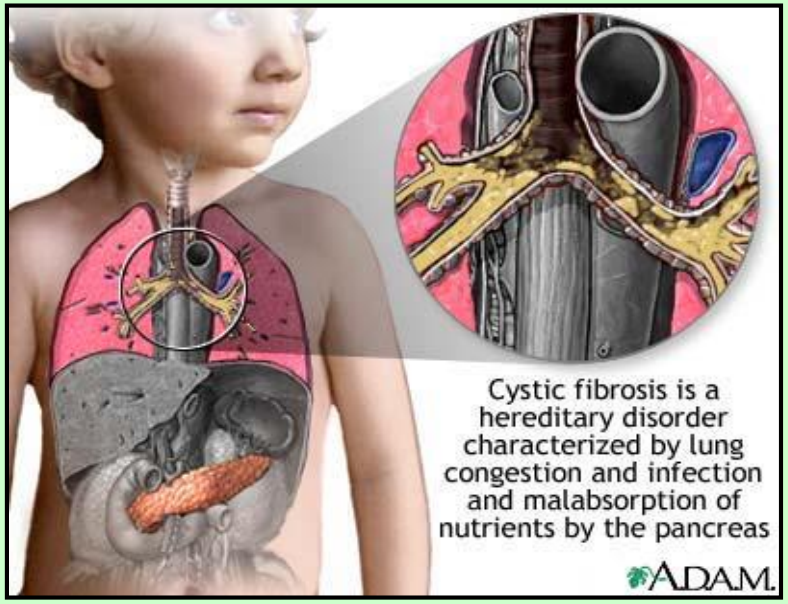
Cystic fibrosis (CF) - inherited autosomal recessive disorder

incidence of 1 in 3 000 live births

carrier frequency of 1 in 25

CF affects roughly 70 000 worldwide

Hallmarks of CF

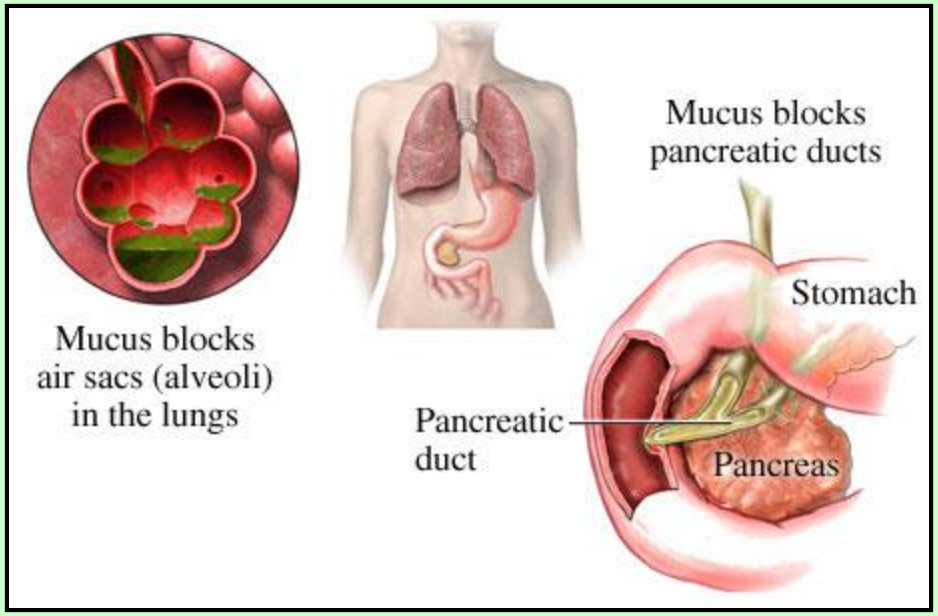


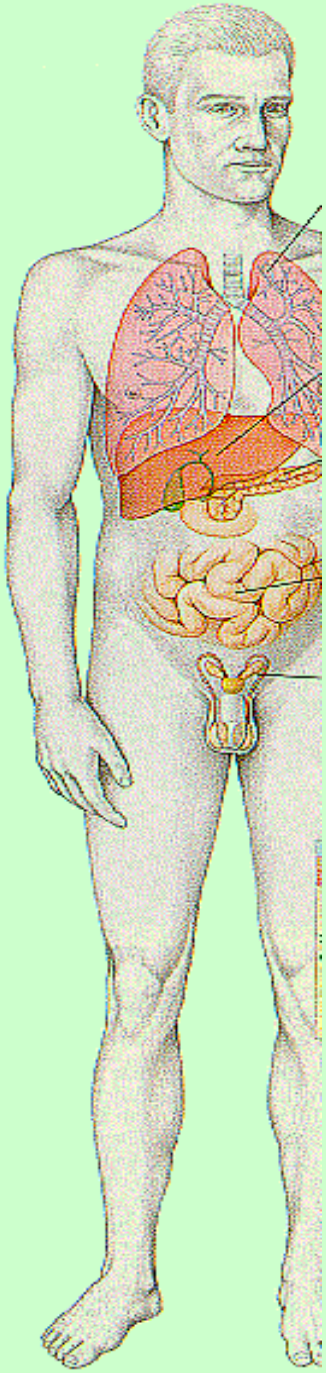
- Very salty-tasting skin
- Appetite, but poor growth & weight gain
- Coughing, wheezing & shortness of breath
- Lung infections, e.g. pneumonia/bronchitis

Clinical Aspects

Cystic fibrosis affects the entire body

- Lungs and sinuses
- GI, liver and pancreas
- Endocrine system
- Reproductive system





Organs Affected by Cystic Fibrosis

AIRWEAYS: Clogging and infection of bronchial passages impede breathing. The infection progressively destroy the lungs.

LIVER: Plugging of small bile ducts impedes digestion and discrupts liver function in perhaps 5% of patients

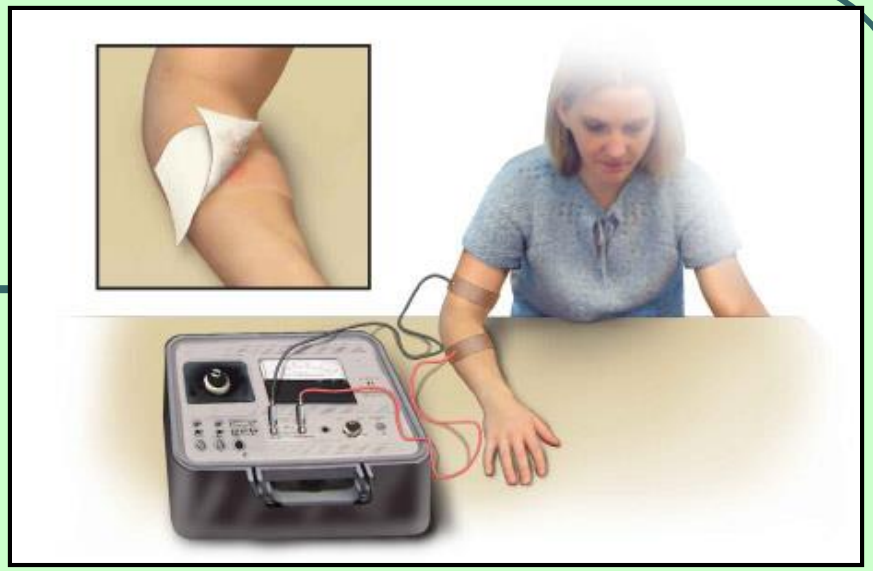
PANCREAS: Oclusion of ducts prevents the pancreas from delivering critical digestive enzymes to the bowel in 65% of patients. Diabetes can result as well.

SMALL INTESTINE: Obstruction of the gut by thick stool necessitates surgerry in about 10% of newborns

REPRODUCTIVE TRACT: Absence of fine ducts, such as the vas deferans, renders 95% of males infertile. Occasionally, women are made infertile by a dense plug of mucus that blocks sperm from entering the uterus.

SKIN: Malfunctioning of sweat glands causes perspiration to contain excessive salt (NaCl)

The Sweat Test



- Measures the concentration of chloride and sodium that is excreted in sweat.
- Two reliable positive results on two separate days is diagnostic for CF.
- Clinical presentation, family history and patient age must be considered to interpret the results.

CFTR gene (cystic fibrosis transmembrane conductance regulator)

Location: 7q31.2

Over 1,000 mutations in CFTR have been found

ΔF508 accounts for just 70% of CF cases

Panel 1: **Frequencies of *CFTR* mutations***

***CFTR* mutation**	**Allele frequency (%)**	***CFTR* mutation**	**Allele frequency**
ΔF508	69·4%	2789+5G→A	0·3%
Unknown	15·7%	R1162X	0·3%
G542X	2·3%	G85E	0·3%
G551D	2·2%	R560T	0·2%
ΔI507	1·6%	R334W	0·2%
W1282X	1·4%	3659ΔC	0·2%
N1303K	1·2%	A455E	0·1%
R553X	0·9%	711+1G→T	0·1%
621+1G→T	0·8%	1898+1G→A	0·1%
R117H	0·7%	2184ΔA	0·1%
3849+10 kbC→T	0·7%	S549N	0·1%
1717–IG→A	0·5%	1078ΔT	0·03%
R347P	0·3%		

*n=17 853.

The ΔF508 Mutation

A 3 base pair deletion called ΔF508 is the most common mutation causing cystic fibrosis

The mutation results in the deletion of a single amino acid (Phe) at position 508.

In Normal CFTR:

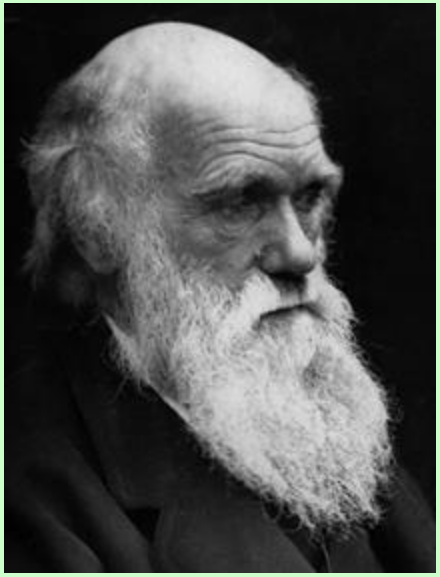
Nucleotide	AAT	ATC	ATC	TTT	GGT	GTT	TCC
Amino Acid	Asn	Ile	Ile	Phe	Gly	Val	Ser
	505			508			511

In ΔF508 CFTR:

Nucleotide	AAT	ATC	ATC	GGT	GTT	TCC
Amino Acid	Asn	Ile	Ile	Gly	Val	Ser
	505					

Benefits of ΔF508

The ΔF508 mutation most likely occurred over 50,000 years ago in Northern Europe.

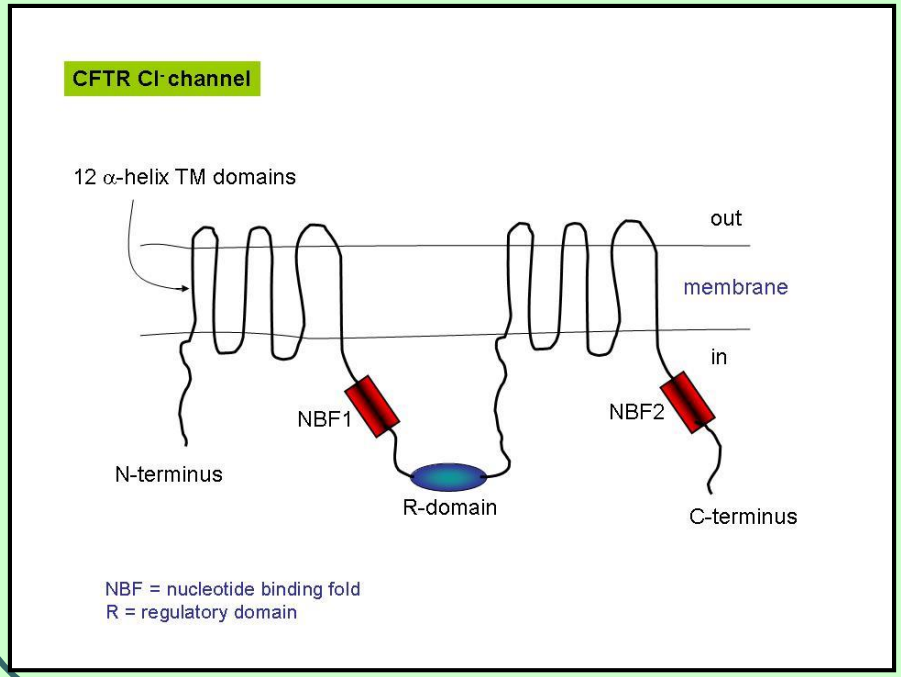


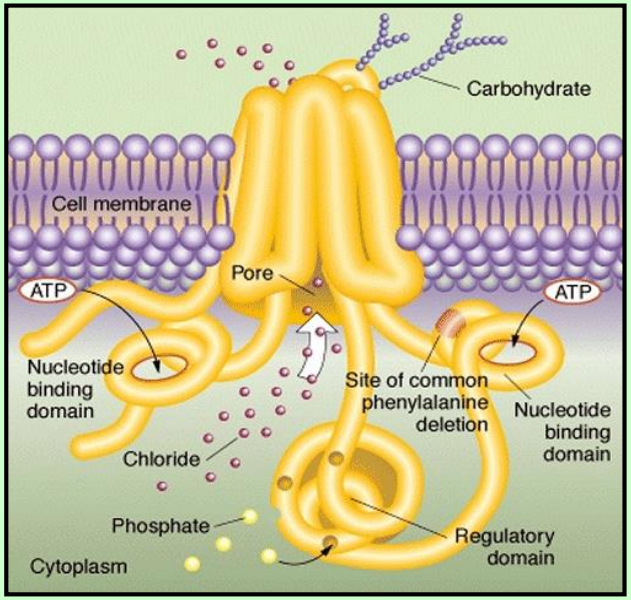
Individuals with two copies of ΔF508 get cystic fibrosis and often cannot reproduce.

Having one copy of ΔF508 reduces water loss during cholera, greatly increasing the chance of survival.

The Function of CFTR

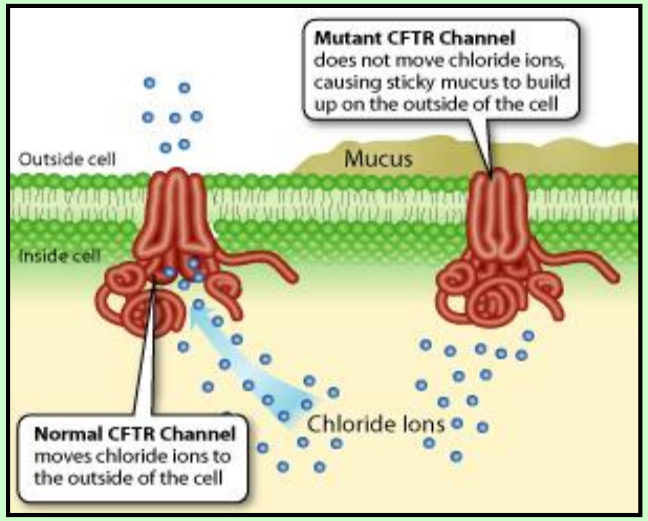
CFTR encodes a 170 kDa, membrane-based protein with an active transport function



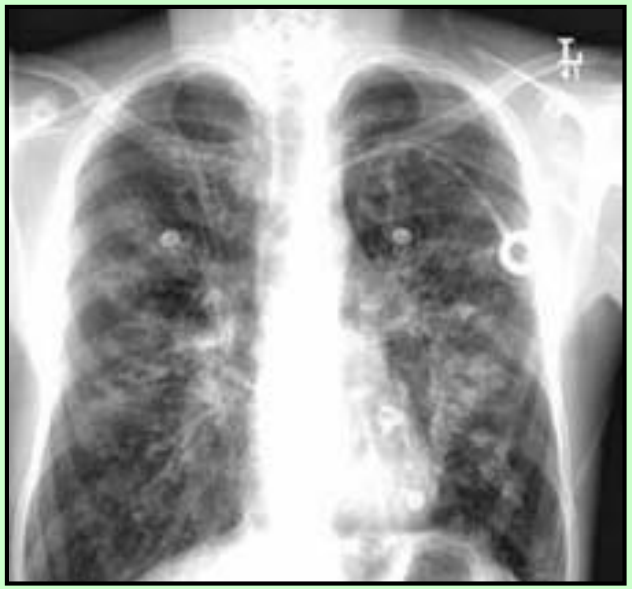


From Mutation to Disease

The mutant form of CFTR prevents chloride transport, causing mucus build-up



Mucus clogs the airways and disrupts the function of the pancreas & intestines.



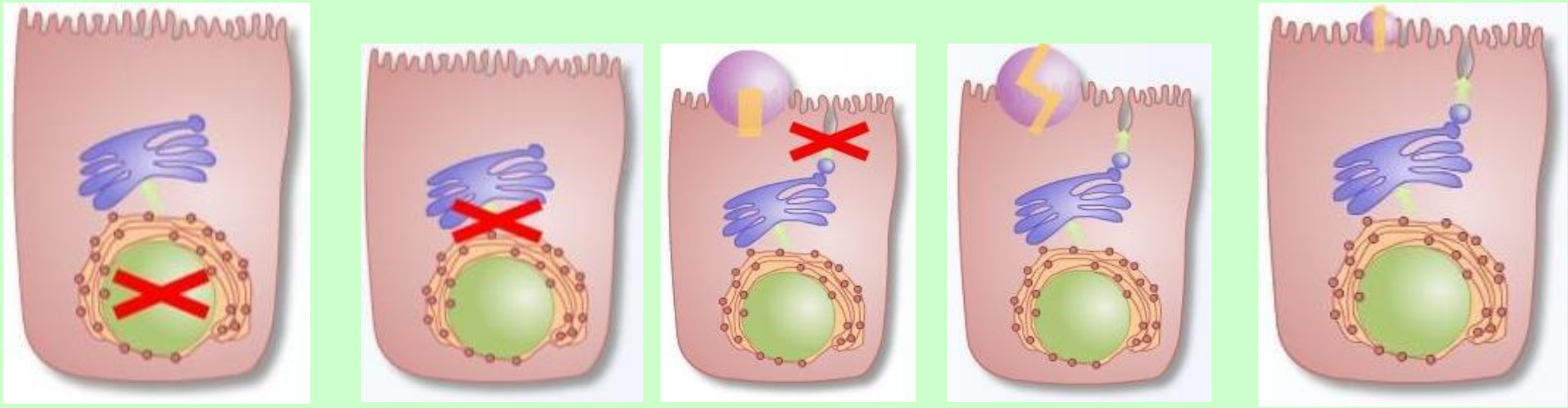
5 Classes of CFTR Mutations

CF Mutations can be classified by the effect they have on the CFTR protein.

Panel 2: **Functional classification of CFTR alleles**

Class	Functional effect of mutation	Allele
I	Defective protein production	G542X, R553X, W1282X, R1162X, 621–1G→T, 1717–1G→A, 1078ΔT, 3659ΔC
II	Defective protein processing	ΔF508, ΔI507, N1303K, S549N
III	Defective protein regulation	G551D, R560T
IV	Defective protein conductance	R117H, R334W, G85E, R347P
V	Reduced amounts of functioning CFTR protein	3849+10KbC→T, 2789+5G→A, A455E
Unknown		711+1G→T, 2184DA, 1898+1G→A

5 Classes of CFTR Mutations

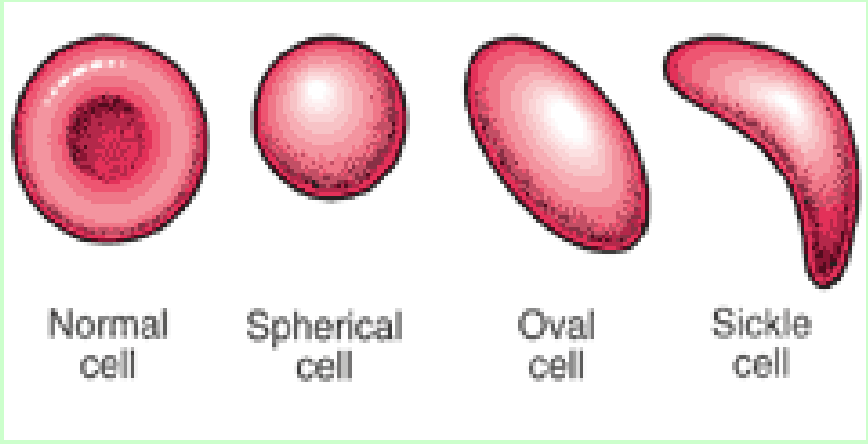


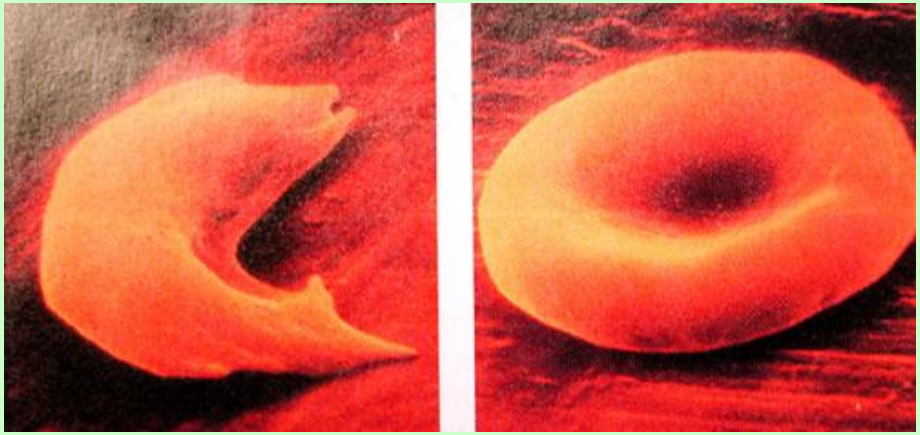
I	II	III	IV	V
Defective Production	Defective Processing	Defective Regulation	Defective Conductance	Reduced Amounts

Sickle Cell Anemia

autosomal recessive inheritance

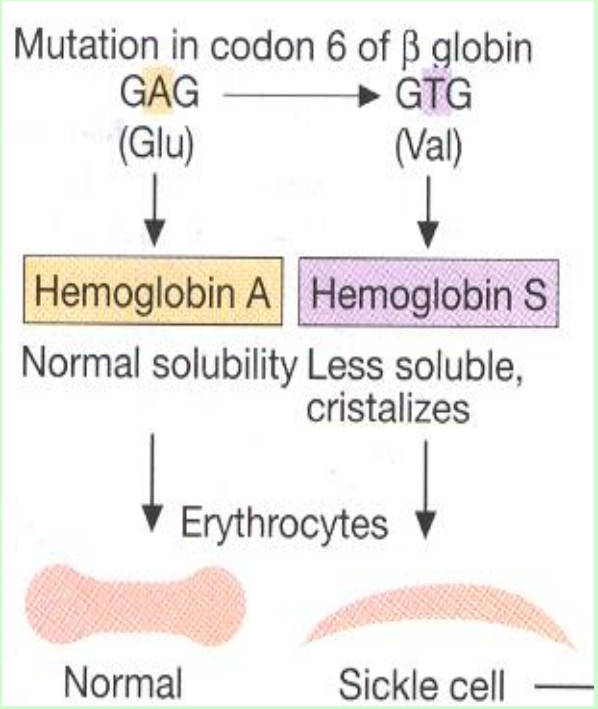
Sickle Cell Anemia

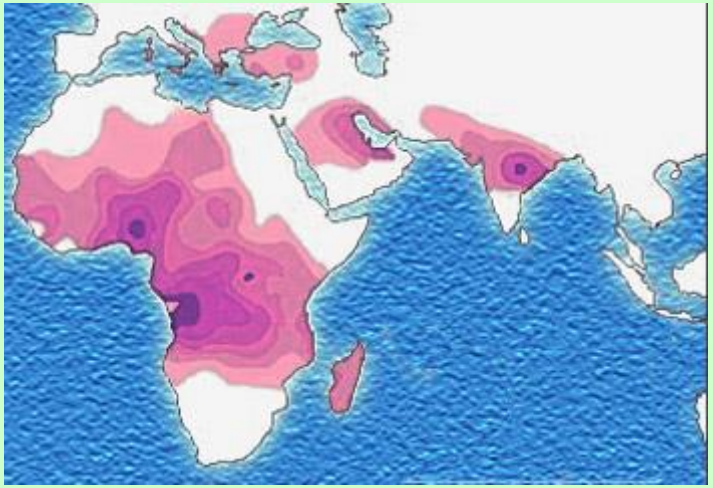




- mutation in the Hemoglobin Beta Gene which can be found in the chromosome 11 - 11p15.5
- haemoglobin A is replaced with what's known as haemoglobin S
- abnormally shapes red blood cells.

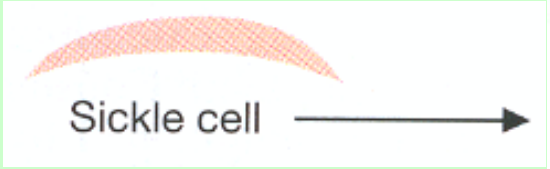
- substitution of the second nucleotide base of codon 6, adenin (A) to thymine (T) changes the codon GAG for glutamic acid to the codon GTG for valine

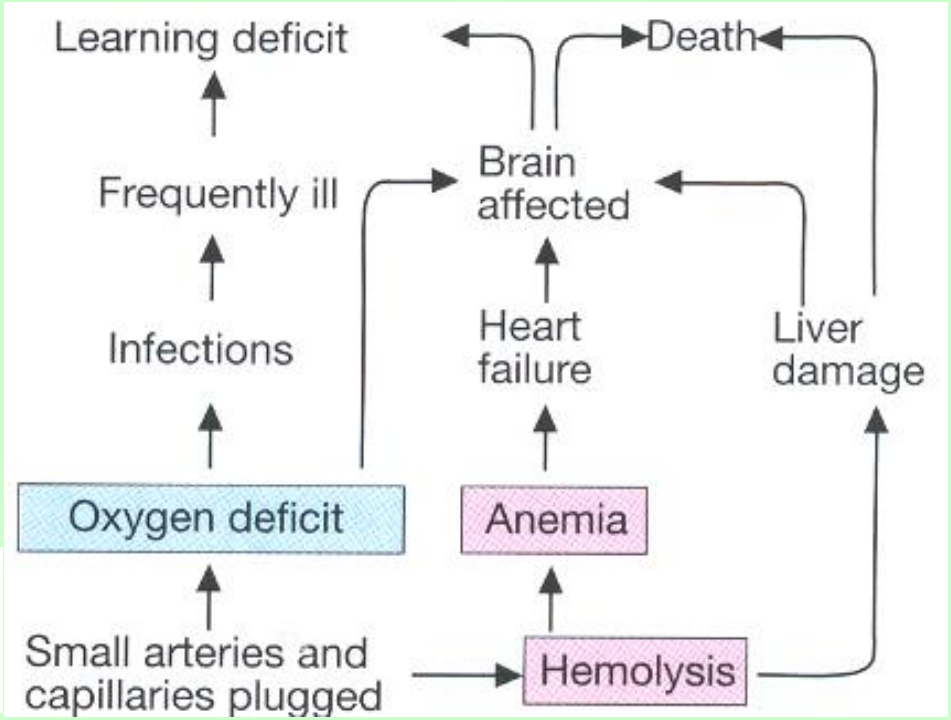




Sickle cells

- Unlike normal erythrocytes, sickle cells are unable to pass through small arteries and capillaries. These become clogged and cause local oxygen deficiency in the tisues, followed by infection. Defective erythrocytes are destroyed (hemolysis). The result is chronic anemia and its numerous sequelae such as heart failure, liver damage and infection





Signs and symptoms of sickle cell anemia

- **Anemia -** sickle cells die in 10 to 20 days, leaving a shortage of red blood cells
- **Episodes of pain-** develops when sickle-shaped red blood cells block blood flow through tiny blood vessels, this can lead to bone and joint damage
- **Painful swelling of hands and feet**
- **Frequent infections**
- **Delayed growth**
- **Vision problems**

Hemophilia A

X linked recessive hereditary disorder

incidence about 1 in 5 000 males

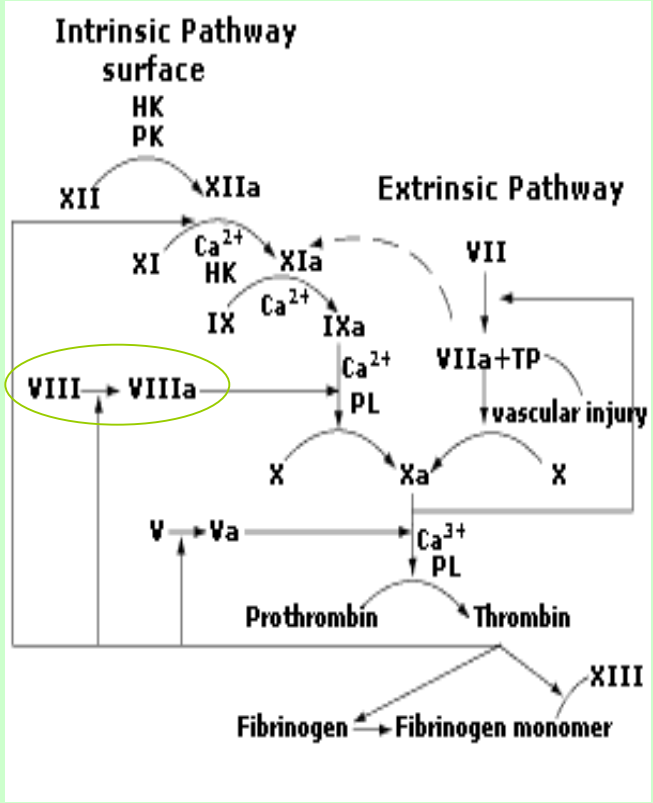
Hemophilia A

- 2 types of hemophilia: A and B
- Hemophilia A: X linked recessive hereditary disorder
- Hemophilia A results from the deficiency of blood coagulation factor VIII, which function as a cofactor in the activation of factor X to factor Xa during the intermediate phase of the coagulation cascade

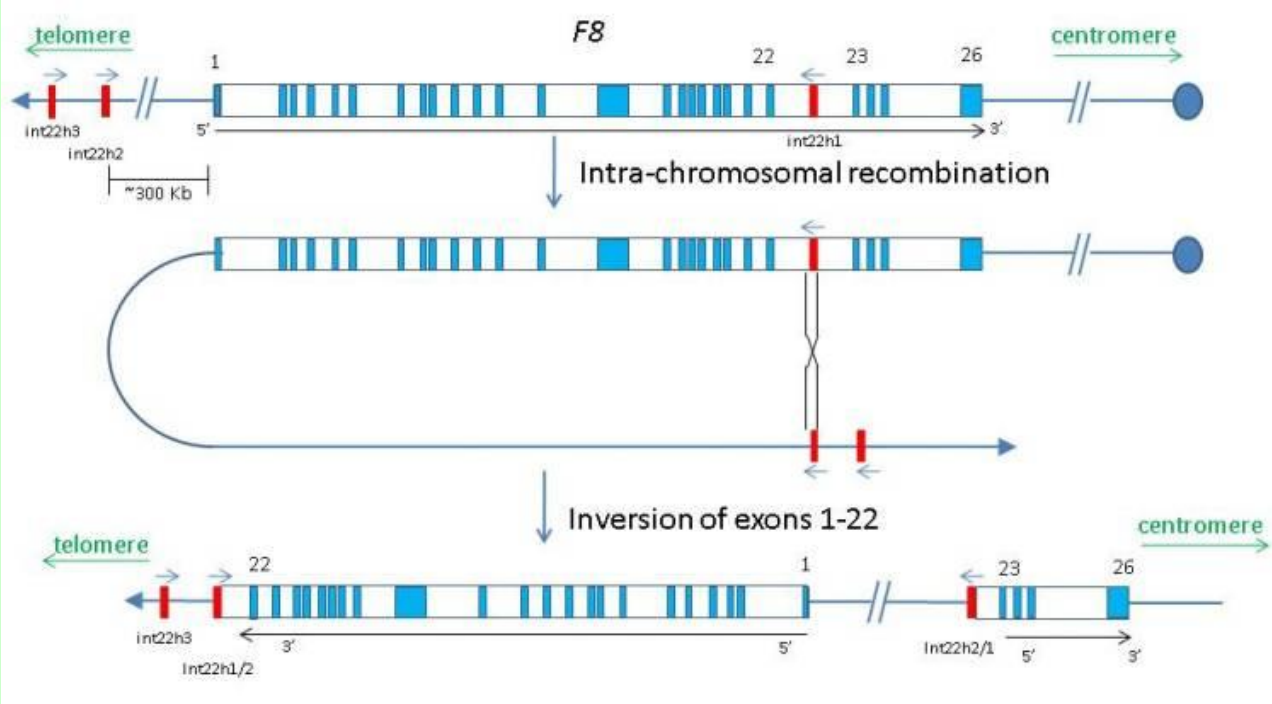
- Bruising and bleeding
- Minor bleeds: early joint and muscle bleeds, bleedig in the mouth and gums, hematuria
- Major bleeds: central nervous systém, severe injury, neck/throat, eye, gastrointestinal, late joints and muscles,…
- Chronic joint deformities from recurrent bleeding

Genetics

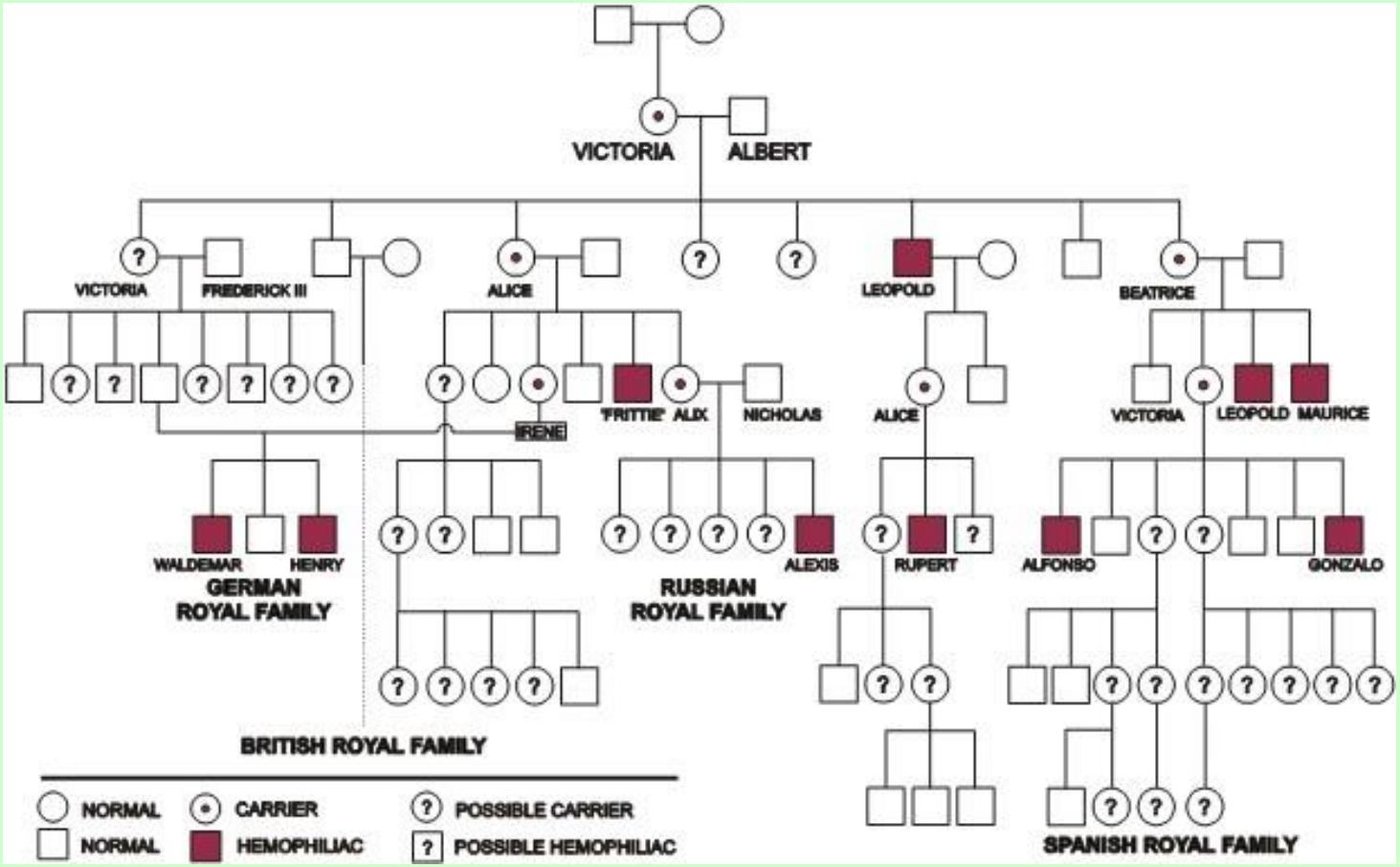
- Factor VIII gene - Xq28, one of the largest genes -186kb, 26 exons. Its large size predisposes it to mutations
- In Hemophilia A there is no uniform abnormality. There are deletions, insertions, and mutations
- Germinal mosaicism



- Aprox 40% of severe hemophilia A is caused by a major inversion in the gene - the breakpoint is situated within intron 22



A „Royal Disease“



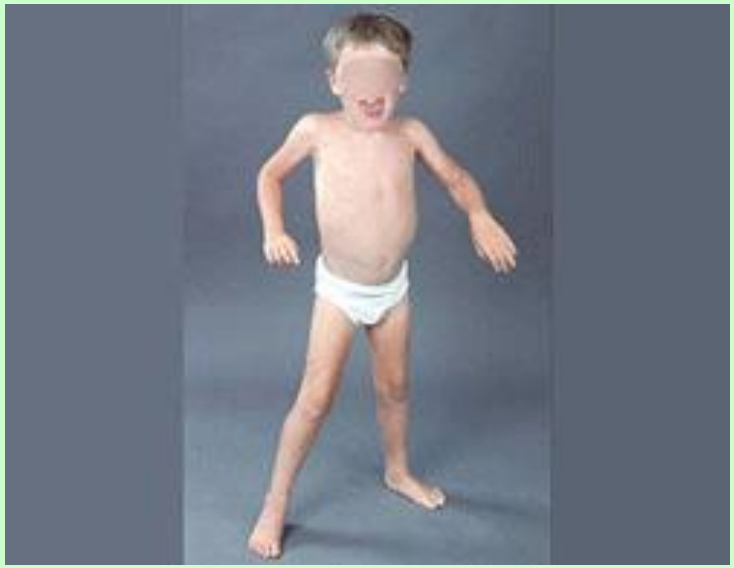
Duchenne Muscular Dystrophy

X – recesive

Occuring in 1 in 3000 males

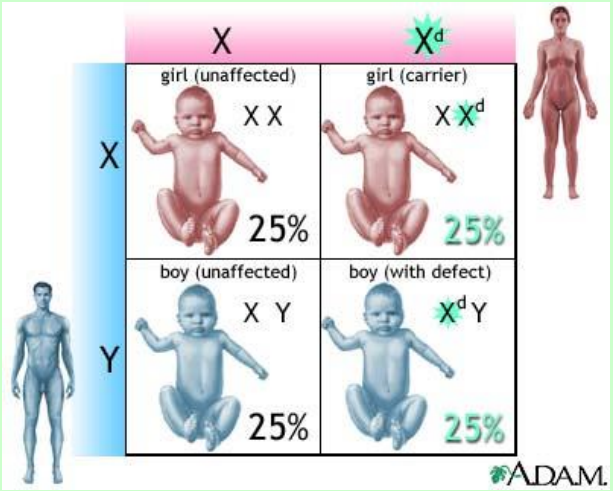
Duchenne Muscular Dystrophy

Occuring in 1 in 3000 males
X – recesive



Duchenne Muscular Dystrophy

- Females carry the DMD gene on the X chromosome.
  - Females are carriers and have a 50% chance of transmitting the disease in each pregnancy.
    - Sons who inherit the mutation will have the disease.
    - Daughters that inherit the mutation will be carriers.
- The DMD gene is located on the Xp 21 band of the X chromosome



- Dystrofin gene: locus Xp21
- 2,4 MB (1% of X chromosome)
- 79 exons
- over 200 types of mutations
- the most frequent mutation:
  - –Deletion of 1 and more exons (65%)
  - –Frameshift mutations
  - – 1/3 patients has *de novo* mutation

Clinical Features - Phenotype of DMD

- Delays in early childhood stages involving muscle use
- Learning difficulties in 5% of patients.
- Speech problems in 3% of patients.
- Leg and calf pain, walk on toes
- curvature of spine
- IQ's usually below 75 points.
- Increase in bone fractures due to the decrease in bone density
- Chest muscles weaken, making
- breathing difficult
- Wheelchair bound by 12 years of age.
- Cardiomyopathy at 14 to 18 years.
- Few patients live beyond 30 years of age.
    - **Reparatory problems and cardiomyopathy leading to congestive heart failure are the usual cause of death.**

DMD Gene and Dystrofin - Function

- The DMD gene encodes for the protein dystrofin, found in muscle cells and some neurons.
  - Dystrofhin provides strength to muscle cells by linking the internal cytoskeleton to the surface membrane.
  - Without this structural support, the cell membrane becomes permeable. As components from outside the cell are allowed to enter the internal pressure of the cell increases until the cell bursts and dies.

Molecular genetic testing of DMD gene

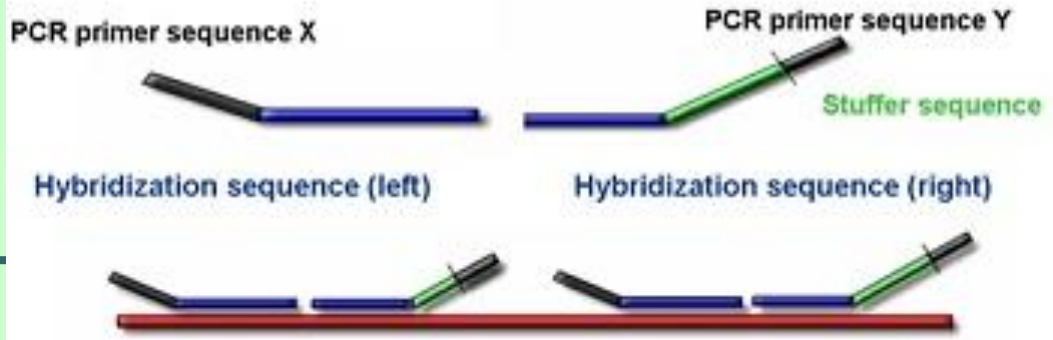
- 1. Detection of exon deletions in hot spot regions of DMD gene (multiplex PCR)
- 2. MLPA
- 3. Sequencing of coding regions of DMD gene
- 4. Indirect DNA diagnostics

MLPA (Multiplex Ligation-dependent Probe Amplification)

- multiplex PCR method detecting abnormal copy numbers of up to 50 different genomic DNA or RNA sequences
- it only requires a thermocycler and capillary electrophoresis equipment
- low cost and technically uncomplicated method
- over 300 probe sets

- five major steps:
- - DNA denaturation and hybridisation of MLPA probes
- - ligation reaction
- - PCR reaction
- - separation of amplification products by electrophoresis
- - data analysis

1. Denaturation and Hybridization

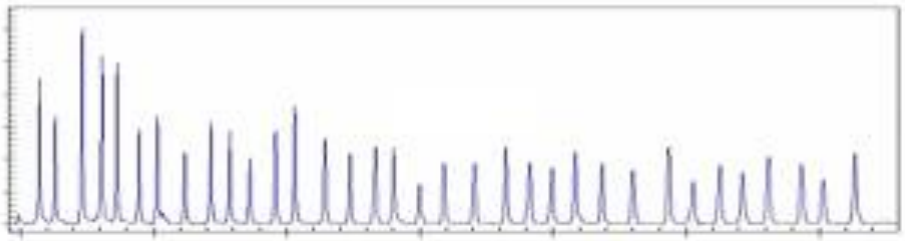


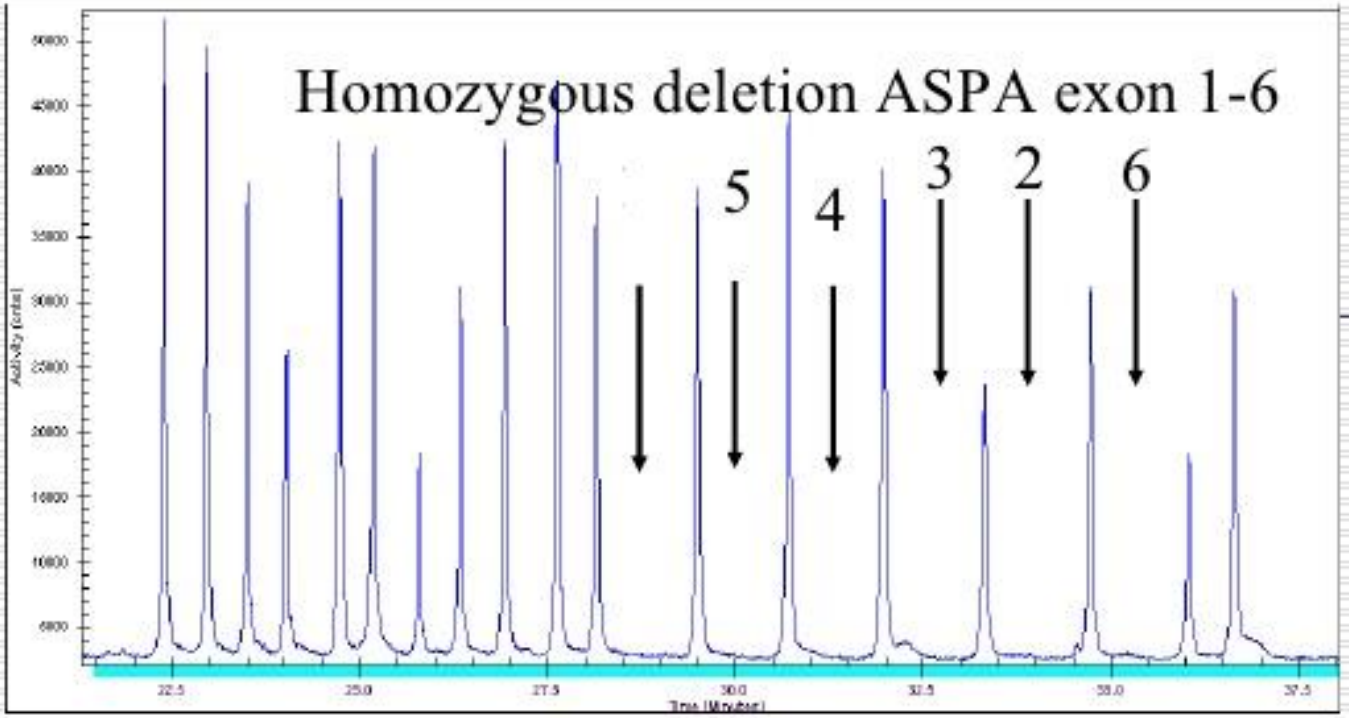
2. Ligation

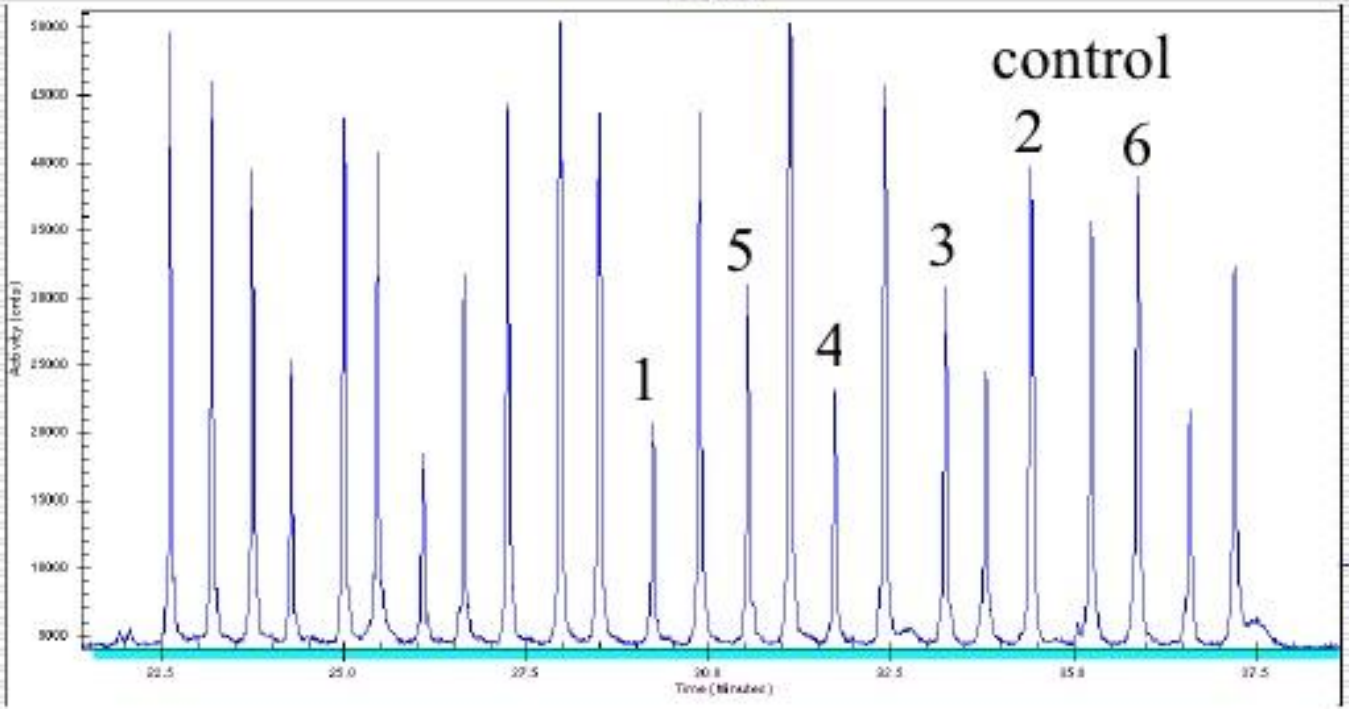
3. PCR with universal primers X and Y

exponential amplification of ligated probes only

4. Fragment analysis







Specificity of MLPA probes is very high.

MRC-Holland b.v.
MLPA

Allelic Variants

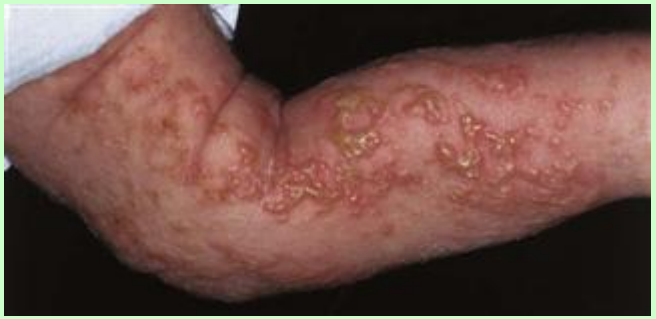
Disease	Mutation	Effect of Mutation	Phenotype
Duchenne Muscular Dystrophy	Very Large Deletions caused by: Stop mutations Splicing mutations Deletions Duplications	Severely Functionally Impaired Dystrophin Protein	As Discussed In Prior Slides
Becker Muscular Dystrophy	Deletion or Duplication That Change In-Frame Exons	Creates A Protein That Is Partially Functional	Same As But Less Sever Then DMD But Onset At Greater Then 7 Years Old
DMD Related Dilated Cardiomyopathy	Effects The Cardiac Muscle Promoter and The First Exon	No Dystrophin Transcriptions Being Carried Out In Cardiac Muscle	Tachycardia (Fat Heart Beat) Leads To Congestive Hear Failure
Limb-Girdle Muscular Dystrophy	In Gene That Encodes Scarcoglycans and Other Proteins of Muscle Cells	Decrease In Scarcoglycans Proteins	Pelvic and Shoulder Girdle Can Look Like DMD or BMD

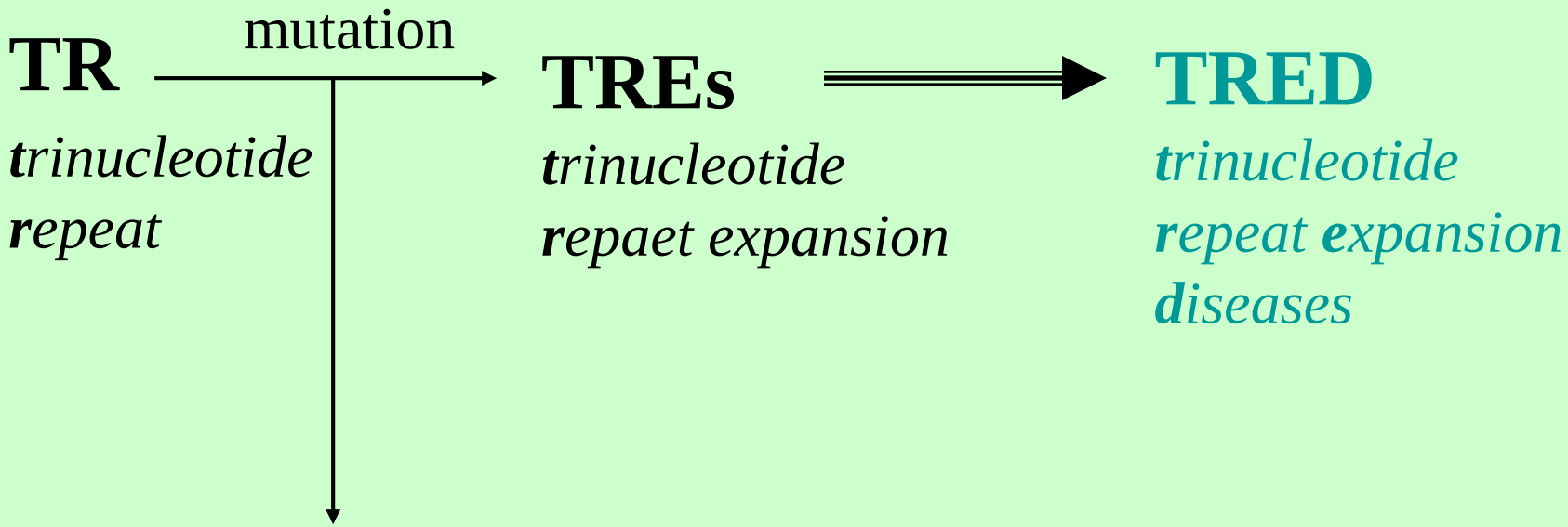
Incontinentia pigmenti

gonosomal dominant inheritance

- affects the skin, hair, teeth, nails, and central nervous systém
- also known as **Bloch–Siemens syndrome**, **Bloch–Sulzberger syndrome** and **nevus pigmentosus systematicus**
- skin abnormalities that begin in childhood. Other symptoms include hair loss, dental abnormalities, eye abnormalities lined or pitted fingernails and toenails. Associated problems can include delayed development, intellectual disability, seizures, and other neurological problems

- IP is lethal in most, but not all, males
- mutations in a IKK-gamma gene (IKBKG) also called NEMO (NF-κB essential modulator)




TR
mutation
TREs
TRED
trinucleotide
repeat
trinucleotide
repaet expansion
trinucleotide
repeat expansion
diseases
expansion
New type of mutation, described 1991

Trinucleotide repeat disorders

- caused by an unusual form of mutation called trinucleotide repeat expansion (TNRE)
  - The term refers to the phenomenon that a sequence of 3 nucleotides can increase from one generation to the next
- These diseases include
  - Huntington disease (HD)
  - Fragile X syndrome (FRAXA)

- Certain regions of the chromosome contain trinucleotide sequences repeated in tandem
  - In normal individuals, these sequences are transmitted from parent to offspring without mutation
  - However, in persons with TRNE disorders, the length of a trinucleotide repeat increases above a certain critical size
    - It also becomes prone to frequent expansion
    - This phenomenon is shown here with the trinucleotide repeat CAG

CAGCAGCAGCAGCAGCAGCAGCAGCAGCAG

***n* = 11**

CAGCAGCAGCAGCAGCAGCAGCAGCAGCAGCAGCAGCAGCAGCAGCAGCAGCAG

***n* = 18**

- In some cases, the expansion is within the coding sequence of the gene
  - Typically the trinucleotide expansion is CAG (glutamine)
  - Therefore, the encoded protein will contain long tracks of glutamine
    - This causes the proteins to aggregate with each other
    - This aggregation is correlated with the progression of the disease

- In other cases, the expansions are located in noncoding regions of genes
  - These expansions are hypothesized to cause abnormal changes in RNA structure
    - Thereby producing disease symptoms

Triplet Repeat Disorders

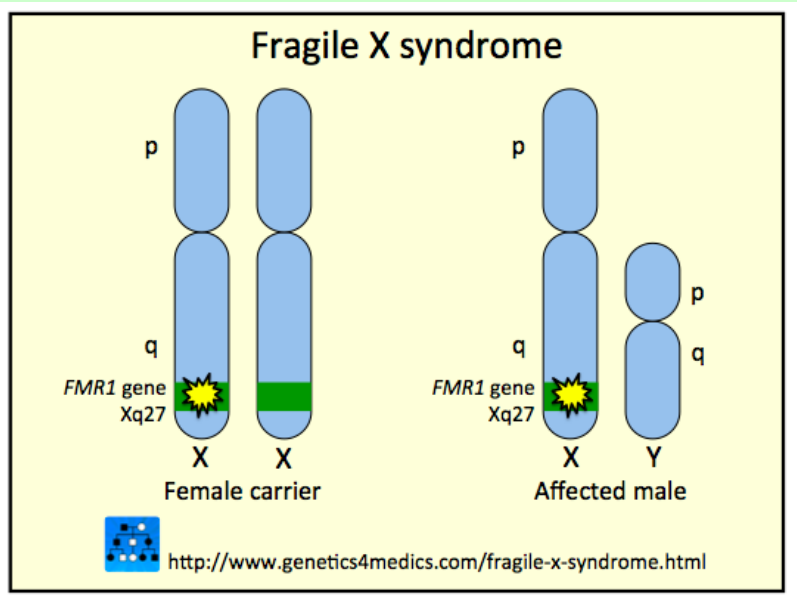
Table 12.7

Triplet Repeat Disorders

Disorder	OMIM	mRNA Repeat	Normal Number of Copies	Disease Number of Copies	Signs and Symptoms (Phenotype)
Fragile X syndrome	309550	CGG or CCG	6–50	200–2,000	Mental retardation, large testicles, long face
Friedreich ataxia	229300	GAA	6–29	200–900	Loss of coordination and certain reflexes, spine curvature, knee and ankle jerks
Haw River syndrome	140340	CAG	7–25	49–75	Loss of coordination, uncontrollable movements, dementia
Huntington disease	143100	CAG	10–34	40–121	Personality changes, uncontrollable movements, dementia
Jacobsen syndrome	147791	CGG	11	100–1,000	Poor growth, abnormal face, slow movement
Myotonic dystrophy type I	160900	CTG	5–37	80–1,000	Progressive muscle weakness; heart, brain, and hormone abnormalities
Myotonic dystrophy type II	602668	CCTG	<10	>100	Progressive muscle weakness; heart, brain, and hormone abnormalities
Spinal and bulbar muscular atrophy	313200	CAG	14–32	40–55	Muscle weakness and wasting in adulthood
Spinocerebellar ataxia (5 types)	271245	CAG	4–44	40–130	Loss of coordination

Fragile X Syndrome

- occurs in about 1 in 4,000 males and 1 in 8,000 females
- expansion of the **CGG** repeat sequence in the ***FMR1*** gene on chromosome Xq27
- *FMR1* gene codes for the FMR protein (FMRP), which is expressed in many cell types, especially in neurons.



- **Expansion size**:
  - **Normal:** 5-44 repeats
  - **Intermediate:** 45-54 repeats. May expand to a premutation allele during intergenerational transmission.
  - **Premutation:** 55-200 repeats. May expand to a full mutation allele, especially during maternal transmission.
  - **Full mutation:** >200 repeats
- Premutation alleles in a female **may (not always!)** expand to full mutation in the subsequent generation.

- **Males with Full mutation**
- **Development:** developmental delay - mainly speech.
- **Behaviour:** Hyperactivity, autistic features, temper tantrums in childhood. Usually shy as adults.
- **Intellectual difficulties:** moderate to severe
- **Physical features**: Prominent forehead, long face, prominent jaw, large ears, joint hypermobility. Large testes (post-pubertal feature)

- **Premutation carriers**
- **Fragile X associated tremor/ataxia syndrome (FXTAS):** Occurs in a proportion of premutation carriers (both males & females). Characterised by **late onset** cerebellar signs (ataxia & intentional tremor) and other neurological features including dementia and cognitive decline.
- **Primary ovarian insufficiency**: Occurs in about 20% of females who are premutation carriers. Characterised by menopause before the age of 40 years.

Huntington disease

- named for George Huntinton, described this disease in 1872
- begin between 30 and 50 years of age, but can start at any age
- About 8% of cases start before the age of 20 years
- Autosomal dominant mutation in either of an individual's two copies of a gene called Huntingtin (HTT, *HD* or *IT15)* - 4p16.3
- expansion of CAG triplet repeats in the gene coding for the Huntingtin protein results in an abnormal protein, which gradually damages cells in the brain
- 4 to 15 in 100,000 people of European descent, affects men and women equally

Classification of the trinucleotide repeat, and resulting disease status, depends on the number of CAG repeats

Repeat count	Classification	Disease status	Risk to offspring
<26	Normal	Will not be affected	None
27–35	Intermediate	Will not be affected	Elevated but <<50%
36–39	Reduced Penetrance	May or may not be affected	50%
40+	Full Penetrance	Will be affected	50%

Symptoms

- **Cognitive:** amnesia, delusion, lack of concentration, memory loss, mental confusion, slowness in activity and thought, or difficulty thinking and understanding
- **Muscular:** abnormality walking, increased muscle activity, involuntary movements, problems with coordination, loss of muscle, or muscle spasms
- **Behavioral:** compulsive behavior, fidgeting, irritability, or lack of restraint
- **Psychological:** delirium, depression, hallucination, or paranoia
- **Mood:** anxiety, apathy, or mood swings
- **Also common:** tremor, weight loss, or impaired voice

Myotonic dystrophy

- Affects 1 in 8000 people
- Autosomal Dominant Disorder with anticipation
  - Myotonia: hyperexcitability of muscle membrane $\rightarrow$ inability of quick muscle relaxation
  - Progressive muscular weakness and wasting, most prominent in cranial and distal muscles
  - Cataracts, frontal balding, testicular atrophy
  - Cardiac abnormalities, mental retardation

- 1). “Mild DM” (adult onset): People often lead active lives and may even be unaware that they have the disorder.
- 2). “Classical DM” (adult onset): People commonly have muscle weakness and wasting, myotonia, hand and wrist weakness and/or foot drop.
- 3). “Congenital Myotonic Dystrophy” (CMD): A very severe form of DM1, often fatal in young children

Myotonic dystrophy 1 and 2

- In DM1, the affected gene is called DMPK (myotonic dystrophy protein kinase) which codes for a myosin kinase expressed in skeletal muscle. The gene is located on the long arm of chromosome 19
- DM2 is similarly caused by a defect of the ZNF9 gene on chromosome 21.

DMPK gene

- gene DMPK (myotonic dystrophy protein kinase gene) - 19q13.3
- CTG repeat expansion in a gene on chr. 19 in 3´ UTR (3´ untranslated region)
- This condition occurs when the CTG segment is abnormally repeated from 50 to 5,000 times. The mutated DMPK gene produces an altered version of messenger RNA that interacts with certain proteins to form clumps within the cell. The abnormal clumps interfere with the production of many other proteins.

- People with the classic features of type 1 myotonic dystrophy, including muscle weakness and wasting beginning in adulthood, usually have 100 to 1,000 CTG repeats.
- People born with the more severe congenital form of type 1 myotonic dystrophy tend to have a larger number of CTG repeats, often more than 2,000.

CNBP gene

- “CCHC-type zinc finger, nucleic acid binding protein.”
- CNBP gene provides instructions for making a protein called CCHC-type zinc finger, nucleic acid binding protein
- tetranucleotide repeat CCTG
- This condition occurs when the CCTG segment is abnormally repeated 75 to more than 11,000 times.