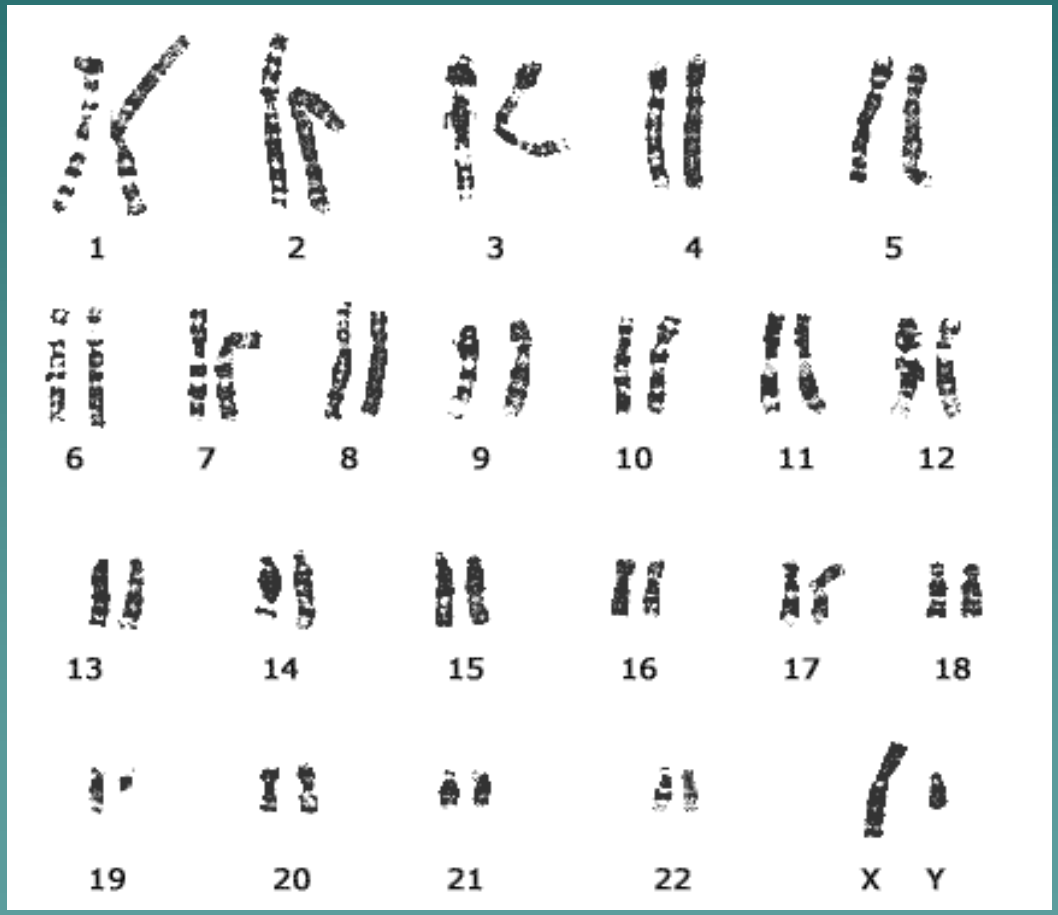
Kerr C, Preston A, Barr A, Biggs R. Further studies on the inheritance of Factor VIII. Br J Haem 1966, 12,212

What are the outcomes if a female who carries haemophilia reproduces?

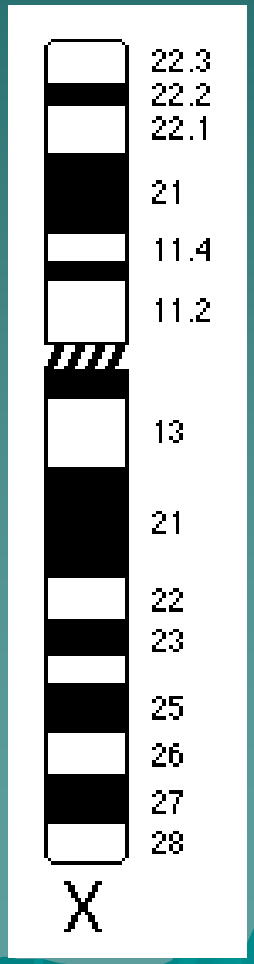


Chromosomes

Male Karyotype



X chromosome



Short arm (p)

← Centromere

Long arm (q)

1975

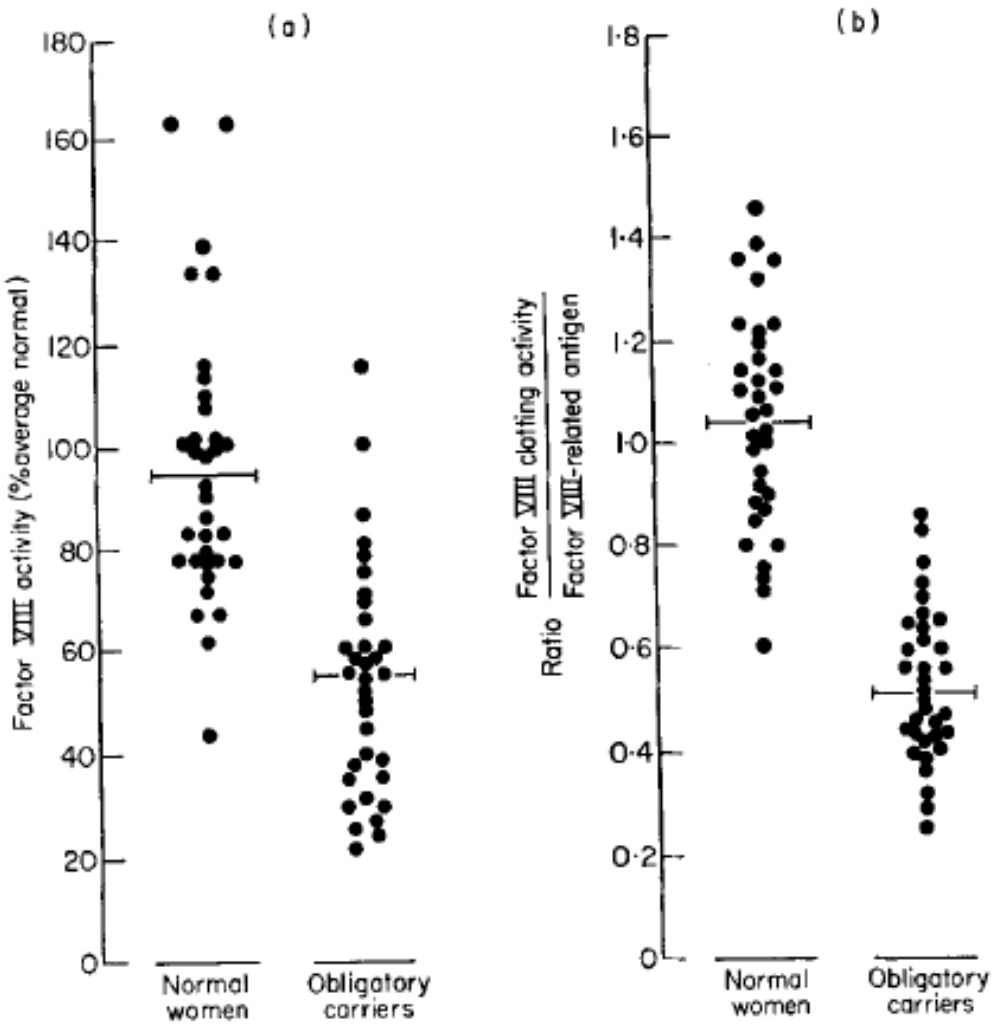
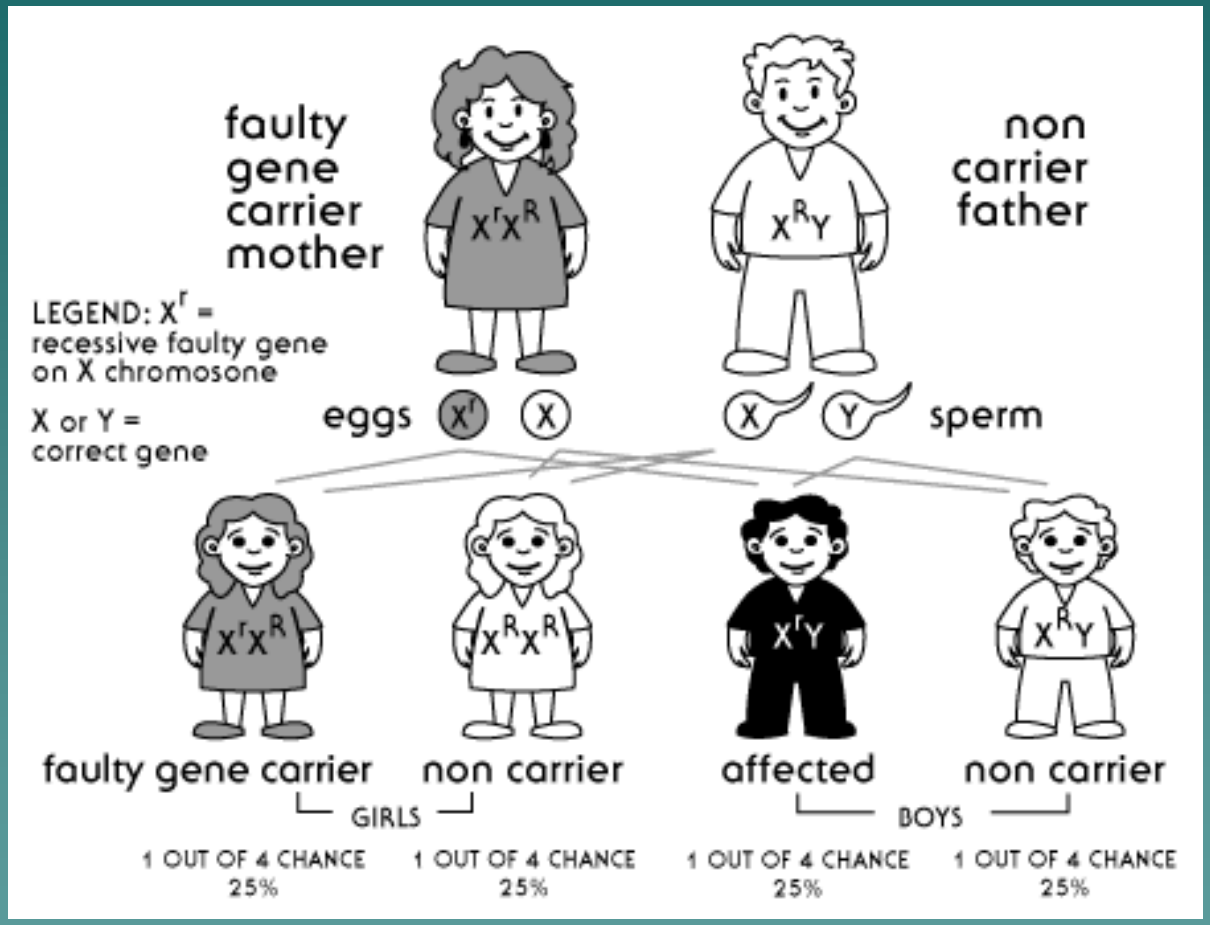


FIG 3. (a) Distribution of values of factor VIII activity in normal women and obligatory carriers of haemophilia. (b) Distribution of values of the ratio of factor VIII activity to factor VIII-related antigen in normal women and obligatory carriers of haemophilia.

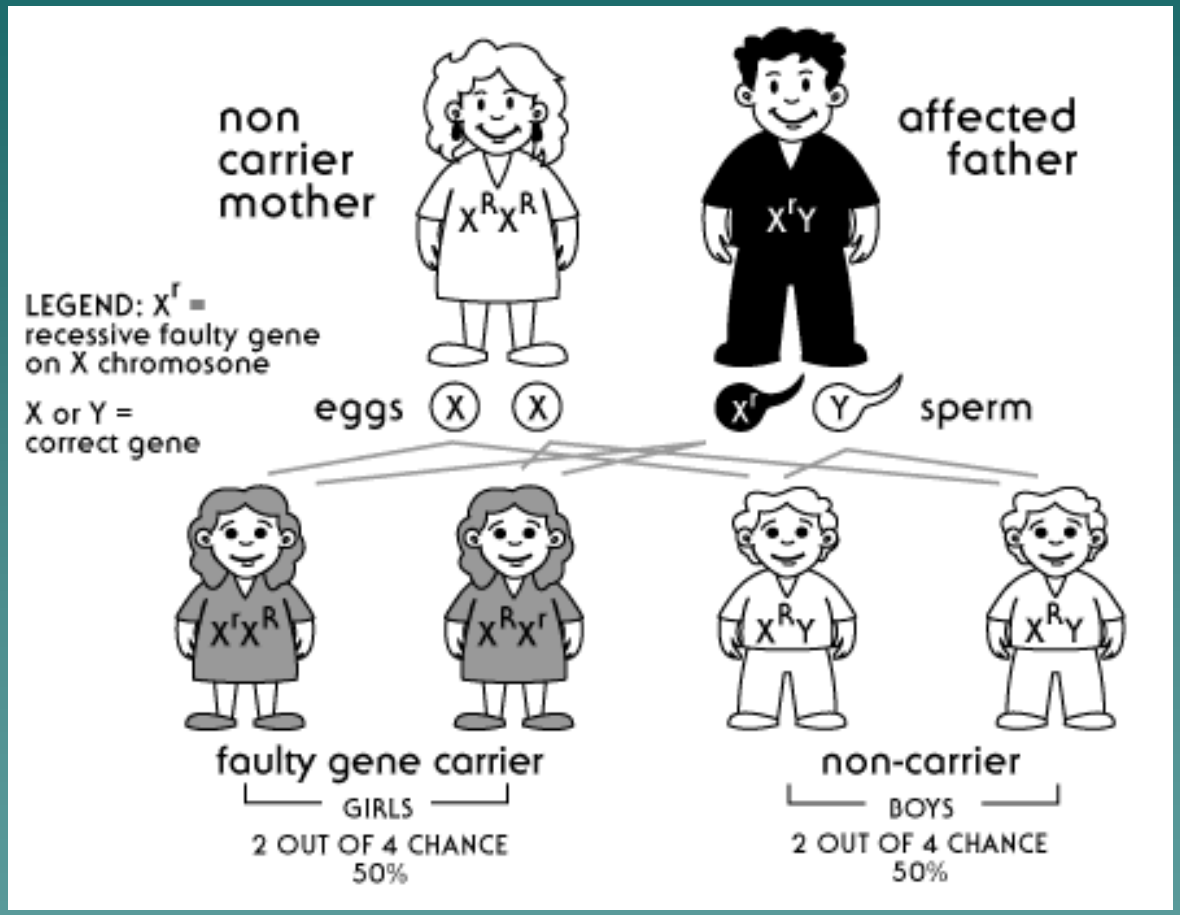
Rizza C,Rhymes I, Austen D, Kernoff P, Aroni S.Detection of carriers of Haemophilia. A 'blind' study. Br J Haem 1975, 30, 447

Carrier female



- Sons have 1 in 2 chance of having haemophilia
  - Daughters have 1 in 2 chance of being carriers

Affected male



- Daughters are obligate carriers
- Sons will all be unaffected