



Gene mapping & gene maps



History of gene mapping

- In 1911, by **Thomas Hunt Morgan**, gene for *eye-color* was located on the X chromosome of fruit fly.
- Shortly after that, **E.B. Wilson** attributed the sex-linked genes responsible for *color-blindness* and *hemophilia* in human beings to be located on the X-chromosome, similar to the many X-linked factors being described by the Morgan group in flies.
- It wasn't until 1968 that an autosomal assignment of linkage was made by **Donahue**---"Duffy" was assigned to Chromosome #1.

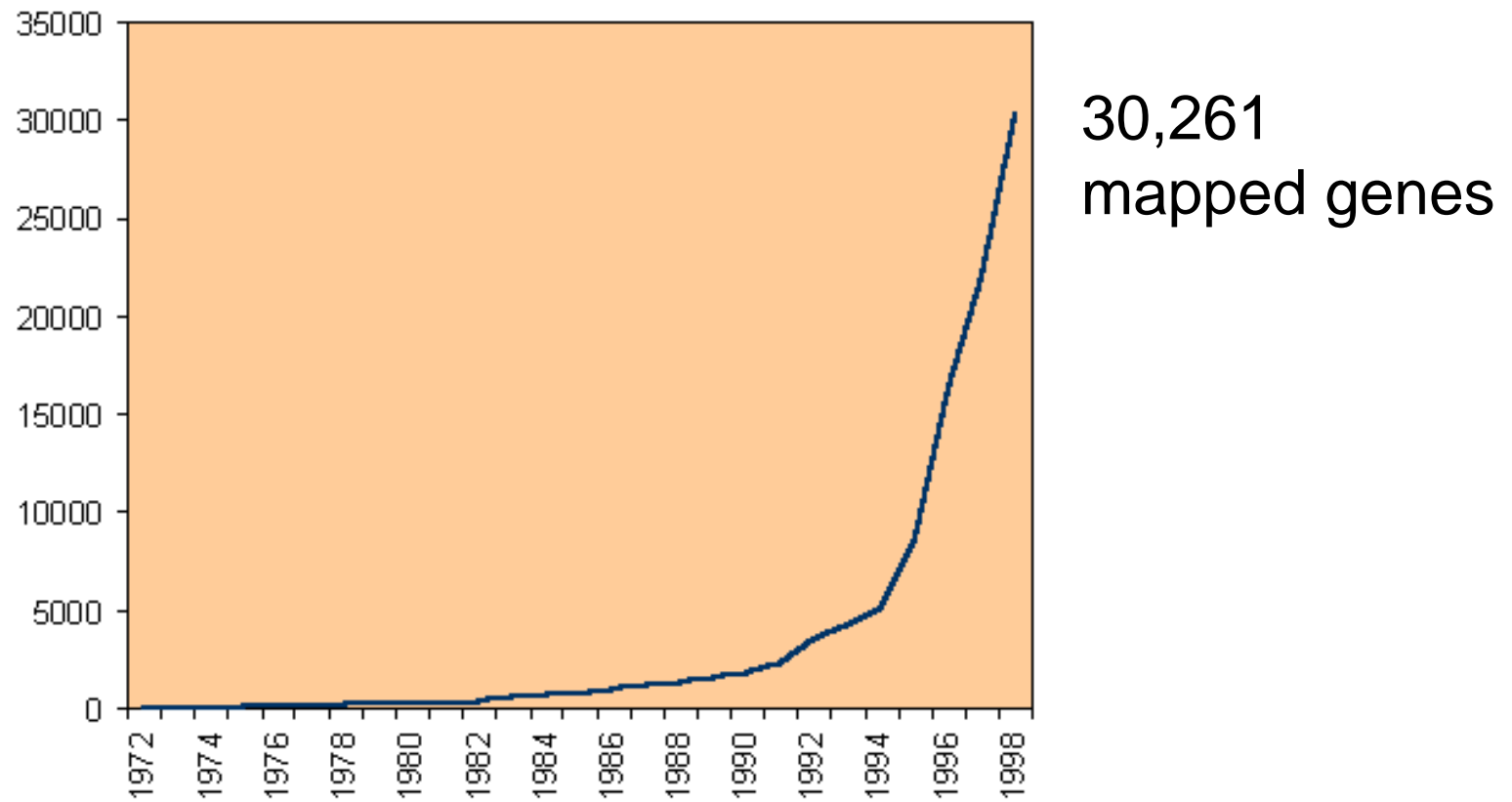
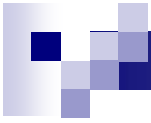


Fig. increase of mapped human genes



Outline

- Gene Mapping
- Techniques
 - in situ hybridization
 - somatic cell hybridization
 - dosage effect
 - chromosome aberration
 - linkage analysis
- Strategies



Gene mapping

- "Gene mapping" refers to the mapping of genes to specific locations on chromosomes.
- It is a critical step in the understanding of genetic diseases.
- There are two types of gene mapping:
 - **Genetic Mapping** - using linkage analysis to determine the relative position between two genes on a chromosome.
 - **Physical Mapping** - using all available techniques or information to determine the absolute position of a gene on a chromosome.



Genetic Mapping

- Requires informative markers – polymorphic and a population with known relationships
- Best if measured between “close” markers.
- Unit of distance in genetic maps = centiMorgans, cM
- 1 cM = 1% chance of recombination between markers



Physical mapping

- Relies upon observable experimental outcomes
 - hybridization
 - amplification
- May or may not have a distance measure.



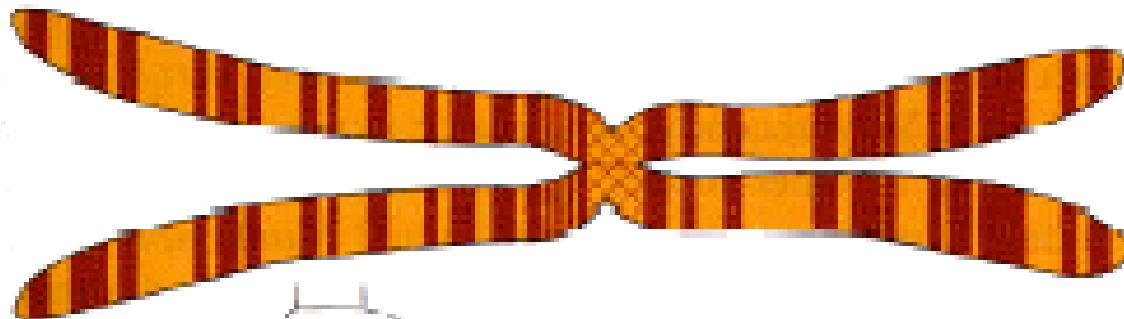
gene mapping

- The ultimate goal of gene mapping is to *clone genes*, especially disease genes. Once a gene is cloned, we can determine its *DNA sequence* and study its *protein product*.
- For example: *cystic fibrosis* (CF) (P249).
- In 1985, the gene was mapped to chromosome 7q31-q32 by linkage analysis.
- Four years later, it was cloned by *Francis Collins* and his co-workers. We now know that the disease is caused by the defect of a chloride channel - the protein product of this disease gene.

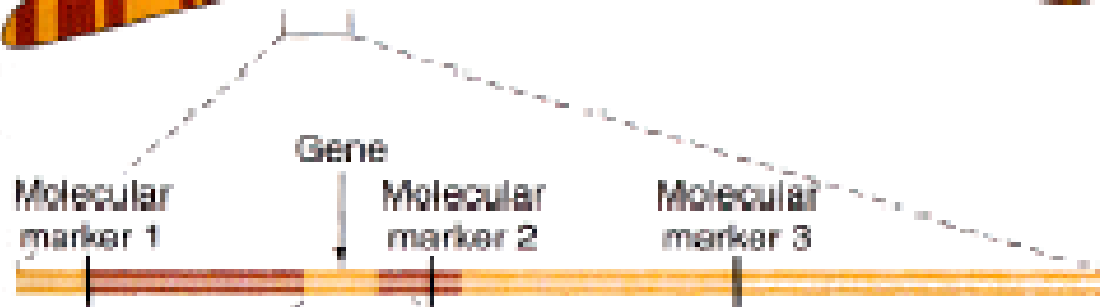


Gene maps

- Genetic map
- Physical map
- Transcription map
- Sequence map



CYTOGENETIC
MAPPING



GENETIC
HIGH-RESOLUTION
MAPPING



PHYSICAL MAPPING

TTAGGTTAAGGTAGTGGTACGGTACGGTGGCTTAT

DNA SEQUENCING

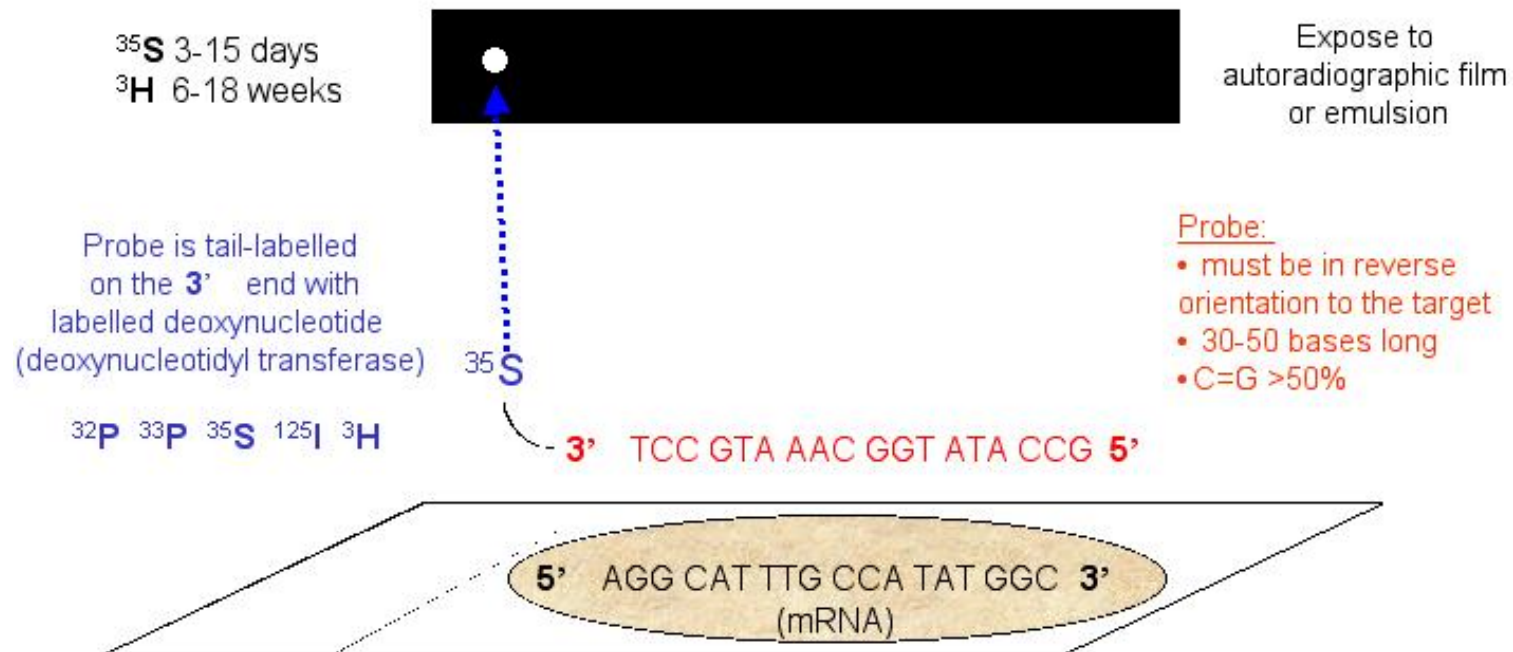


Technique 1

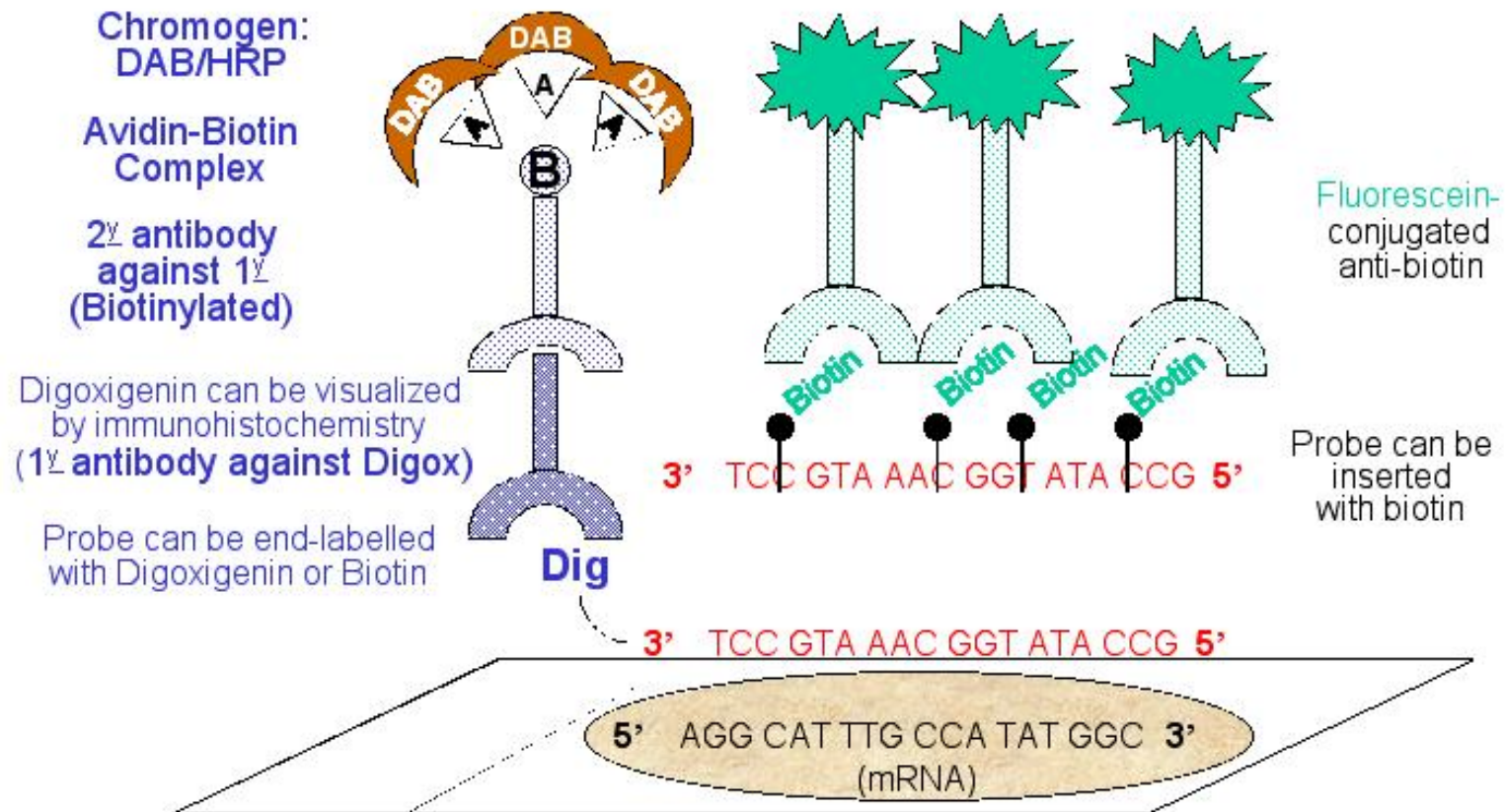
--- Gene mapping by In situ Hybridization

- The method which involves hybridizing labeled DNA (or RNA) probes directly to metaphase chromosomes.

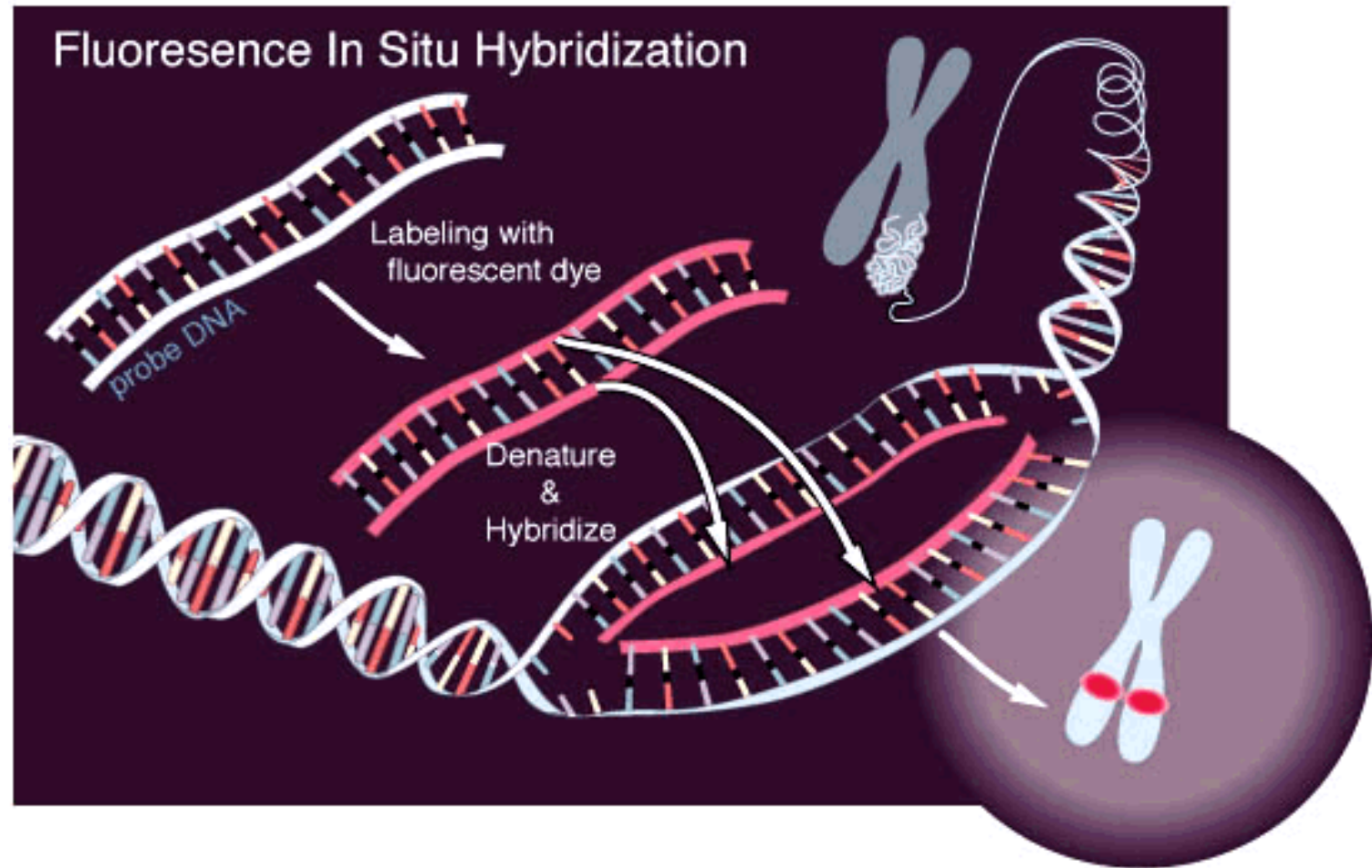
In Situ Hybridization using Radiolabelled Probes



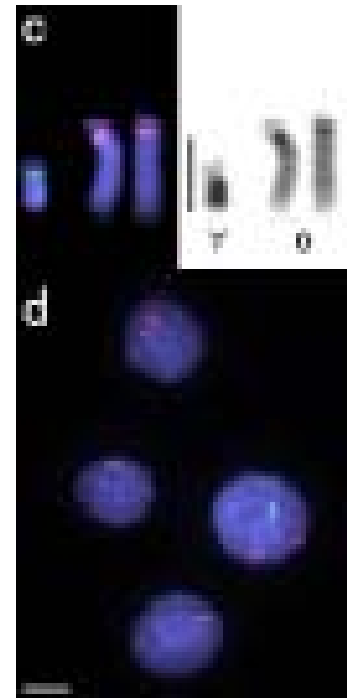
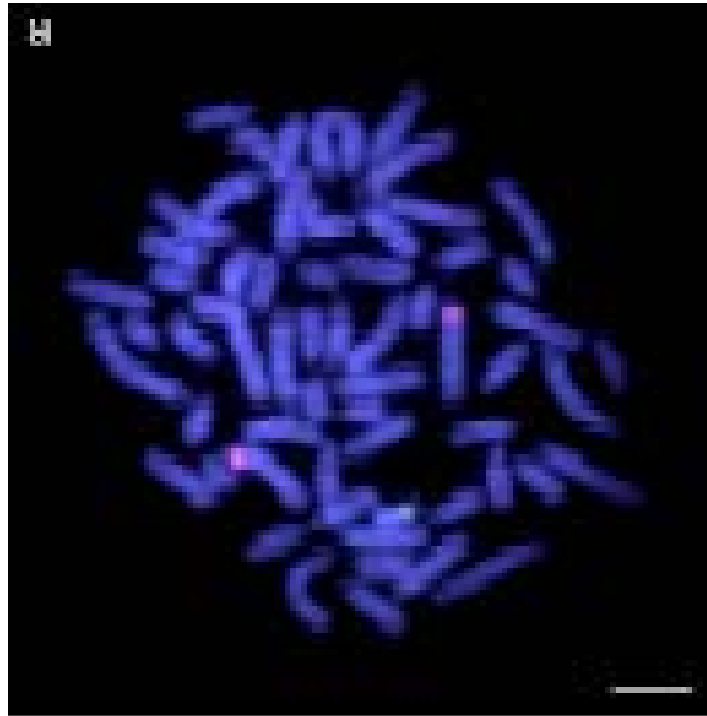
In Situ Hybridization using Non-Radiolabelled Probes



FISH



FISH (fluorescence in situ hybridization)



D6Z1 (red)
DYZ1 (green)



Specialties

- Simple, convenient and intuitionistic
- Only for known genes or known DNA segments
- Cannot obtain high-resolution maps on metaphase chromosomes or pre-metaphase chromosomes



Technique 2

--- Gene mapping by somatic cell hybridization

- The method which to fuse somatic cells from different species.



Somatic cell hybridization (cell fusion)

- **An experimentally based segregation technique used to map genes to human chromosomes.**
- **Cells from two different species (e.g. humans and rodents) are artificially fused together.**
- **These culture lines are developed by mixing human and mouse cells in the presence of the Sendai virus.**
- **The virus facilitates the fusing of the two cell types to form a hybrid cell.**



Chromosome loss of hybrid cells

- **For a reason that is not entirely known, most, but not all, human chromosomes are randomly lost from the hybrid cell lines.**
- **Usually a few human chromosomes are retained.**
- **Because the human and mouse chromosomes can be distinguished by chromosome staining techniques, it can be determined which human cells are retained with a specific cell line.**

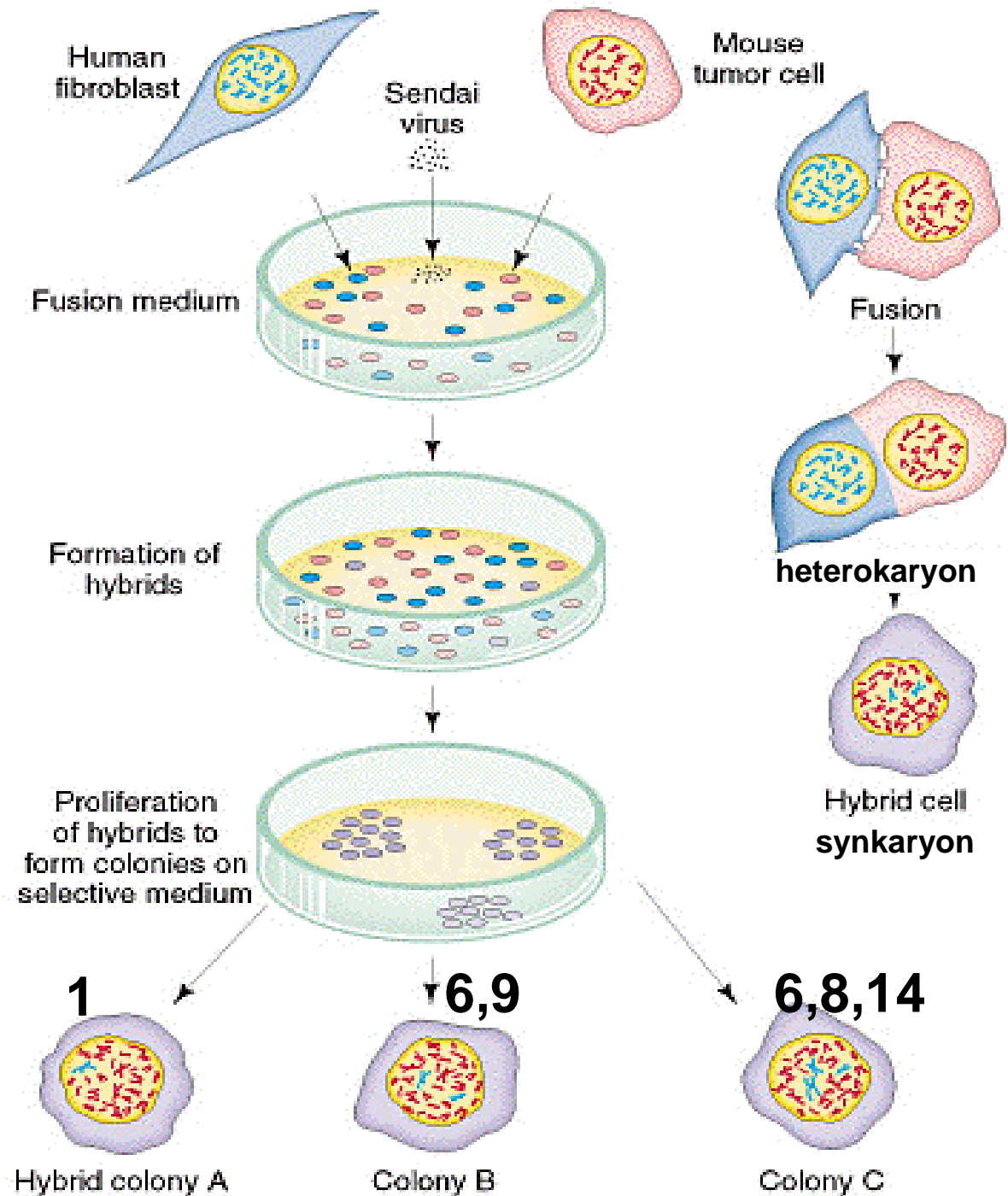
Exposure to virus or to chemical agent leads to cell fusion

A cell with two nuclei forms (heterokaryon)

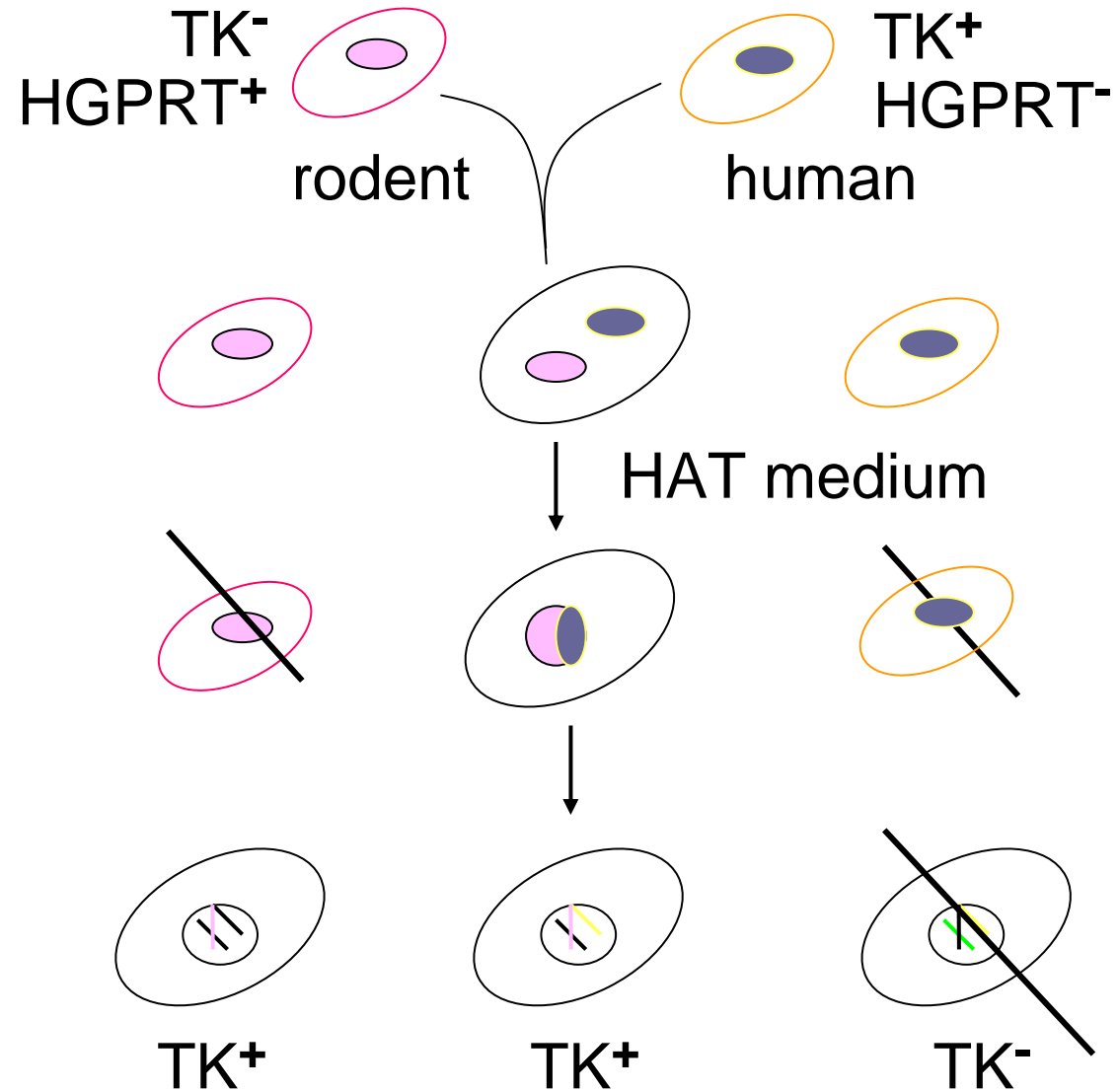
The nuclear membranes then fuse

Each cell then randomly loses some human chromosomes

Colonies arise from these cells... e.g.



Exemple:



Miller located
TK gene onto
chromosome
17



Panel of clonal hybrids

Hybrid cell lines	Human chromosomes remained	Gene products			
		A	B	C	D
23	1, 2, 3, 4	—	+	—	+
34	1, 2, 5, 6	+	—	—	+
41	1, 3, 5, 7	+	+	—	+

- A --- chromosome 5
- B --- chromosome 3
- D --- chromosome 1
- C --- not on these chromosomes involved



Specialties:

- Simple, convenient and intuitionistic;
- Only for analyzing the known genes or their products;
- Cannot map genes accurately;
- Because of the homologies between human genes and rodent genes, this method cannot succeed in mapping some of the genes.



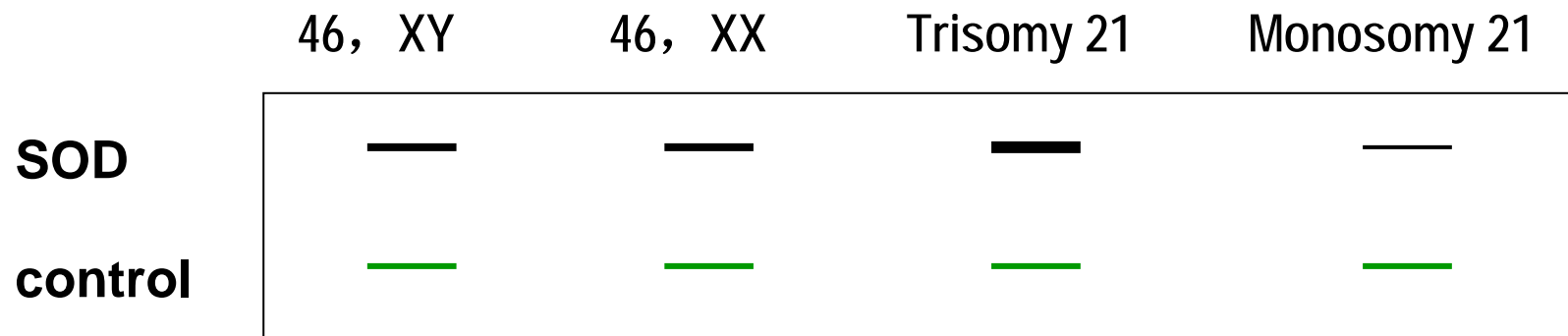
Technique 3

--- Gene mapping by gene dosage using patient cells

- The method which to detect dosage differences in either gene products or gene sequences themselves between patients' cell lines containing different numbers of copies of a particular gene.

dosage effect

- The gene dosage strategy was originally used to assign genes to chromosome 21 by detecting levels of enzyme activity in cell lines from patients with Down syndrome that were 1.5-fold higher than levels in cell lines from chromosomally normal persons.
- i.e. gene for SOD (superoxide dismutase)



- At the DNA level, the dosage approach has been used increasingly to assign DNA markers to the X chromosome.

	46, XY	46, XX	47, XXY	49, XXXXY
Probe 1	—	—	—	—
Probe 2	—		—	—
Probe 3	—	—	—	—



Specialties:

- Simple and convenient;
- For the known genes or their protein products;
- The cell lines involving numerical chromosomal aberrations are infrequent naturally.



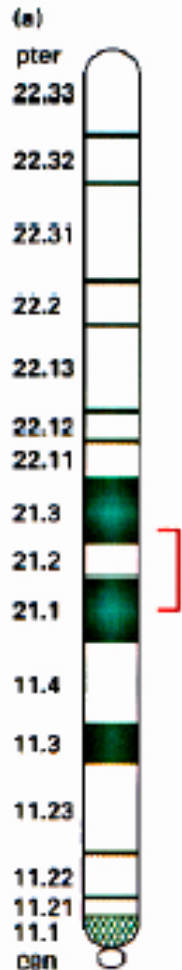
Technique 4

--- Gene mapping by chromosomal aberration

- To detect directly chromosomal aberration involving genes which may lead to particular disease.

Example: DMD (Duchenne muscular dystrophy)

① X - A translocation



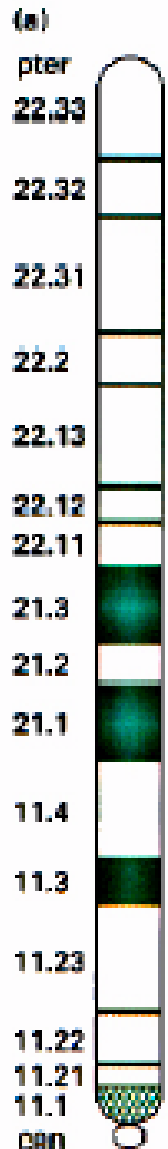
DMD, X-linked recessive inheritance, is very rare in female.

Karyotype analysis of several affected female indicated common X-A translocation.

Although these translocations involved different autosomes, their broken points on X chromosomes were commonly located on **Xp21**.

This indicated that the broken points were inner of the gene for DMD.

② Microdeletion in X chromosome



In one well-studied example, a boy with no known family history of any genetic disease presented with 4 ordinarily distinct X-linked conditions: DMD, chronic glaucomatous disease (CGD), retinitis pigmentosa (RP), and a rare red blood cell phenotype.

Careful cytogenetic analysis revealed a small but detectable deletion in band **Xp21.2**.

Normal X

DMD

CGD

RP



Patient B.B.





Specialties:

- Simple, convenient and intuitionistic;
- For mapping the unknown genes;
- Most of the individuals with (structural) chromosomal aberrations are eliminated naturally.



Technique 5

--- Gene mapping by linkage analysis

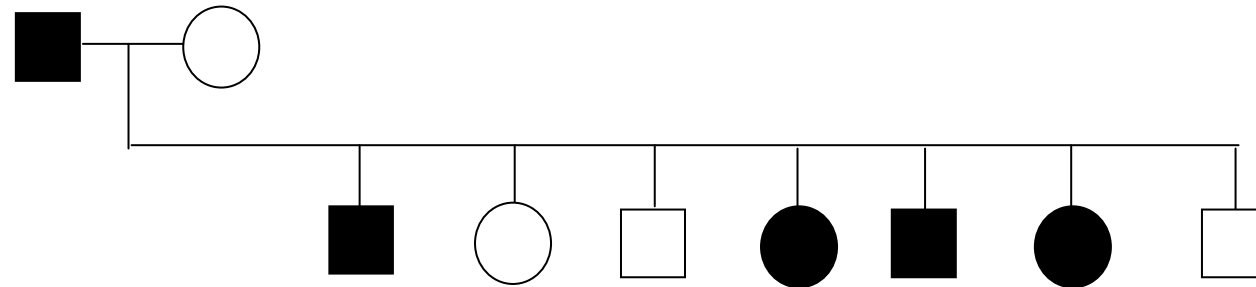
- Linkage analysis is a method of mapping genes that uses family studies to determine whether two genes show linkage when passed on from one generation to the next.



Linkage analysis

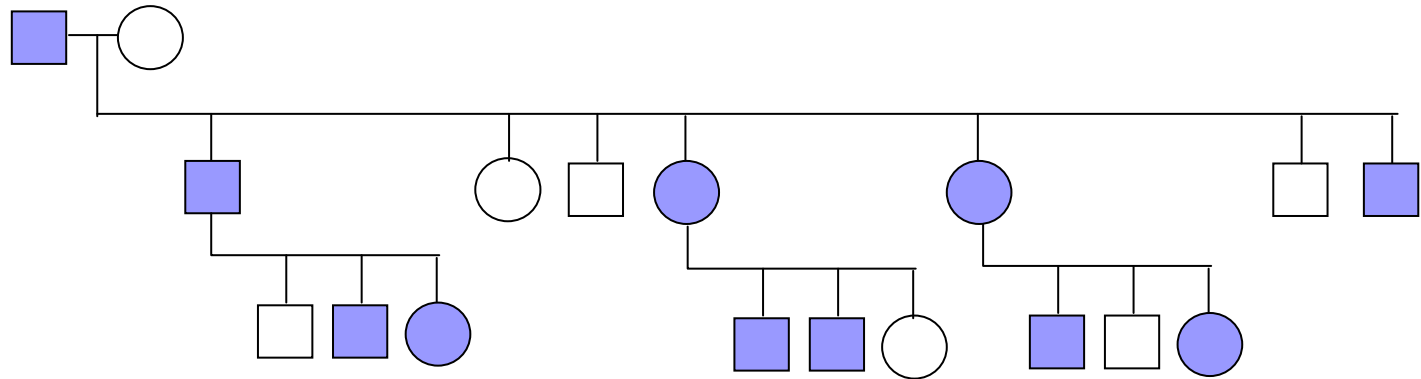
- Mapping by genetic linkage analysis differs from mapping by physical methods because physical mapping relies on having a laboratory method to localize a gene by FISH or by somatic cell hybridization.
- In contrast, linkage analysis is a tremendously important and powerful approach in medical genetics because *it is the only method that allows mapping of genes, including disease genes, that are detectable only as phenotypic traits.*

Linkage



Faulty gene	Dd	dd	Dd	dd	dd	Dd	Dd	Dd	dd
Locus A	21	22	22	12	12	22	22	22	12
Locus B	12	34	13	24	23	14	24	13	23


Mapping BDB by Linkage Analysis



Disease locus	Dd	dd	Dd	dd	Dd	Dd	dd	dd	Dd	Dd	Dd	dd	Dd	Dd	dd	Dd	dd	Dd
D9S938	21	34	23	33	23	22	14	13	24	22	22	42	23	21	31	21	14	24
D9S123	13	22	12	24	13	14	32	12	12	13	14	23	12	13	23	13	32	12

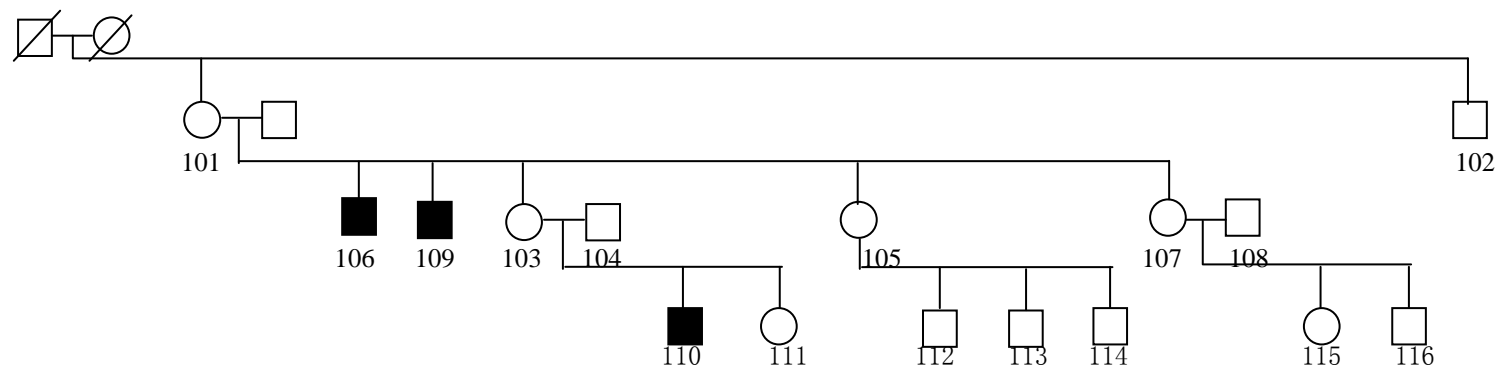
D9S938 : tightly linked to disease locus

**D9S123 : observed 1 recombinant out of 16,
RF=1/16=6.25%**



*How to know the linkage between any locus
and the faulty gene?*

- Genome scanning
- The 10 cM (centiMorgan) limit



DXS1068	12	2	1	2	21	2	2	22	21	1	2	1	21	1	12	1	1
DXS6810	21	1	2	2	11	2	1	21	12	1	2	2	12	1	12	1	1
DXS8080	32	1		3	12	1	2	12	13	1	3	3	13	1	13	1	2
DXS1003	22	1	2	2	12	2	2	22	12	1	2	2	12	3		1	2
DXS1039	32	4	2	2	42	1	2	12	43	4	3	3	43	4	43	4	2
DXS7132	31	3	1	1	31	3	1	31	33	3	3	3	31	3	33	3	2
DXS6789	22	1	2	2	12	3	2	32	12	1	2	2	12	2	21	1	3
DXS6799	23	2	3	3	23	4	3	43	22	2	2	2	23	2	23	2	1
DXS1231	11	2	1	1	21	2	1	21	21	2	1	1	21	2	21	2	1
DXS8020	33	1	3	3	13	2	3	23	13	1	3	3	13	2	23	1	4
DXS1191	31	2	1	1	21	2	1	21	23	2	3	3	21	3	31	2	2
DXS1230	13	2	3	3	23	2	3	23	21	2	1	1	23	2	23	2	1
GTN	23	3	3	3	33	1	3	13	32	3	2	2	33	3	33	3	3
DXS6797	32	2	2	2	22	2	2	22	23	2	3	3	22	3	32	2	1
DXS456	42	2	2	4	22	3	2	32	24	2	4	4	22	2	22	2	1
DXS1059	22	1	2	2	12	3	2	32	12	1	2	2	12	2	22	1	2
DXS1072	21	1	1	2	11	1	1	11	12	1	2	2	11	1	11	1	1
DXS6804	15	3	5	1	35	4	5	45	31	3	1	1	35	2	25	3	4
DXS425	21	3	2	2	31	1	1	11	32	3	2	2	31	3	31	3	1
DXS1220	21	3		2	31	3	1	31	32	3	2	2	31	2	21	3	3
GRIA3	41	3	4	4	31	3	1	31	34	3	4	4	31	2	21	3	1

图5、中国山东MRXN家系精细定位的连锁分析结果
(各位点按从Xpter-qter的顺序排列, 黑体表示携带致病基因的染色体)



LOD score: are two loci linked?

- The statistical term that is generally calculated to evaluate the significance of linkage results is called a **LOD score** (or Z).
- The LOD score compares the likelihood of obtaining the test data if the two loci are indeed linked, to the likelihood of observing the same data purely by chance.

$$\text{LOD} = \log_{10} \frac{\text{likelihood of data if loci linked at a particular } \theta}{\text{likelihood of data if loci unlinked (} \theta=0.5)}$$

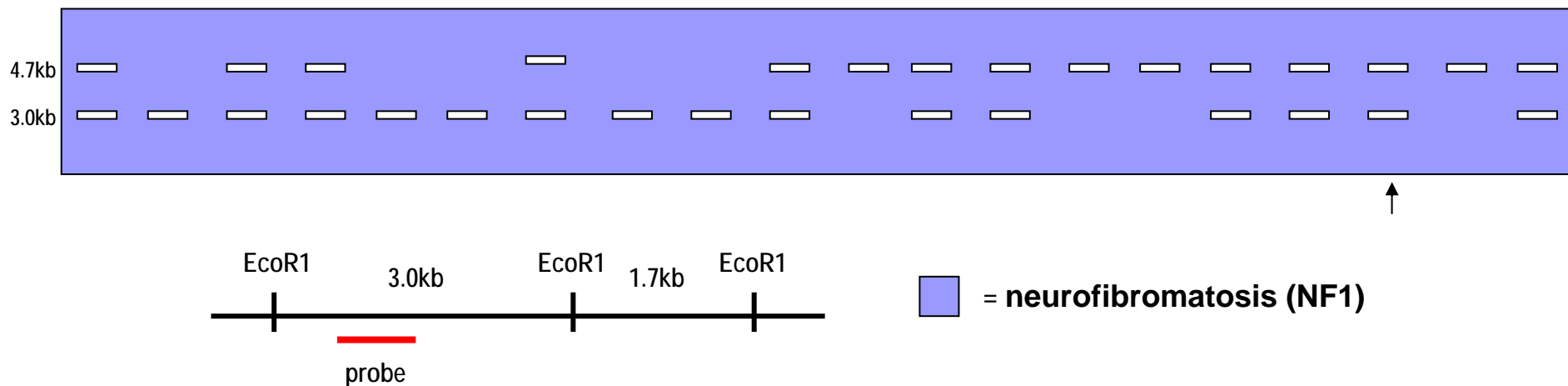
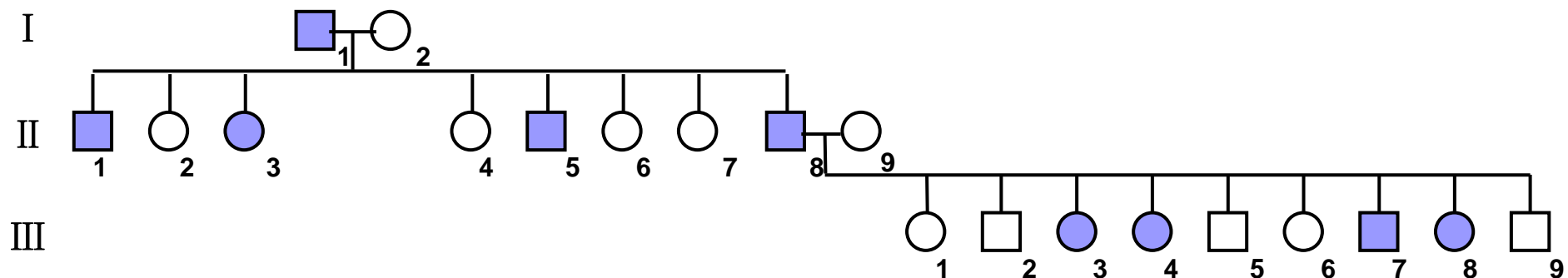
Likelihood is similar to probability and is very dependent on the distance between the two test loci.

LOD score $\geq +3$, definitely linked

LOD score $\leq -2 \pm$, exclusion of linkage

LOD score = ± 1 , linked uncertainly

A pedigree of a family in which AD neurofibromatosis (NF1)



- Linkage analysis of NF1 with a probe on chromosome 17.



LOD Score Calculation (1)

- Let us now calculate the LOD score at $\theta=0.05$.

$$\text{LOD} = \log_{10} \frac{\text{likelihood of data if loci linked at } \theta}{\text{likelihood of data if loci unlinked}}$$

$$= \log_{10} \frac{\text{likelihood of data if loci linked at } \theta = 0.05 \text{ [}\frac{1}{2}\text{(phase 1) + } \frac{1}{2}\text{(phase 2)]}}{\text{likelihood of data if loci unlinked}}$$

$$= \log_{10} \frac{\frac{1}{2}(\mathbf{0.475}^{16} \times 0.025) + \frac{1}{2}(\mathbf{0.025}^{16} \times 0.475)}{0.25^{17}}$$

$$= \log_{10} \mathbf{1442.2} = \mathbf{3.16}$$

LOD Score Calculation (2)

- Let us now calculate the LOD score at $\theta=0.1$.

$$\begin{aligned} \text{LOD} &= \log_{10} \frac{\text{likelihood of data if loci linked at } \theta}{\text{likelihood of data if loci unlinked}} \\ &= \log_{10} \frac{\text{likelihood of data if loci linked at } \theta = 0.1 \text{ [}\frac{1}{2}\text{(phase 1) + } \frac{1}{2}\text{(phase 2)]}}{\text{likelihood of data if loci unlinked}} \\ &= \log_{10} \frac{\frac{1}{2}(\mathbf{0.45^{16}} \times 0.05) + \frac{1}{2}(\mathbf{0.05^{16}} \times 0.45)}{0.25^{17}} \\ &= \log_{10} \mathbf{607} = \mathbf{2.78} \end{aligned}$$



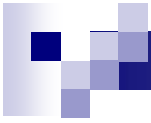
LOD Score Calculation (3)

- If there had been *on recombinants*, for comparison, it is instructive to consider the LOD score for this pedigree at $\theta = 0$.

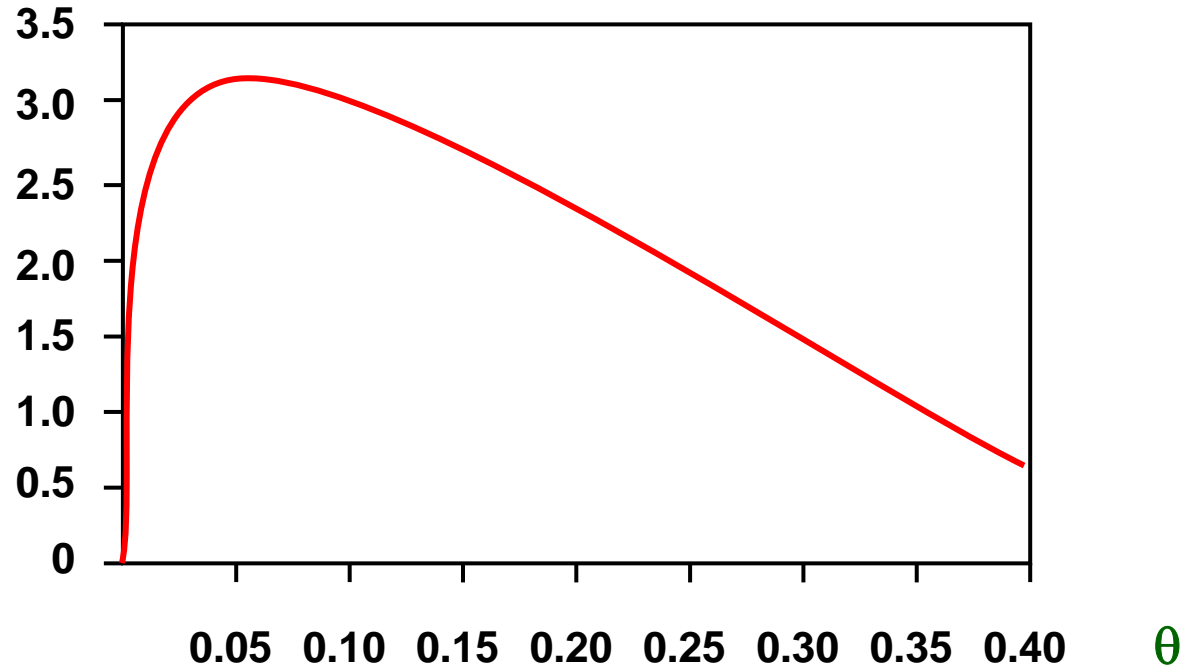
$$\text{LOD} = \log_{10} \frac{\text{likelihood of data if loci linked at } \theta}{\text{likelihood of data if loci unlinked}}$$

$$= \log_{10} \frac{\frac{1}{2}(0.5^{17}) + \frac{1}{2}(0^{17})}{0.25^{17}}$$

$$= \log_{10} 65536 = 4.82$$



LOD



$\theta =$	0	0.05	0.10	0.15	0.20	0.30	0.40	\hat{Z}	$\hat{\theta}$
LOD	$-\infty$	3.16	3.08	2.86	2.57	1.82	0.89	3.165	0.059

- Computer calculation of LOD scores for the linkage analysis of NF1 and the RFLP marker.

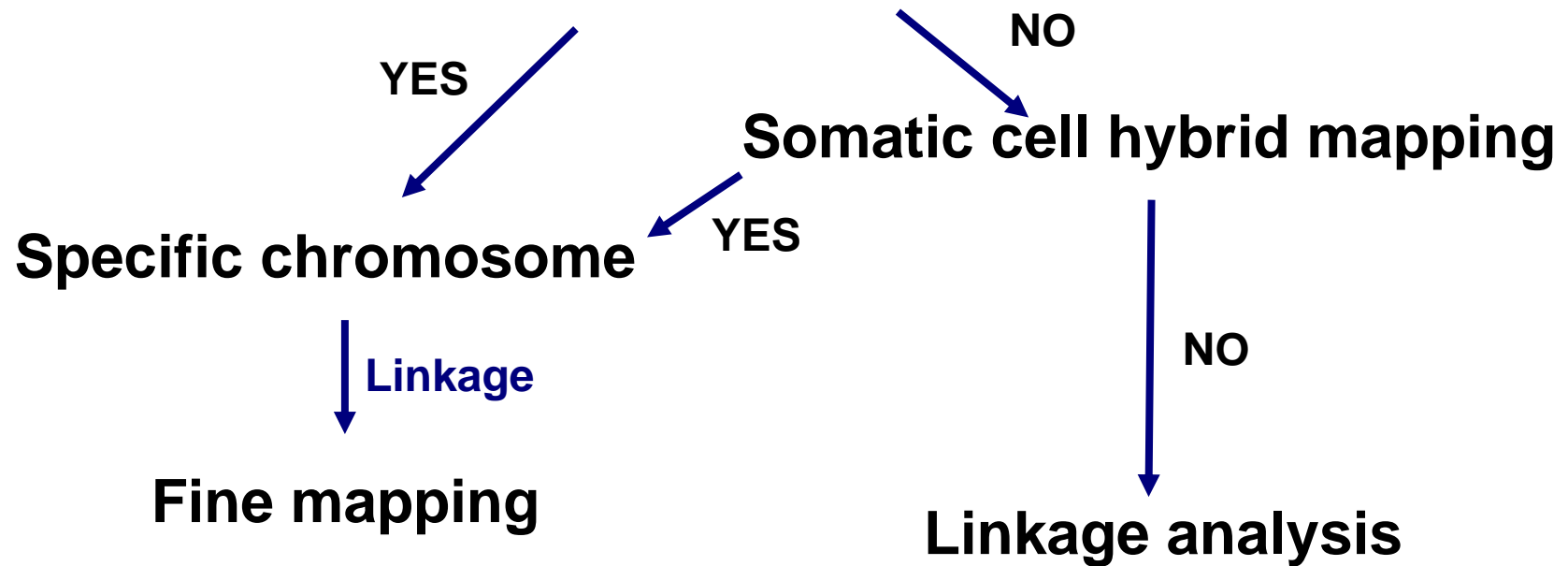


Summary for common methods

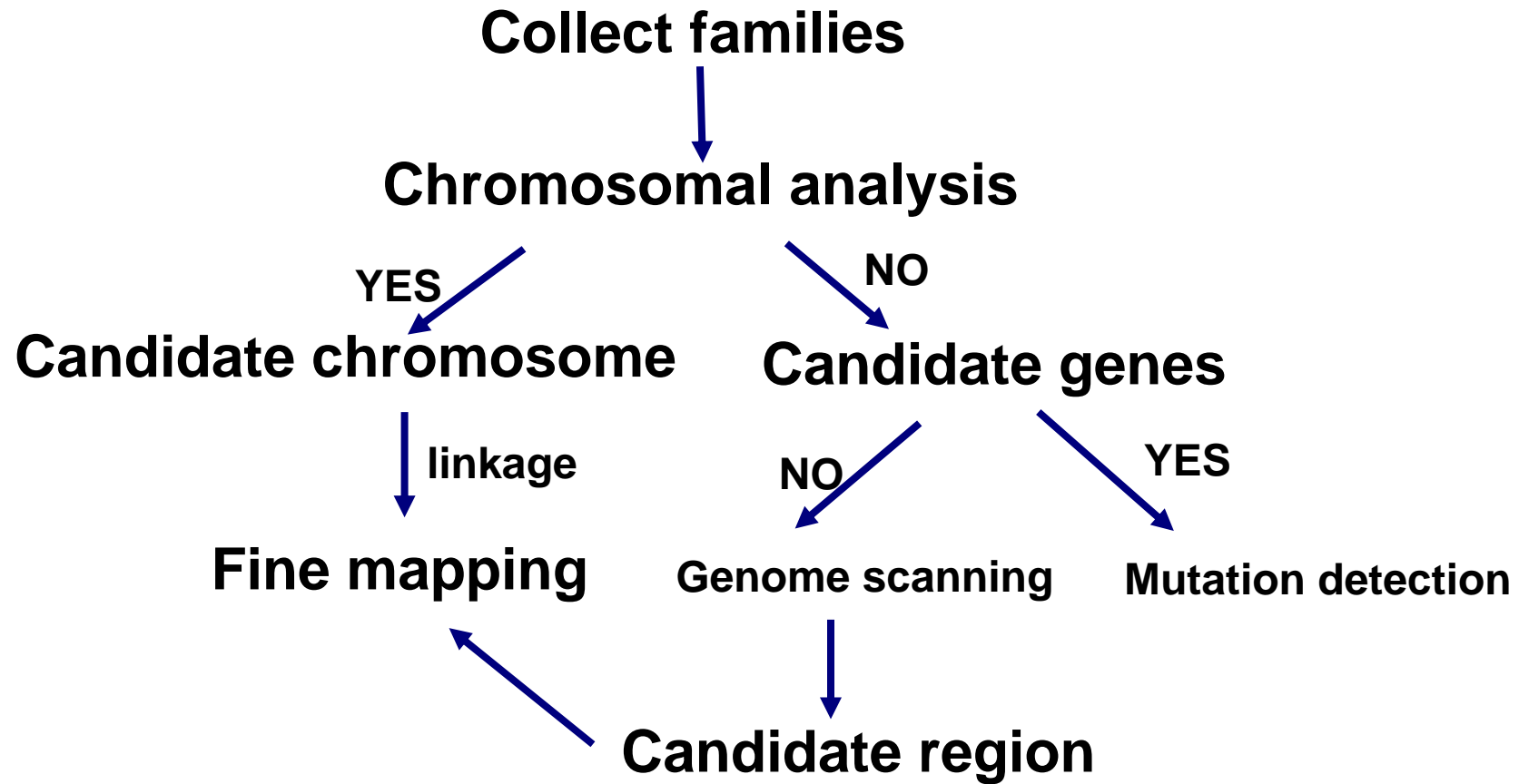
Methods	Known genes	Known protein	Disease genes
in situ hybridization	+	-	-
somatic cell hybridization	+	+	-
dosage effect	+	+	-
chromosome aberration	-	-	+
linkage analysis	+	+	+

Strategies - Mapping a Known Gene

In Situ Hybridization



Strategies - Mapping a disease gene



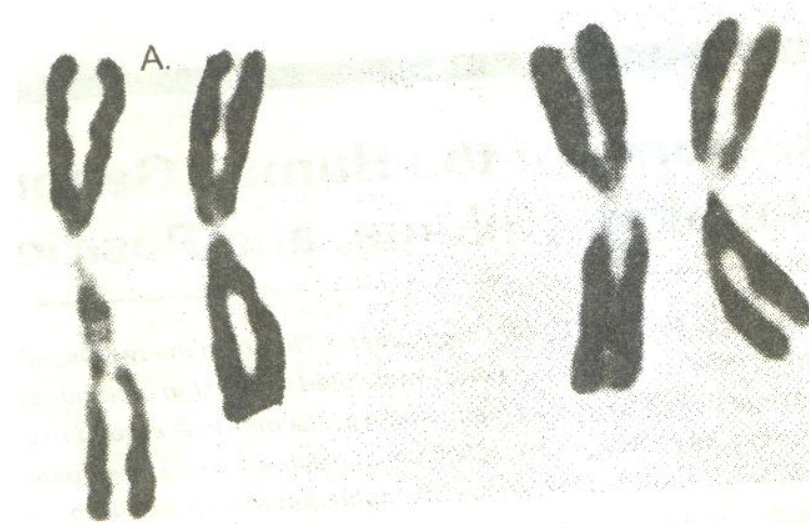





Gene mapping--*Duffy blood group*

- sex-linked inheritance
 - hemophilia and color-blindness
- In 1968, the first autosomal assignment of linkage was made.
- Donahue, an associate of McKusick, observed a peculiar microscopically-visible stretch of chromatin on his own largest chromosome (Chromosome #1).

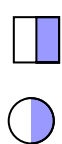
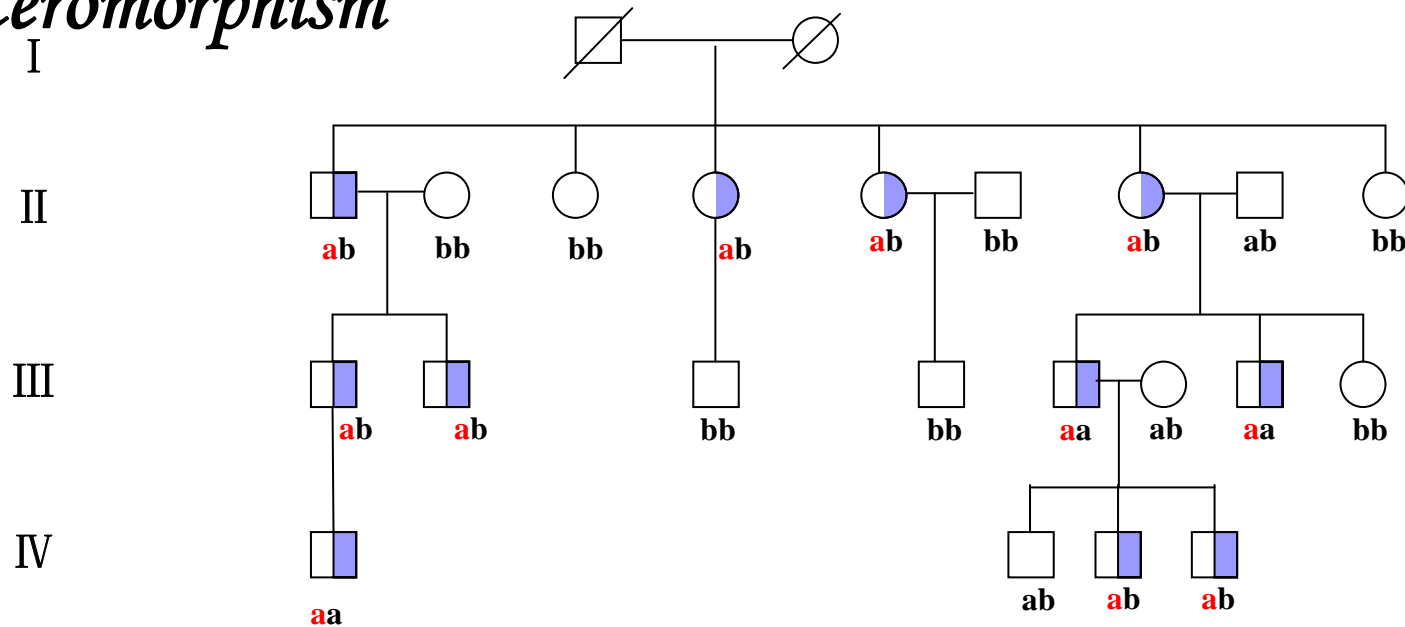
A pair of number 1 chromosomes from two different individuals



- These studies, done before the advent of chromosomes banding, detect a remarkable increase in length below the centromere, referred to as a heteromorphism, in one of the pair of chromosomes on the *left*.
- The chromosomes on the *right* show the more usual morphology.

- 
- Looking at a number of blood factors Donahue found that in both himself and his relatives, an allele of the Duffy blood factor was linked to this observable physical change in their chromosome #1.

The pedigree shows the pattern of inheritance for the Duffy blood group and the chromosome 1 heteromorphism



Presence of the heteromorphism on chromosome 1

a and b

The Duffy blood group genotypes

- Although the individuals in generation I are of unknown status, in the rest of the pedigree it can be seen that the chromosome with the heteromorphism universally travels with the **a** allele at the Duffy locus.

Thus **"Duffy"** was assigned to **Chromosome #1**.





DMD (*Duchenne Muscular Dystrophy*)

- Duchenne muscular dystrophy is the most common and the best known muscular dystrophy. It follows an aggressive and progressive course.
- The disease is inherited in an X-linked manner although up to 30% of cases represent new mutations. The gene for Duchenne muscular dystrophy is the dystrophin locus at Xp21, involving 79 exons.
- The incidence is 30 per 100,000 live born males.

- --- **Genetics**
- --- **Clinical features**
- --- **Investigations**
- --- **Management**
- --- **Prognosis**



Genetics

- The disrupted genetic locus is Xp21.2, which is the site of the dystrophin gene. DNA probes have shown deletions to be the cause in 10% of cases.



clinical features

- Features of Duchenne's muscular dystrophy appear in the first five years of life and consist of:
 - delayed walking - after 18 months
 - frequent falls
 - lordotic waddling gait
 - difficulty in climbing stairs
 - Gower's sign
 - weakness is symmetrical:
 - iliopsoas, gluteal and quadriceps are affected first
 - then, pretibial muscles
 - then, pectoral and shoulder girdle
 - wasting of most affected muscles
 - pseudohypertrophy of the calves
 - there is an association with reduced intelligence and cardiac abnormalities
 - orthopaedic abnormalities

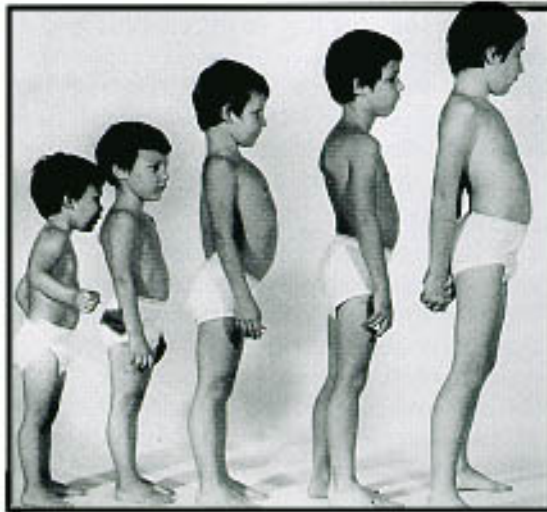
- Children present between the ages of 3 and 6. Often, the patient is bedridden by 12 and the condition is frequently fatal by the age of 20.



late features

- Initially the limbs are weak, wasted and flaccid.
- Later fibrous contractures occur in the muscles which antagonise the more severely affected groups. Contractures are seen in:
 - ankle plantar-flexors
 - hamstrings
 - hip flexors
- Tendon reflexes are lost.
- Bones become osteoporotic.

- Posture changes during progression of DMD





Clinical features in female carriers

- Dystrophin mutations may have clinically detectable effects on female carriers. The most common feature is a proximal myopathy, which may be severe. Mild abnormalities are seen on muscle biopsy, **EMG** (Electromyography) or **CK** (creatine kinase) measurement in over 80% of carriers.
- Females may be severely affected because:
 - they have an XO genotype (Turner's syndrome)
 - Lyonisation results in asymmetrical X chromosome inactivation
 - they have structural chromosomal aberrations such as X-A translocations



Investigations

- The diagnosis of DMD is suggested by the finding of serum **CK** 30 to 200 times higher than normal, myopathic changes on **EMG** and characteristic findings on muscle biopsy.
- Note that the serum CK is raised before the muscular weakness becomes clinically evident.
- Note that the serum creatinine is also slightly raised in female carriers.
- It is now possible to do chromosomal analysis of the deleted part of the genome - chromosome Xp21 - and get a genetic confirmation of the disease.
- **ECG (Electrocardiograph)** changes
 - tall R waves in the right-sided chest leads (V1, V2)
 - Q waves in the left sided chest leads (V5, V6) and limb leads
 - PR interval may be reduced




management

- Management is aimed at maintaining mobility for as long as possible. This includes the use of calipers, wheelchair and adaptation of the home environment.
- Educational needs can be special and are determined both by ability and physical handicap.
- Genetic counselling is of paramount importance.

Cody Joseph Namesnik



- 
- **Parents:** *Joseph G. & Diane C. Namesnik*
 - **Place of Origin:** *Milky Way Galaxy, Earth. Northern Hemisphere, North America. United States of America, Arizona, Phoenix.*
 - **Date of Birth:** *February 14th of Nineteen Hundred & Seventy-Eight*
 - **Race:** *Human "Disabled American"*
 - **Marital Status:** *single/available*
 - **Appearance:** *Ash blonde hair usually short, Brown eyes, thin build, weight unknown, Height 5', 4"*
 - **Health Status:** *Healthy/Mentally Stable w/ Duchennes Muscular Dystrophy "DMD" (Also known as Pseudohypertrophic) In Power Wheel-chair. Respiratory Support 24/7 "ventilator", Tracheostomy tube "airway tube in Trachea", Gastrostomy Tube "G-Tube" (feeding tube in stomach)*
 - **Interests:** *Computers "hardware/software" , Electronics ,Internet/Instant Messaging Chat , Computer Graphics , RPG , Animals , Reading , Sci-Fi , Movies , Psychology , Sociology , Evolution , Science ,Cooking , Gardening.*

MyFriends WΛEΠΙCΟΥC





prognosis

- Duchenne's muscular dystrophy is insidiously progressive. There is often an inability to walk by puberty, and death usually occurs by the age of 20.





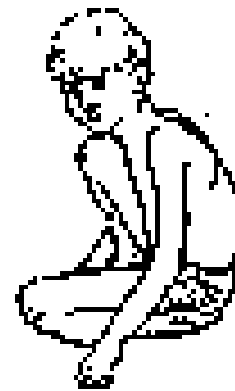
waddling gait

- A waddling gait is the style of walking that is seen in a patient with proximal myopathy.
- It is characterised by:
 - a broad-based gait with a duck-like waddle to the swing phase
 - the pelvis drops to the side of the leg being raised
 - forward curvature of the lumbar spine
 - marked body swing
- This gait may also be seen in patients with congenital hip dislocation and pregnancy.



Gower's sign

- Gower's sign is seen in children with Duchenne's muscular dystrophy.
- On attempting to stand from the ground the child:
 - begins with both hands and feet on the floor
 - then works his way up the legs with his hands until in an upright posture



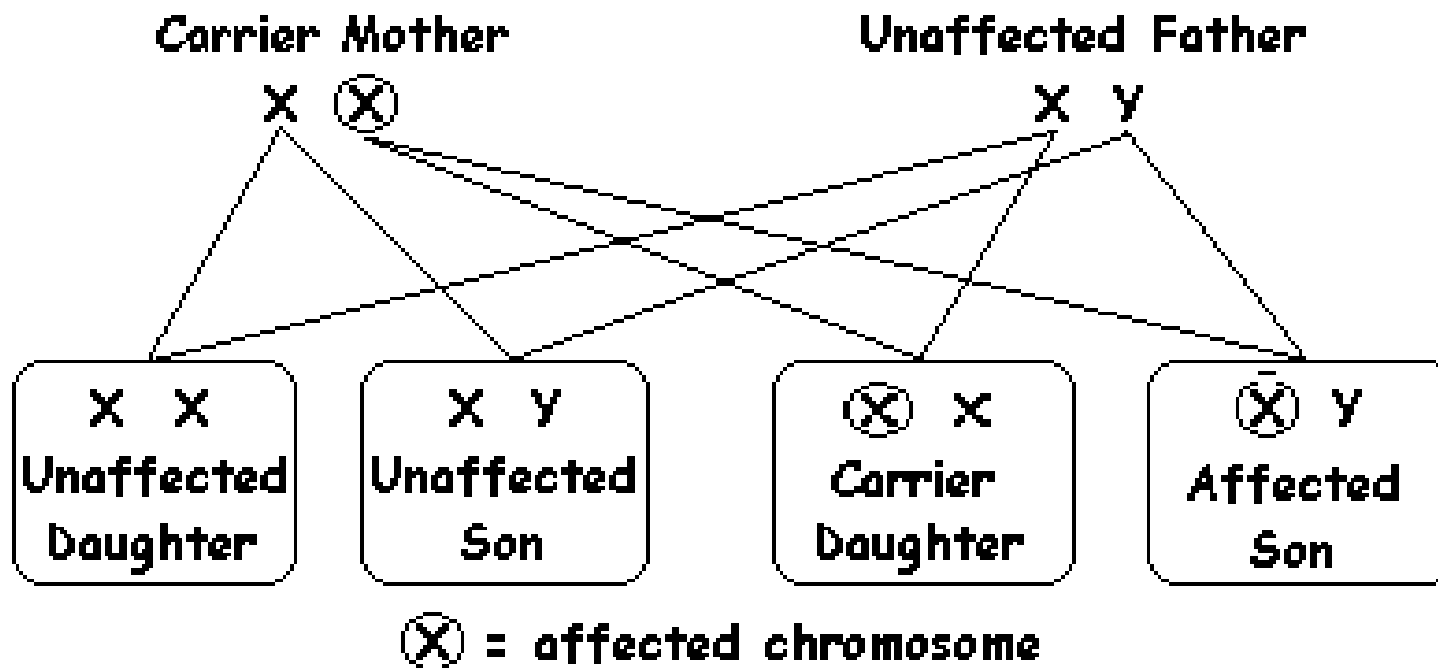
pseudohypertrophy of muscles

- Pseudohypertrophy is a term usually used to refer to the calf muscles in DMD, where the muscles appear hypertrophied while they are in fact weak.



X-linked recessive pattern

- DMD can be *inherited* in the X-linked recessive pattern or can occur when the dystrophin gene spontaneously *mutates*.



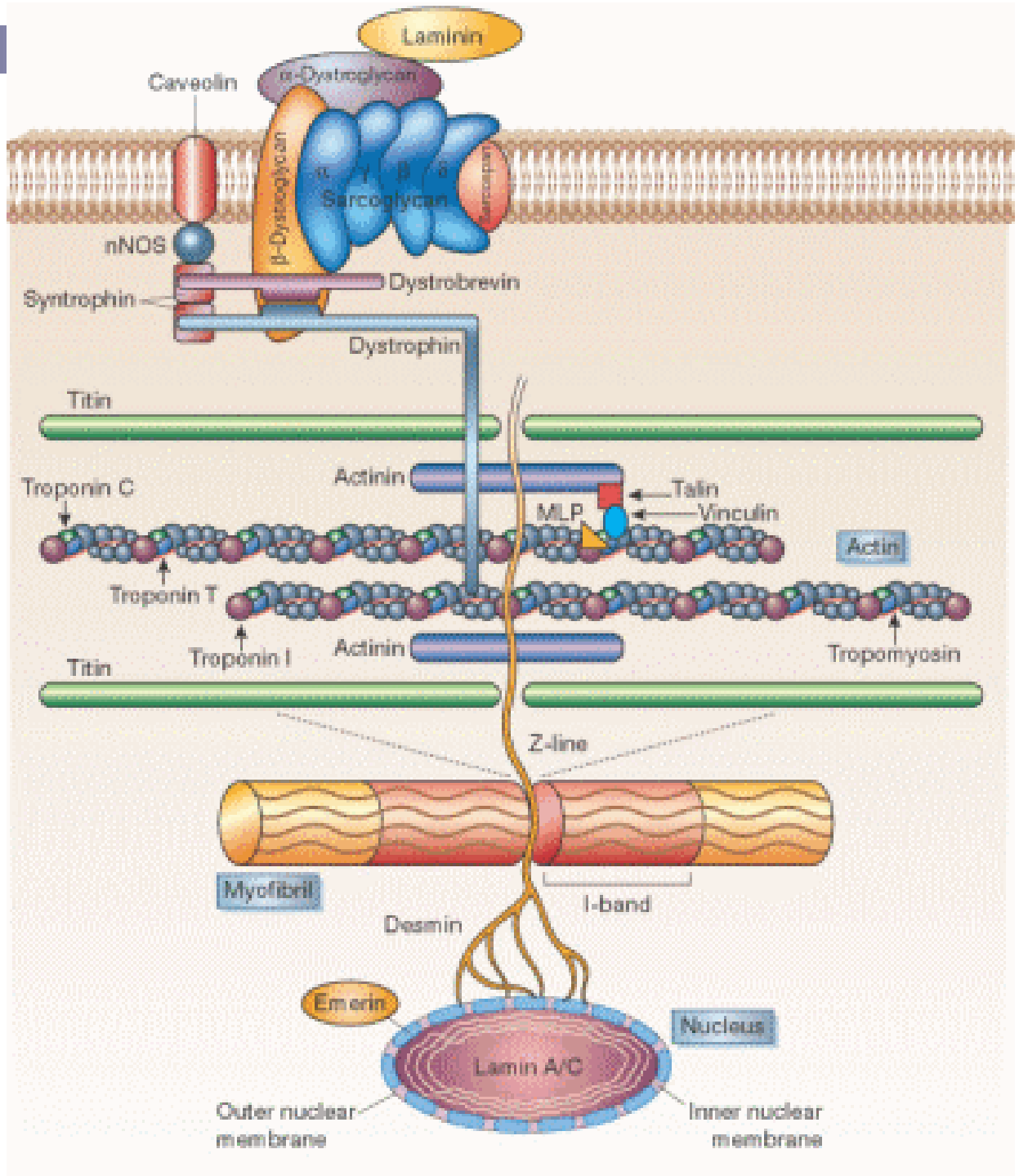
X-LINKED RECESSIVE INHERITANCE



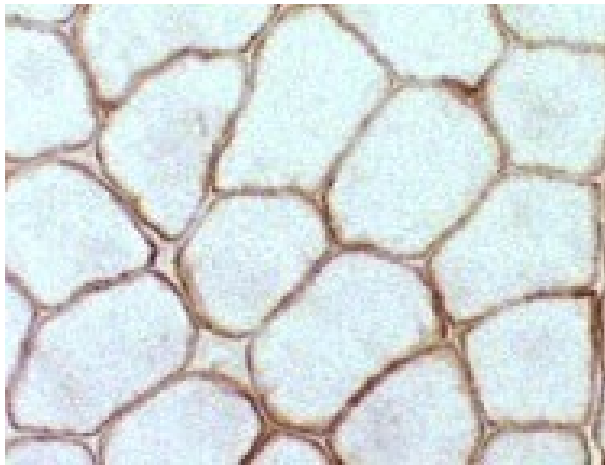
Dystrophin

- Dystrophin has 3685 encoded amino acids which can be separated into four domains:
 - (1) N-terminal domain -240 amino acids
 - (2) 25 triple helical segments similar to the repeat domains of spectrin
 - (3) the cysteine-rich segment similar in part to the entire COOH domain of alpha-actin
 - (4) C-terminal -420 amino acids.

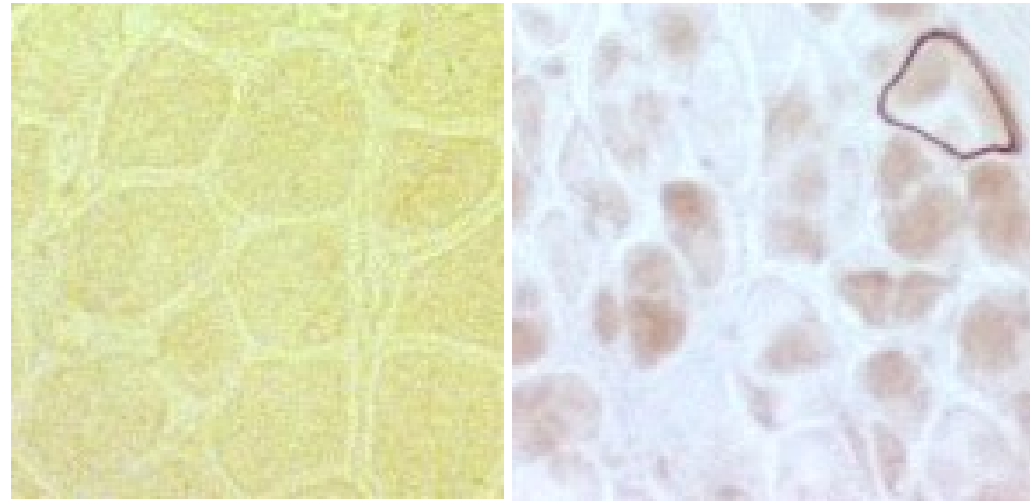
Dystrophin complex:



DMD: *Dystrophin staining*



- **Normal dystrophin staining** around the rim of muscle fibers.



- **Absent dystrophin: DMD**
Left: No staining around the rim of muscle fibers.
Right: No staining of most muscle fibers.
One "revertant" fiber with dystrophin staining.

