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صدق الله العظيم

Clinical Features and Molecular Diagnosis of

Most Common Hereditary Diseases

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Our objectives

Describe the clinical features and molecular diagnosis of most common hereditary diseases:

- Mongolism,
- Turner syndrome,
- Triple X syndrome,
- Klinefelter syndrome

INTRODUCTION

- **Medical genetics** : the diagnosis and management of hereditary disorders.

- **Chromosomes**
  - Autosomes (22 pairs)
  - Sex chromosomes(1Pair)

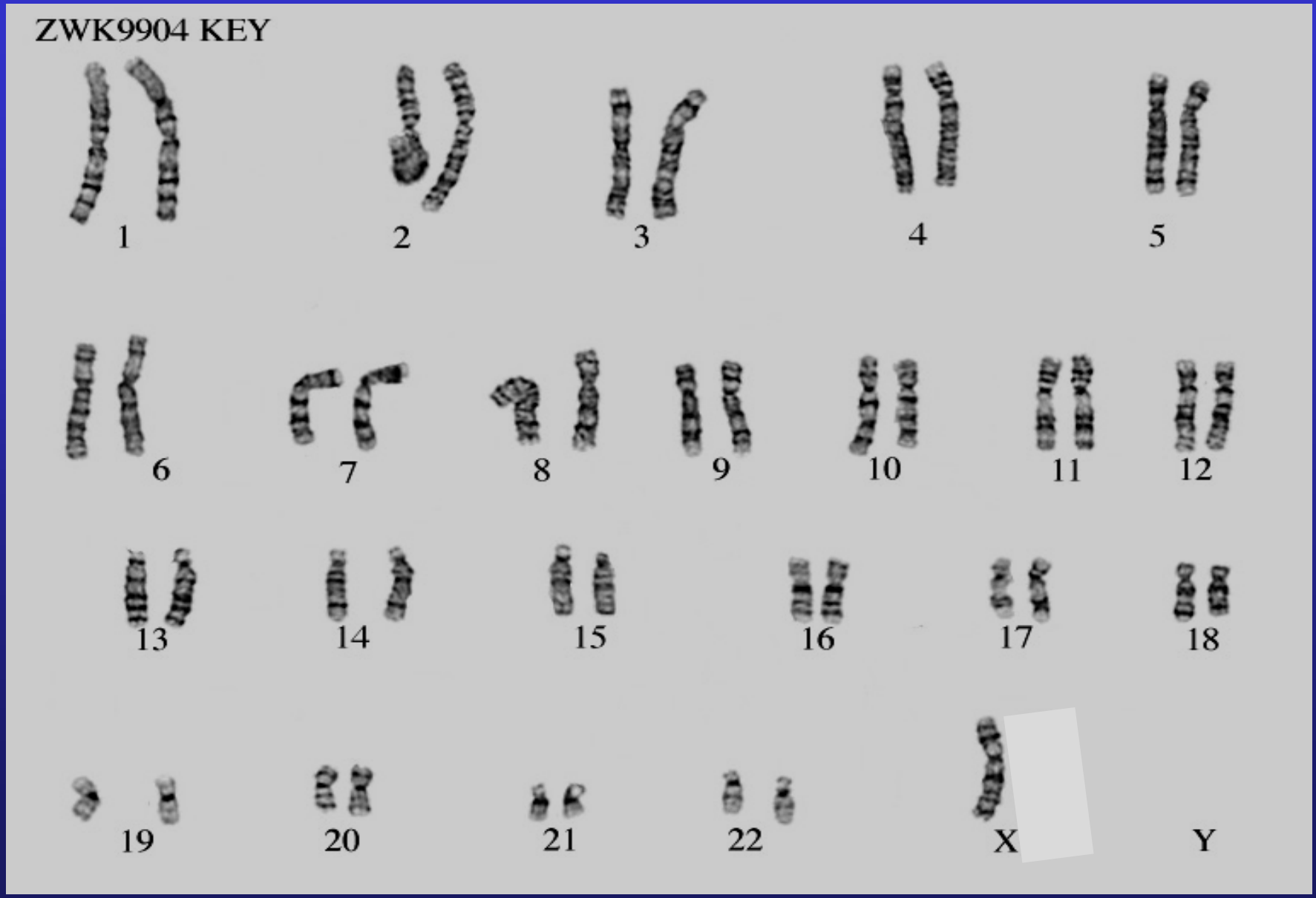
Key Words

DNA	The molecule which contains genes. Shaped like a double helix
Chromosomes	Found in the nucleus. Made up of long coils of DNA therefore contain genes
Gene	A section of DNA molecule. Part of the arm of a chromosome
Allele	Genes which control the same characteristic but have different outcomes
Dominant	The allele which determines which characteristic will appear. It dominates the recessive allele.

Key Words

Phenotype	The outward characteristics of an organism
Genotype	The genetic make-up of an organism
Homozygous	When the genotype for a characteristic has identical alleles, such as BB or bb
Heterozygous	When the genotype for a characteristic has different alleles, such as Bb
Mutation	A change in a gene or chromosome ie DNA

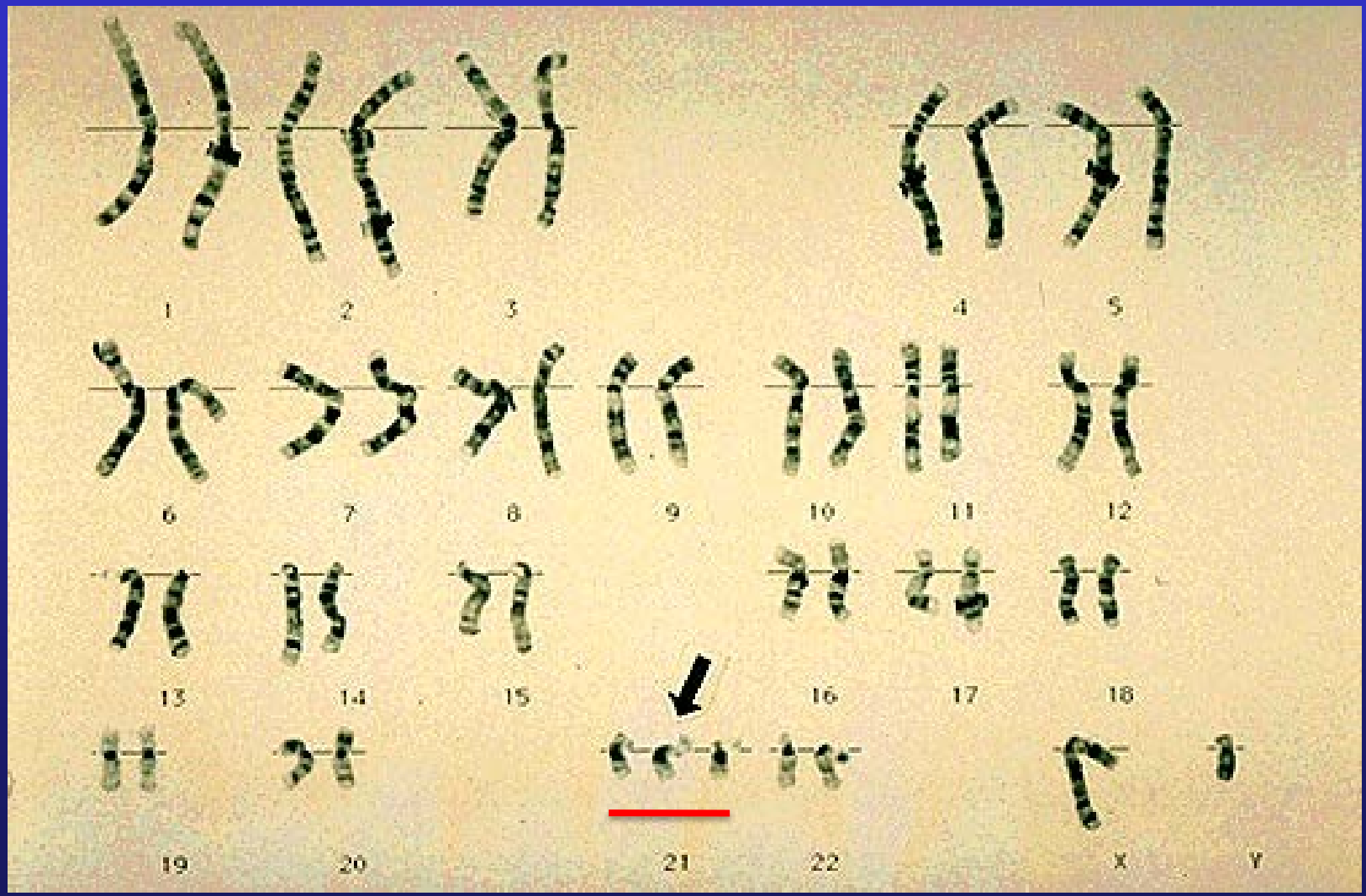
Chromosomes



Chromosomal Abnormalities

- Extra chromosome
  - Trisomy 21(Down syndrome) 47+21
  - Trisomy 18(Edwards' Syndrome) 47+18
  - Trisomy 13(Patau's Syndrome) 47+13
  - Kleinfelter's Syndrome (47XXY)
- Less Chromosome
  - 45XO(Turner syndrome)

Trisomy-21(Down's Syndrome)



DOWN SYNDROME

DOWN SYNDROME

Definition:

Down syndrome is the commonest numerical autosomal disorder due to trisomy 21 due to:

Non disjunction	**Mosiacism**	**Translocation**
in 95% of cases (called regular Mongol)	**in 1% of cases**	**in 4% of cases**

A. Non disjunction

A/E: non disjunction (failure of the chromosomes to disjoin normally) occurring during gametogenesis (meiosis).

⇓

Production of a gamete with an extra chromosome 21

Source of extra chromosome:

maternal in 90% of cases (paternal in 10%).

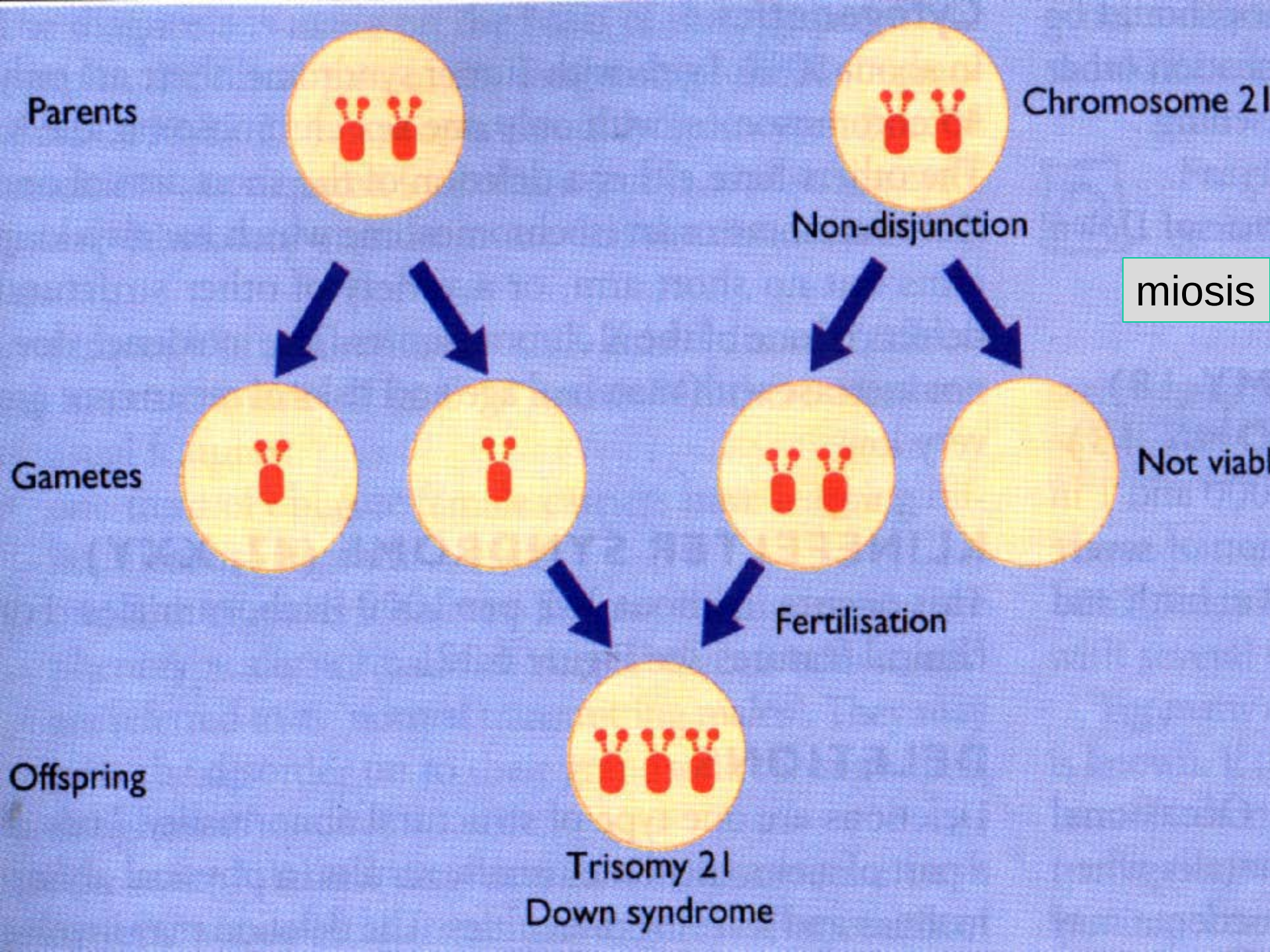
Recurrence rate:

incidence increase with increasing maternal age.

(1/100 if age > 35 y.)

Chromosomal study:

for the baby show 47 (+ 21).


Parents
Chromosome 21
Non-disjunction
miosis
Gametes
Not viabl
Fertilisation
Offspring
Trisomy 21
Down syndrome

B- Mosiac Down syndrome:

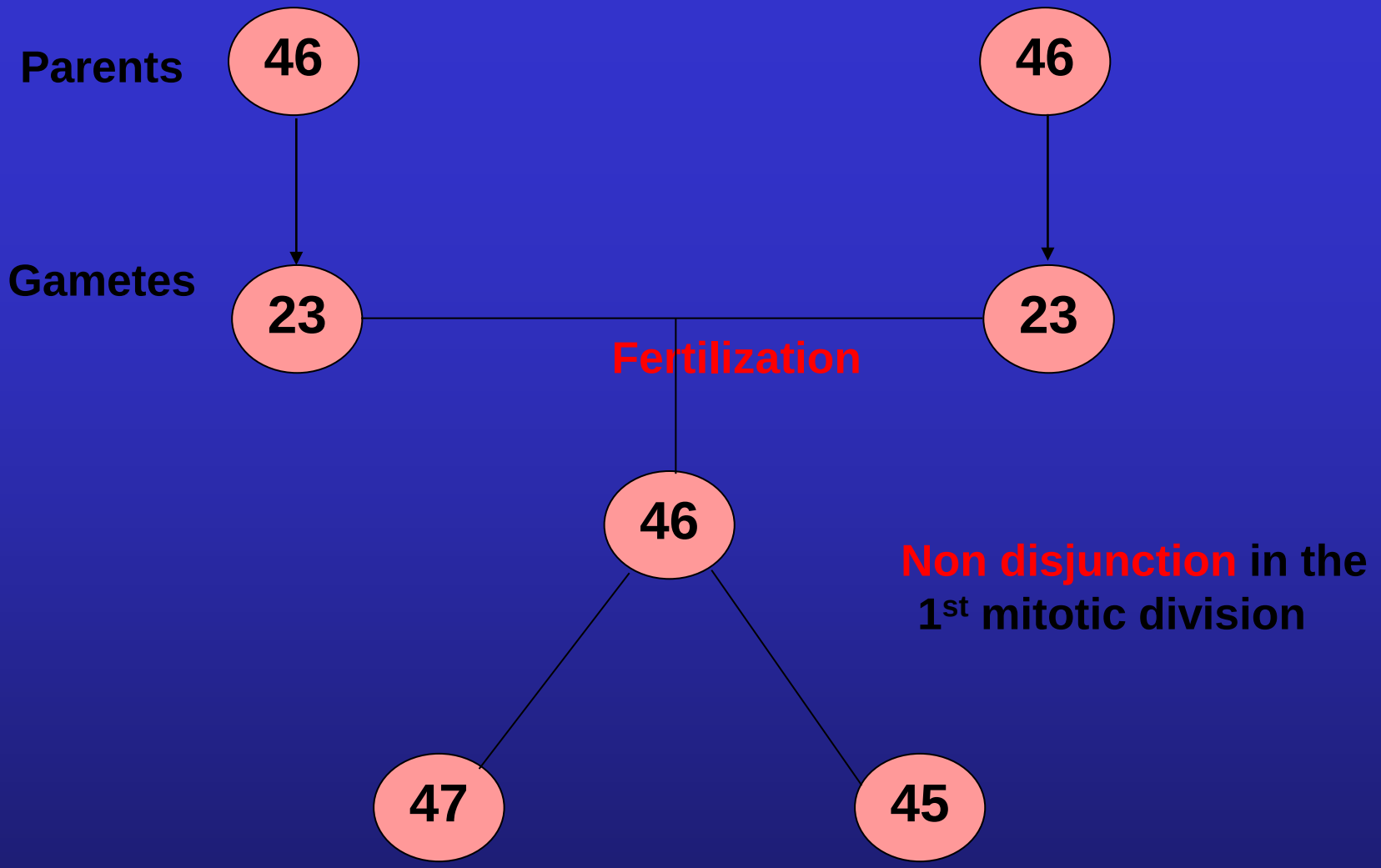
Etiology:

non disjunction occurring post fertilization during the 1st or the 2nd mitotic divisions.

Chromosomal study: **for the baby show either**

- **2 cell lines: if non disjunction occurred in the 1st mitotic division.**
  → 47 (+ 21) + 45 (- 21)

- **3 cell lines: if non disjunction occurred in the 2nd mitotic division**
  → 47 (+ 21) + 45 (- 21) + 46


Parents
46
46
Gametes
23
23
Fertilization
46
Non disjunction in the 1st mitotic division
47
45
Cells with 3 copies of chromosome 21
Cells with one copy of chromosome 21

C- Translocation Down syndrome:

Etiology:

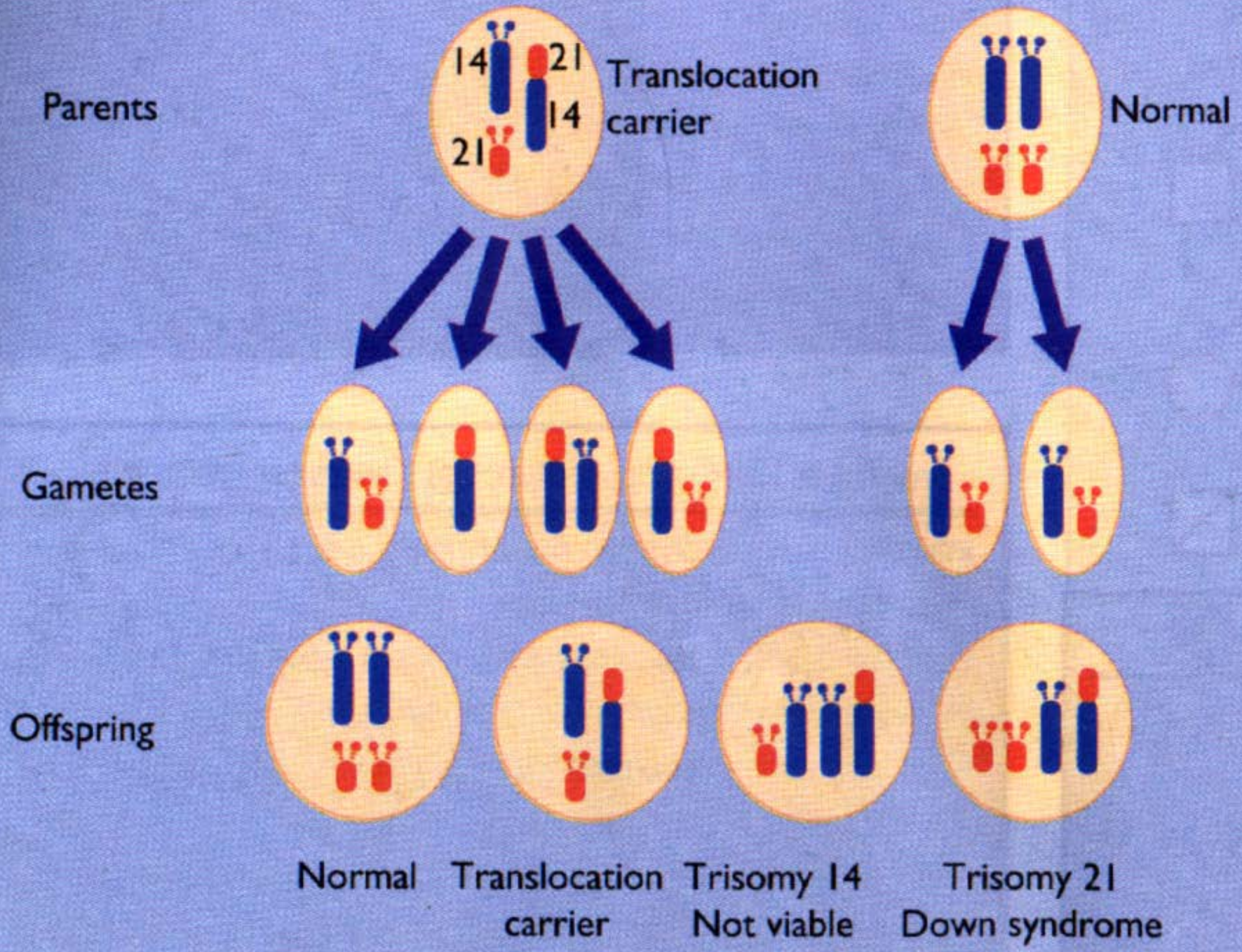
Chromosome 21 is translocated onto another chromosome it occurs only with another acrocentric chromosome i.e 14, 15, 21, 22.

How?

- The 2 short arms of the acrocentric chromosomes are very short, so easily lost & contain no essential genetic material.

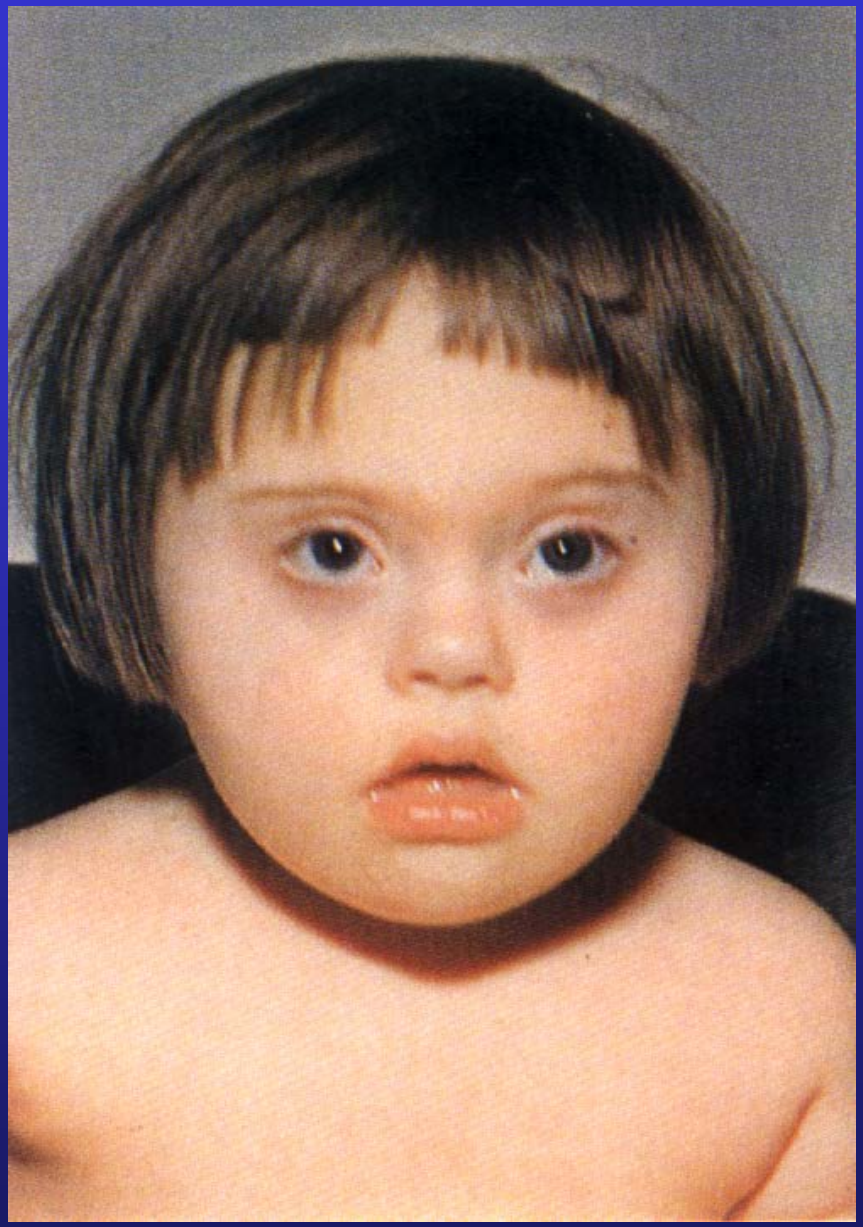
- The 2 long arms fuse together making one long chromosome.

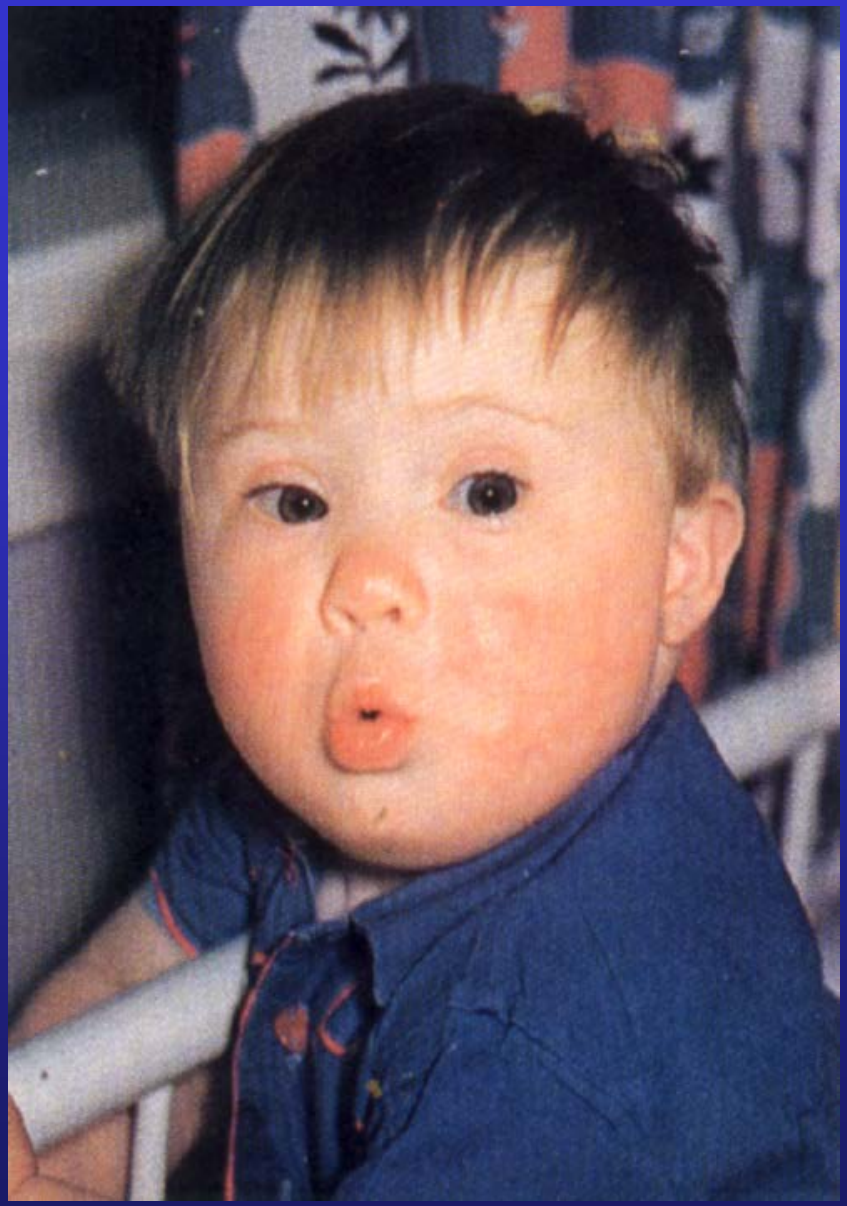
- If this translocation occur in a parent somatic cells he's called balanced translocation Carrier.

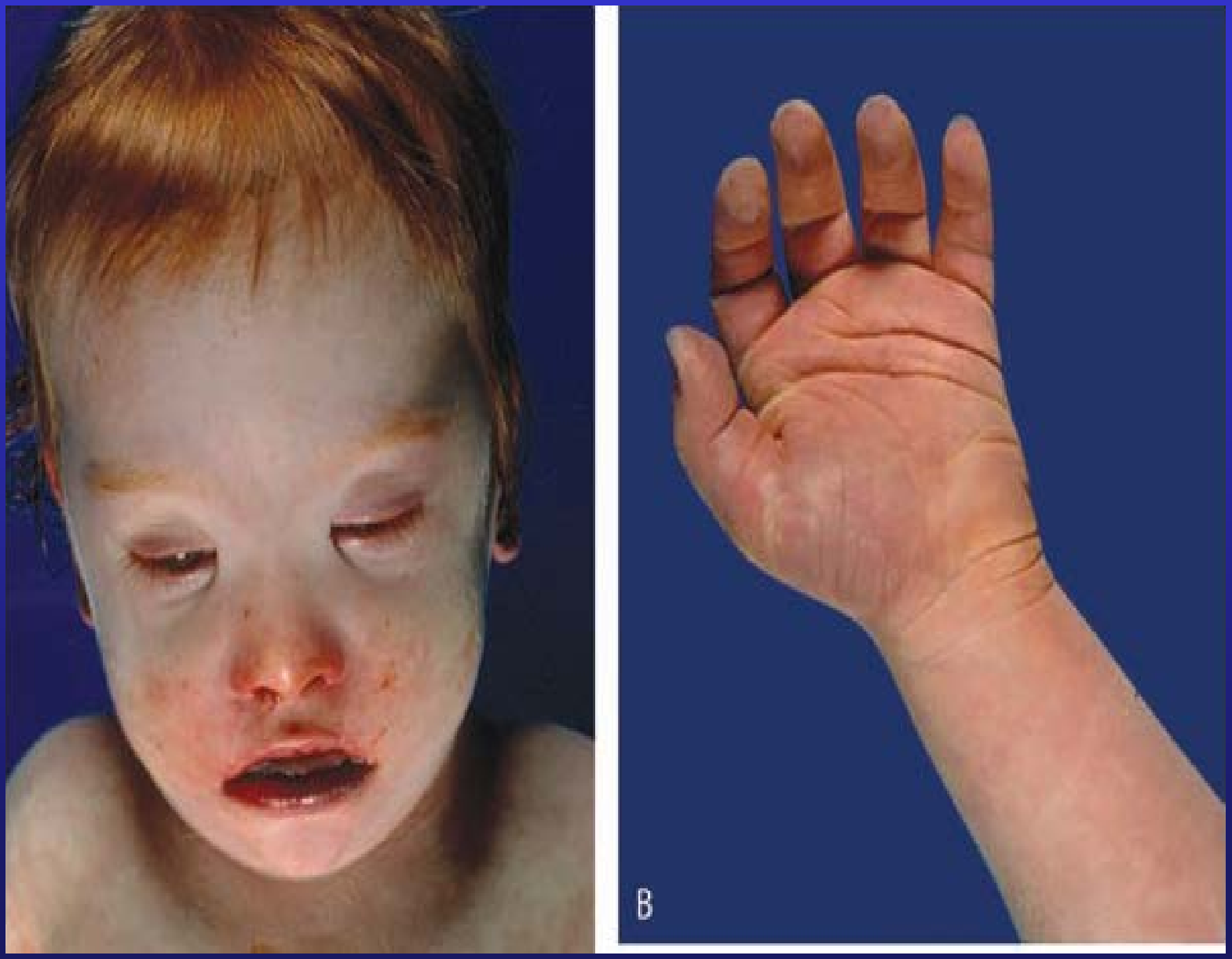

Parents
14
21
21
14
Translocation carrier
Normal
Gametes
Offspring
Normal
Translocation carrier
Trisomy 14 Not viable
Trisomy 21 Down syndrome

Down's Syndrome

- 1 in 600 births
- Short, broad nose
- Epicanthal fold
- Small oral cavity
- Large, furrowed tongue
- Large, irregular teeth
- IQs from 20 to 50



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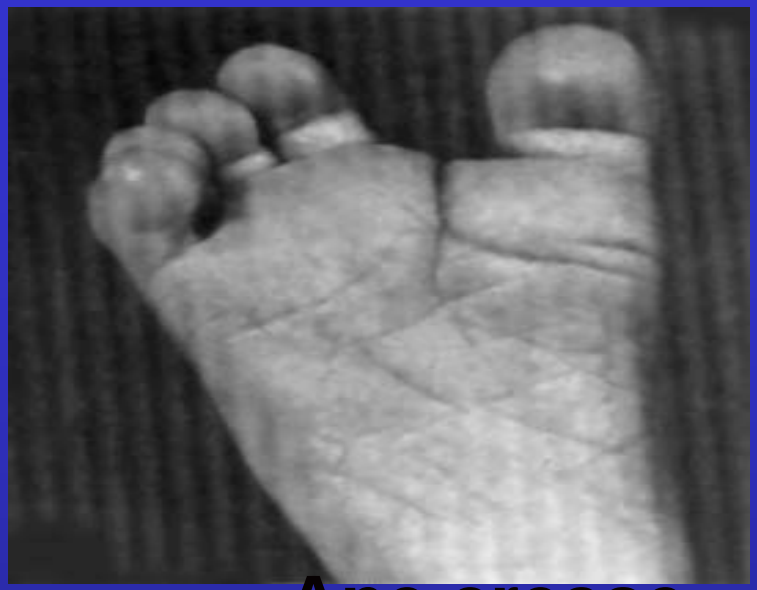
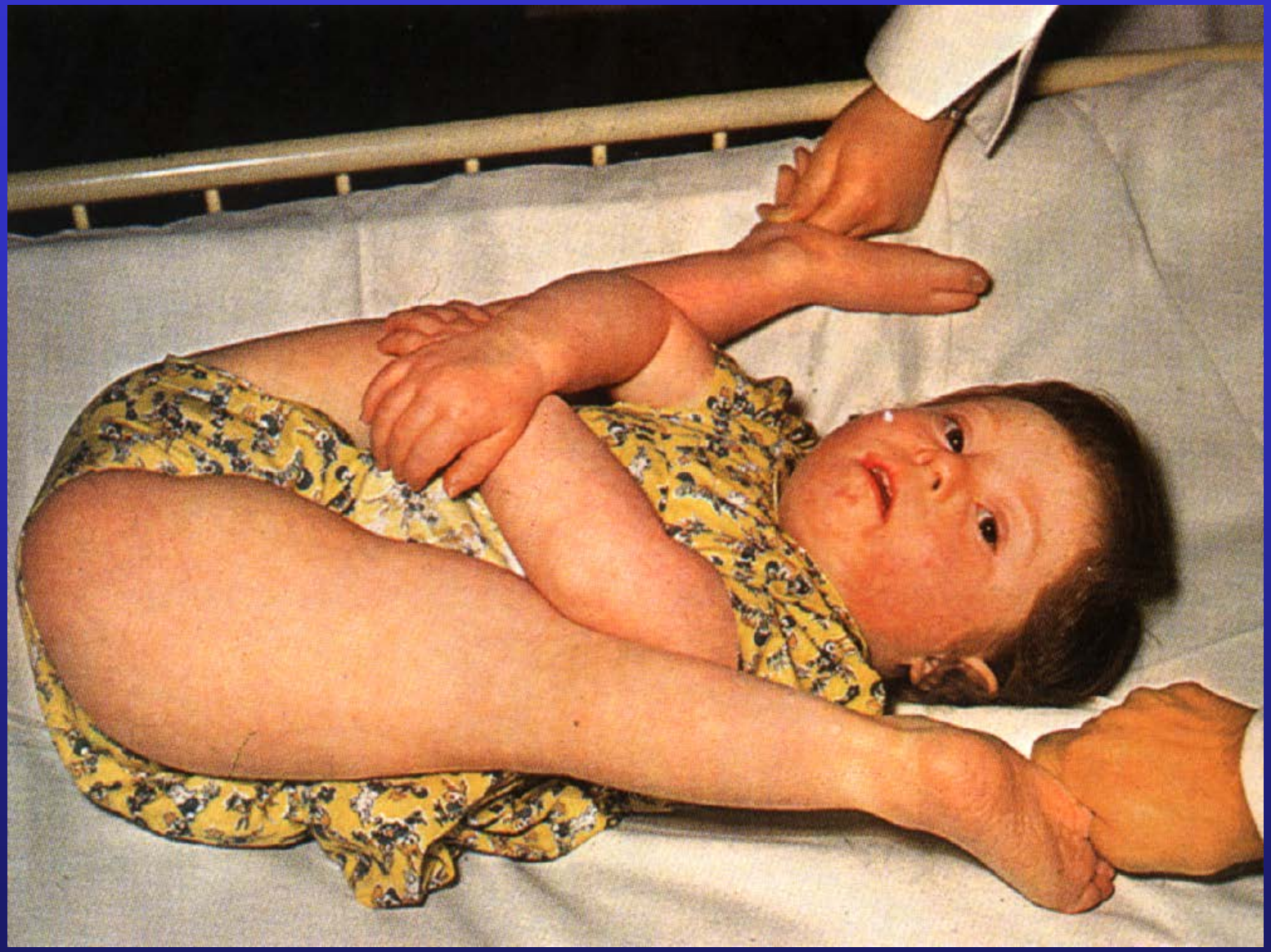
Simian crease



Ape crease

Scrotal tongue



Hypotonia: acrobat sign

Congenital Heart Disease in DS

- Atrioventricular Septal Defect
  - AVSD, AV-canal, ECD
  - 70% of all AVSD's in DS
  - 60% of CHD in DS
  - 2.8% of CHD in non-DS
  - AVSD constitutes spectrum of defects
- Atrial Septal Defect
- Ventricular Septal Defect
- Tetralogy of Fallot
- Patent Ductus Arteriosus

Influence of Down syndrome on Congenital Heart Disease

- DS **not** associated with left heart obstructive lesions, muscular VSD's, situs inversus, situs ambiguus, and transposition of the great arteries
- DS **not** associated with premature atherosclerotic heart disease

Complications & Cause of Death:

- **Mental retardation** → accidental trauma
- **Renal anomalies**
- **Acute leukemia (***20 times more common***)**
- **Congenital heart disease leads to:**
  - recurrent heart failure.
  - recurrent chest infection
- **Congenital GIT anomalies:**
  - Dudenal atresia
  - Hirschsprung disease.
  - Imperforate anus
- **Endocrinal :**
  - Hypothyroidism
  - Diabetes mellitus
  - Addison disease.
- **Immunodeficiency:**
  → recurrent infections in chest, skin, otitis media

Investigations

1- Karyotyping:

- **For the baby to:**
  - **Decide the type of Down syndrome.**
  - **Detect the risk of recurrence.**
- **For the parents if the baby translocation type.**

2- For suspected anomalies e.g.

- **Echocardiography**
- **CBC**
- **Abdominal sonar & X-ray**

3- Prenatal diagnosis:

1- Fetal ultrasound at 15-20 weeks → Nuchal pad thickness ≥ 6mm

2- Triple screen can suspect up to 60% of fetuses with Down:

- Low maternal α feto protein.
- Low unconjugated estriol
- Elevated human chorionic gonadotropin.

3- Karyotyping

for maternal amniotic fluid cells or chorionic villous sample for early diagnosis in suspected cases

Management of Down syndrome

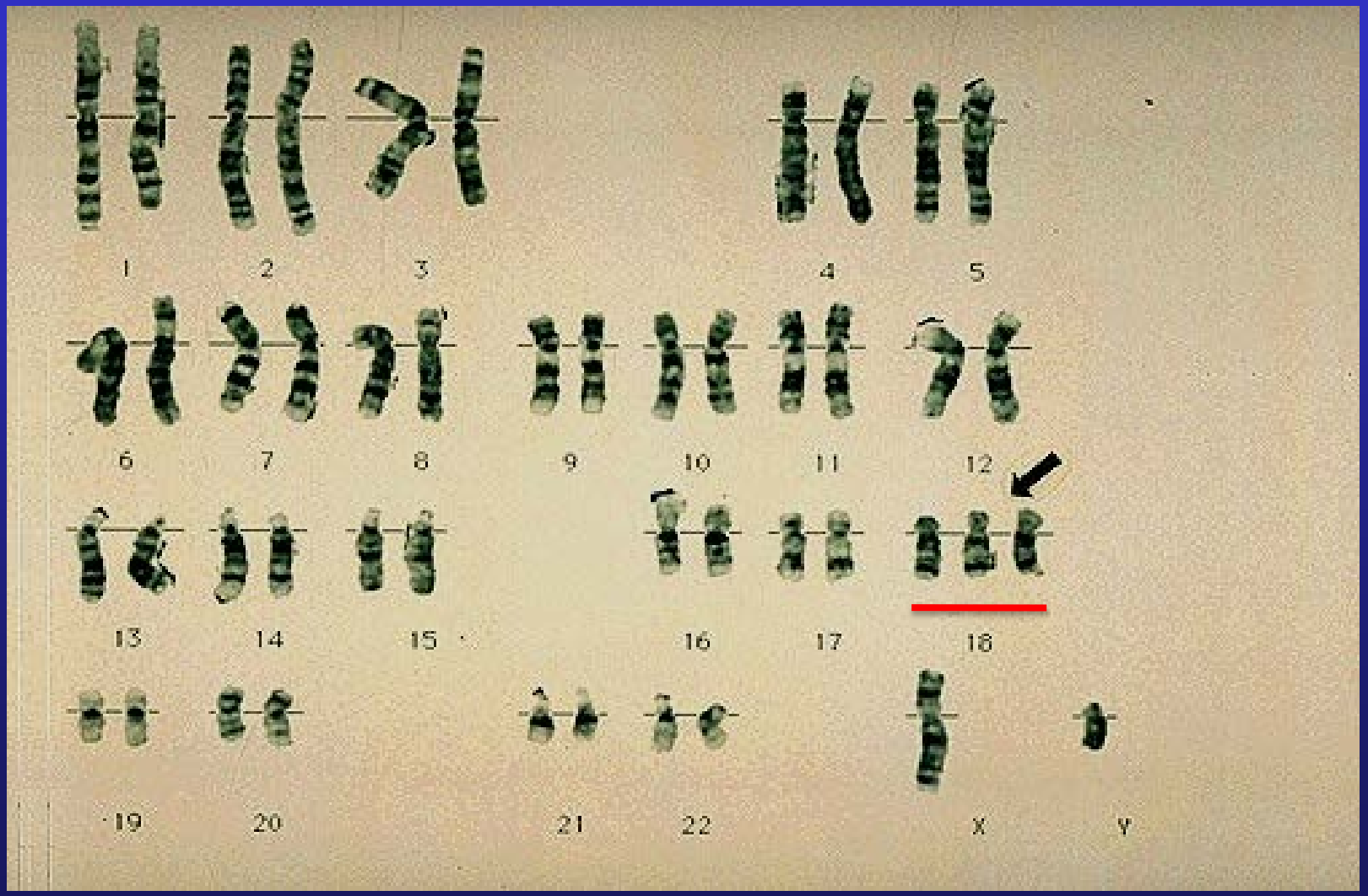
1- Genetic counseling of the parents and education of them about the case and possible progression.

2- General health support e.g :
good nutrition, vaccination, vitamin supply, …

3- Management of complications.

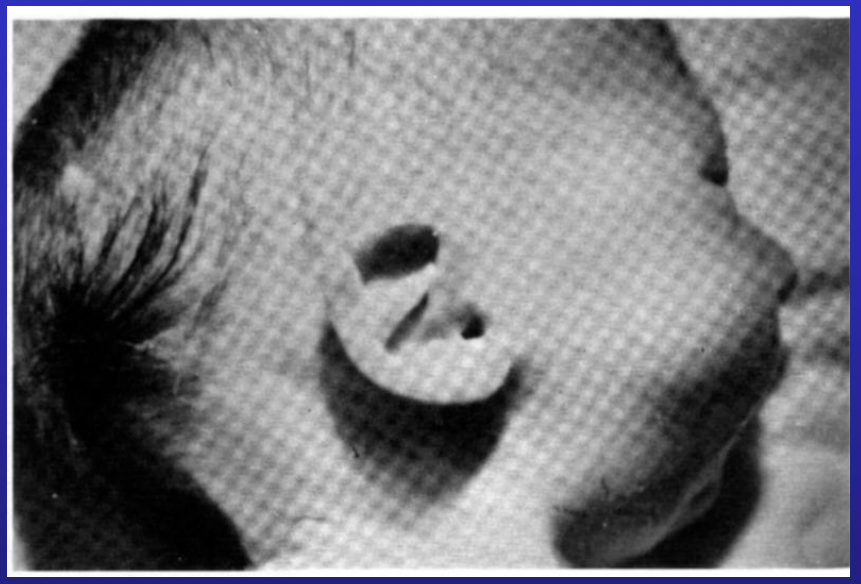
4- Rehabilitation as any case of mental retardation.

Trisomy 18 (Edwards' Syndrome)



Edwards' Syndrome

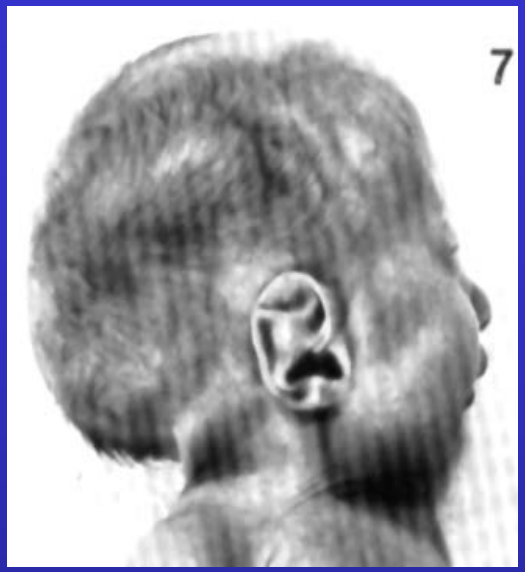
- 1 in 11,000 births
- Small mouth, jaw(Micronathia)
- Low-set, malformed ears
- Clinched fist, index finger overlapping 3rd, 4th fingers
- Rocker-bottom feet
- Heart defects
- Hearing loss
- 90% die by age 1



Edward's Syndrome

Other clinical picture:

- Microcephaly with prominent occiput.
- Mental retardation.
- Hypertonia.
- Congenital anomalies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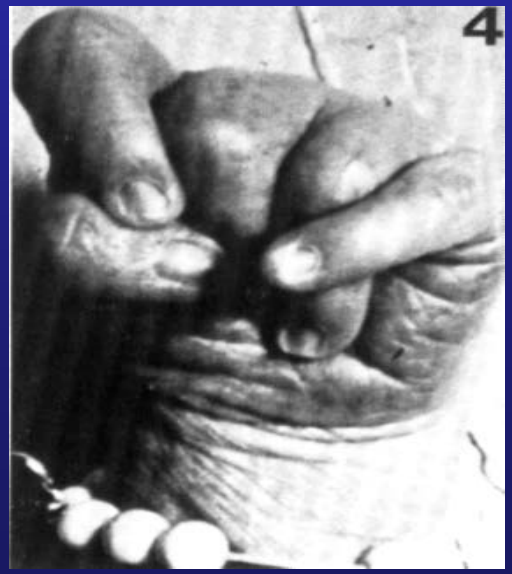
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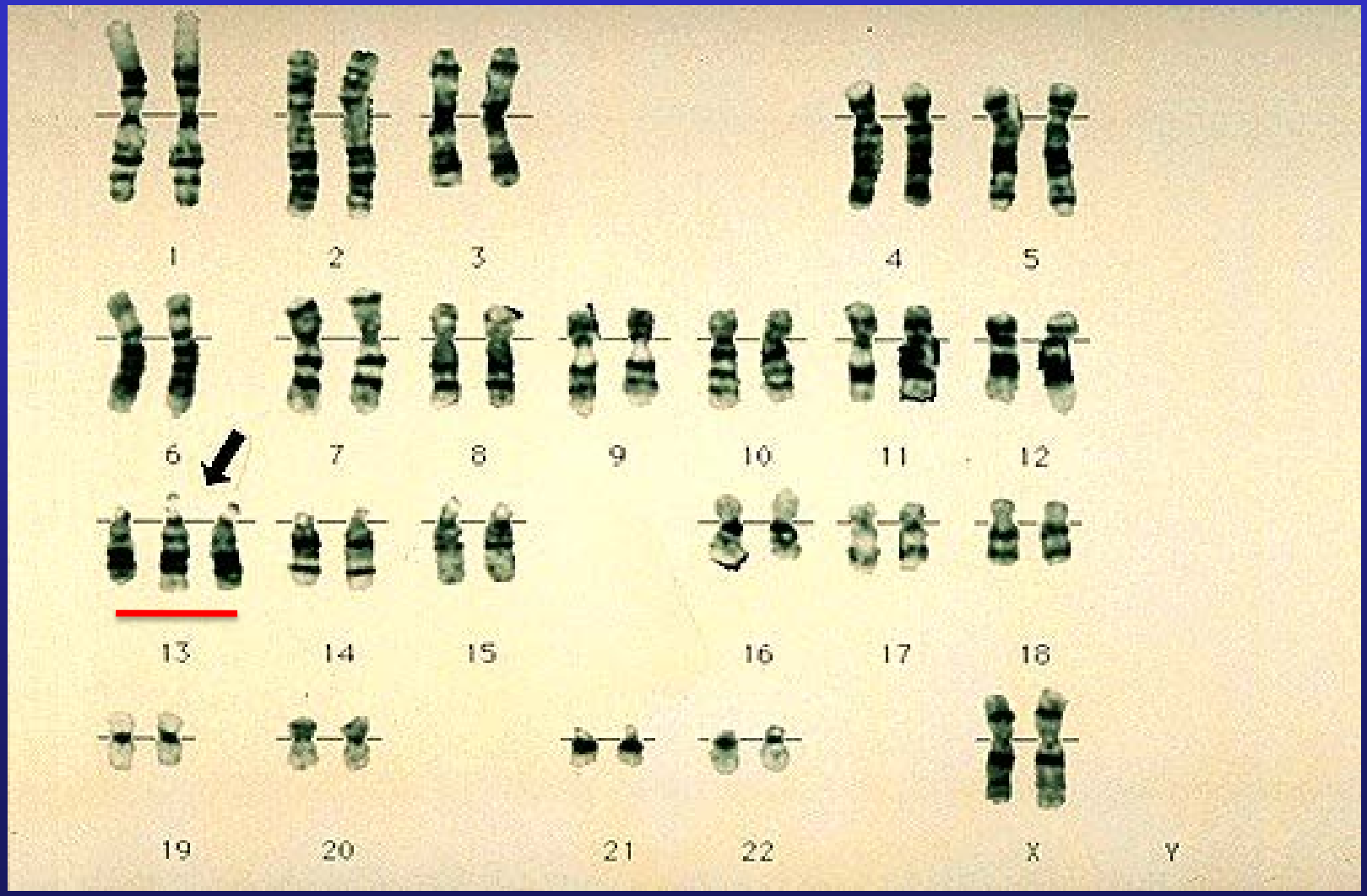
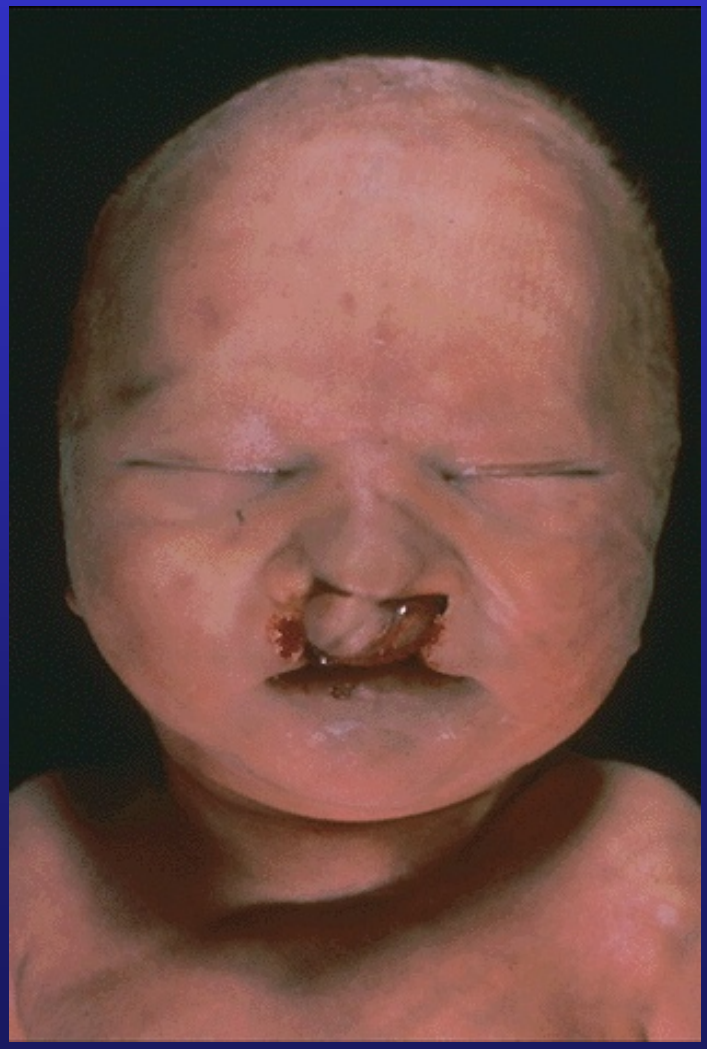
Trisomy 13 (Patau's Syndrome)

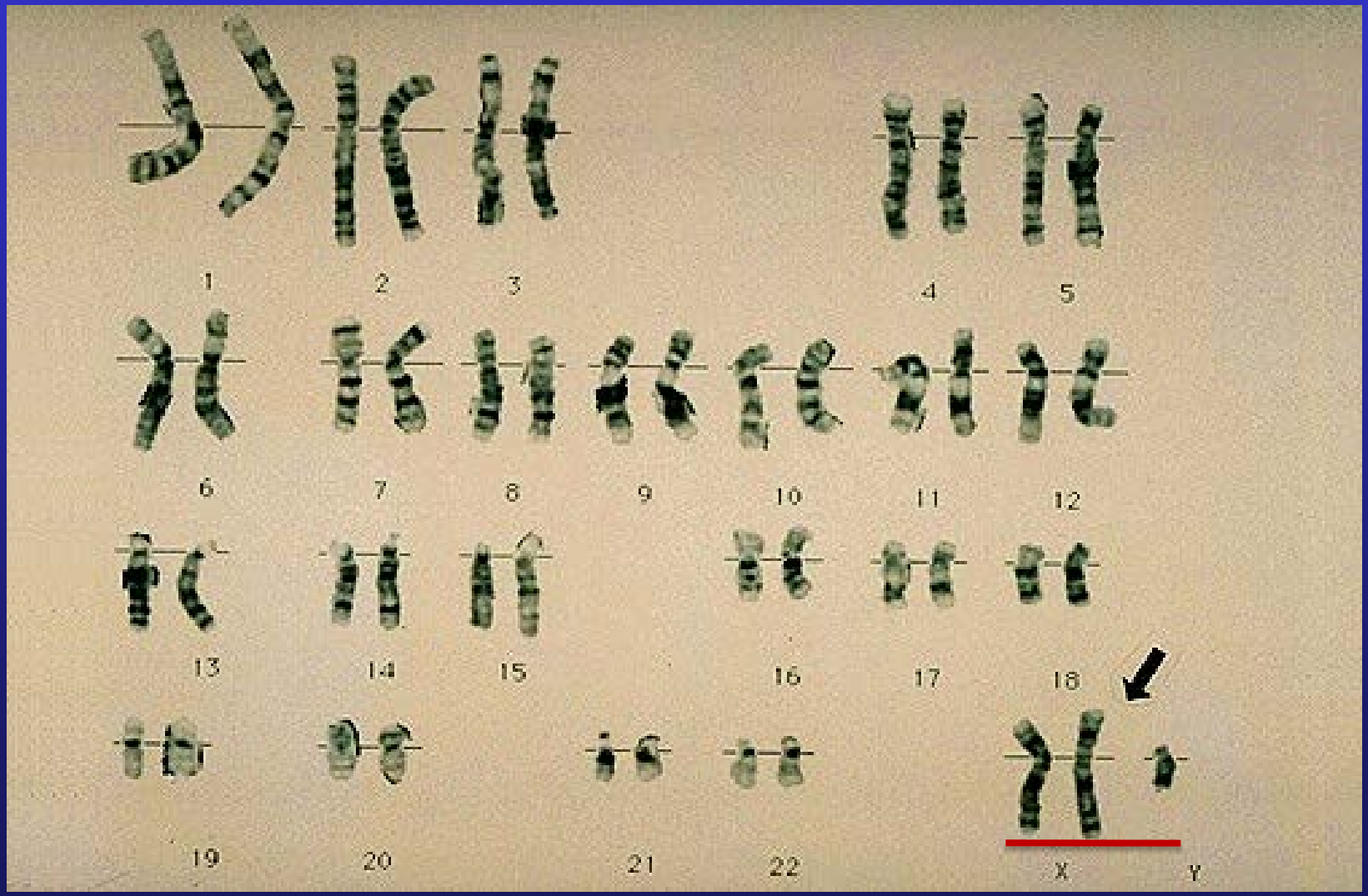


Patau's Syndrome

- 1 in 20,000 births
- Structural defect in brain
- Small head, eyes(Micropthalmia)
- Cleft lip, palate
- Polydactyly
- Heart defects
- 75% die in first year
- 100% by age 6



Kleinfelter's Syndrome



KLEINFELTER SYNDROME

Etiology:

Extra X-chromosome in a male ⇒ (47, XXY) due to non disjunction may be many x-chromosomes e.g 48, XXXY, …..

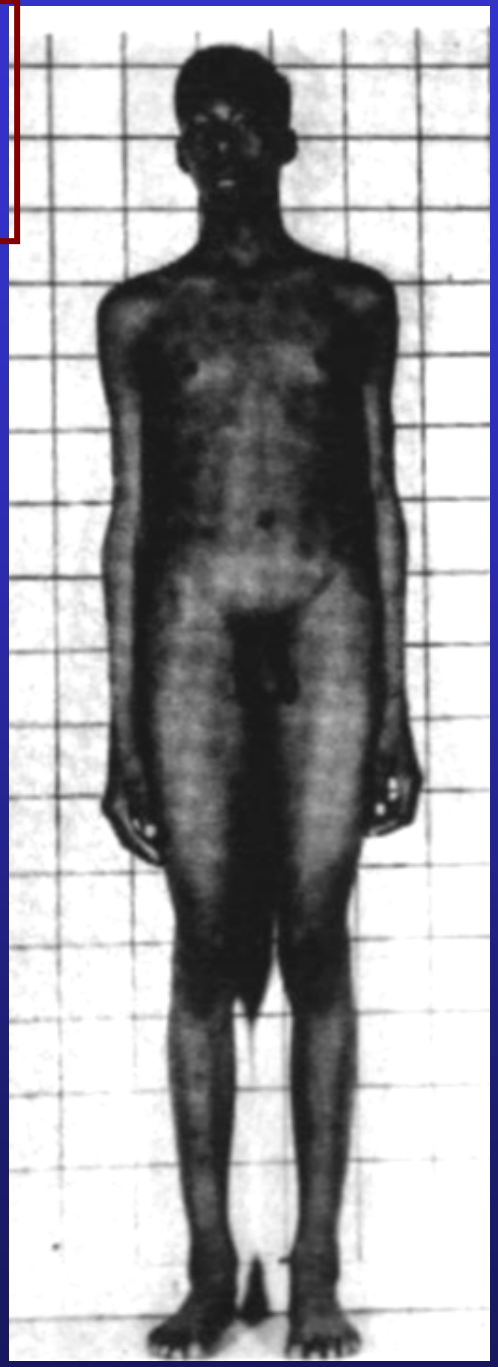
Clinical picture:

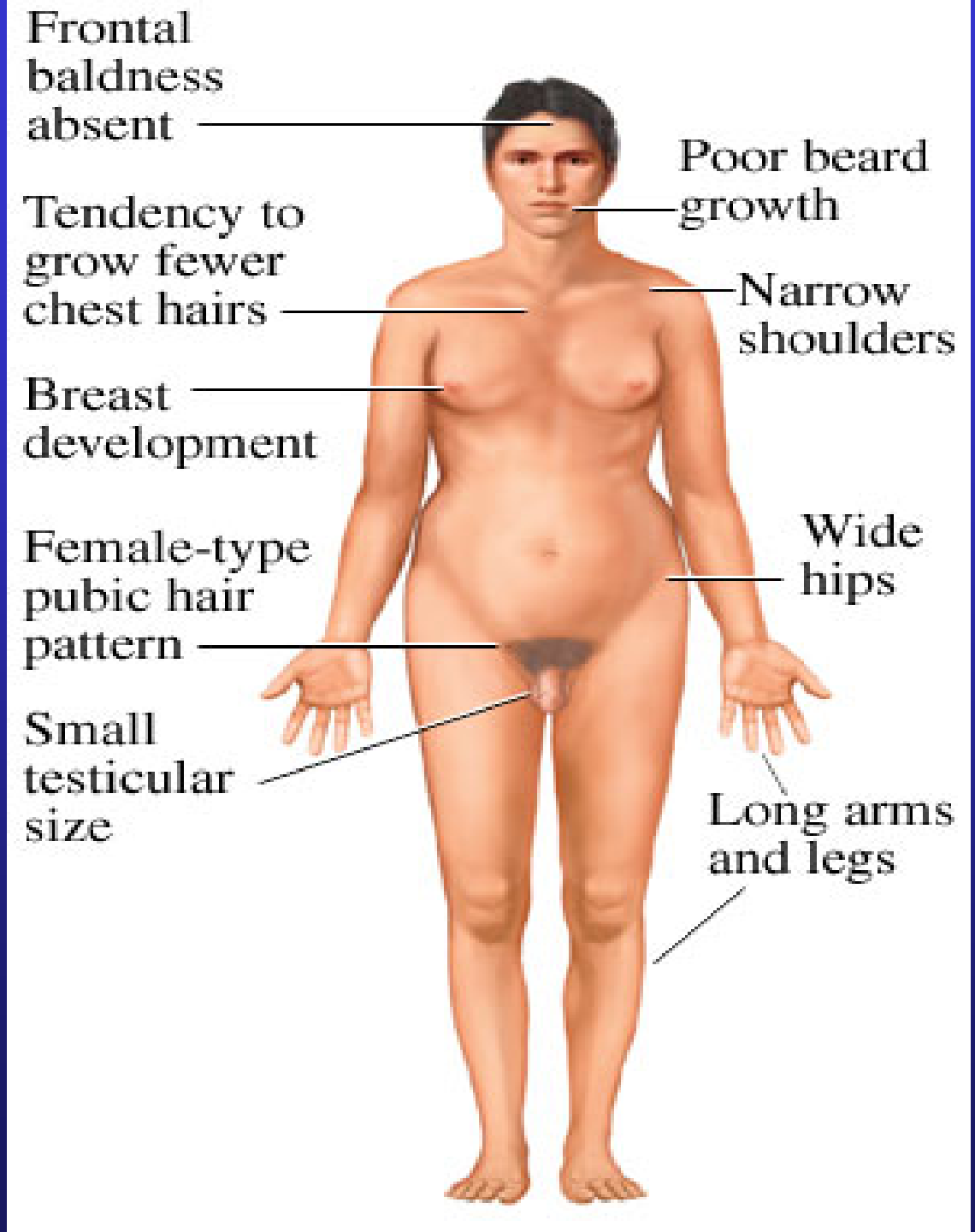
- Mental retardation (more severe with increased number of X chromosomes).
- Gyneacomastia.
- Atrophic testis.
- Tall stature.

- Buccal smear → chromatin (Barr) body +ve

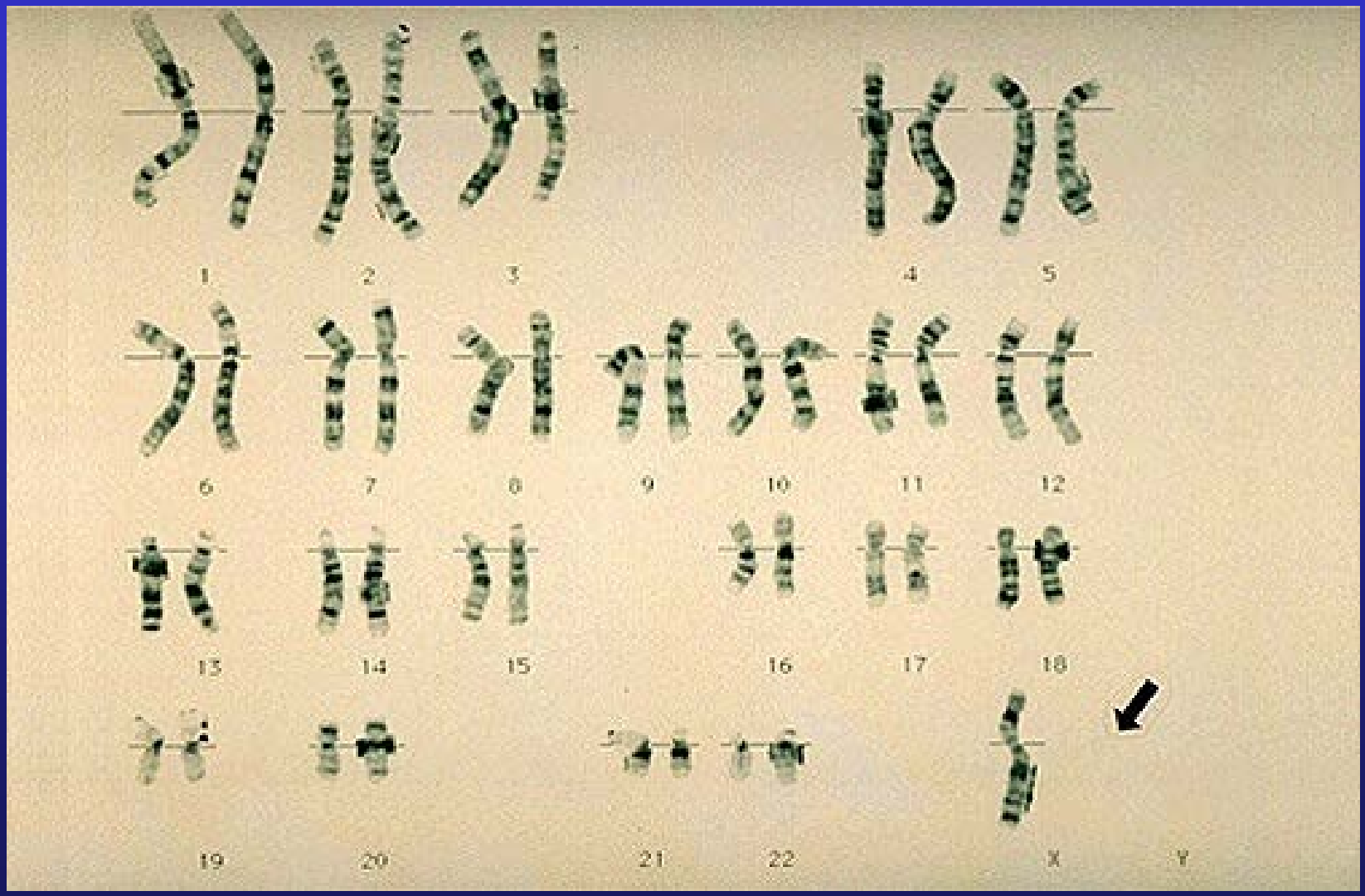
Kleinfelter's Syndrome

- 1 in 500 males
- Taller than average
- Gynaecomastia
- Small testicles(Hypogonadism), high-pitched voice, female hair distribution
- Altered body proportions, hips slightly larger than normal
- IQ about 90(usually in normal range)




Frontal baldness absent
Tendency to grow fewer chest hairs
Breast development
Female-type pubic hair pattern
Small testicular size
Poor beard growth
Narrow shoulders
Wide hips
Long arms and legs

Turner's Syndrome



TURNER SYNDROME

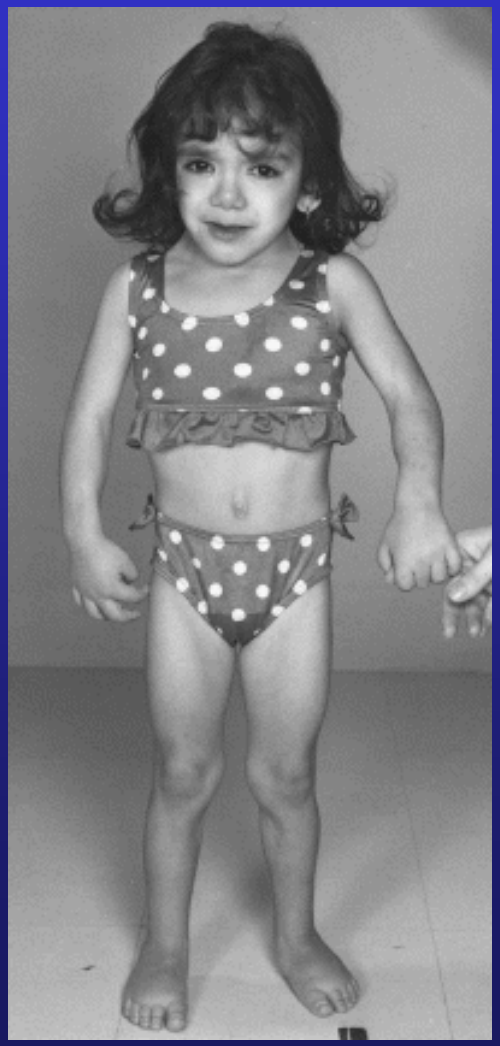
Etiology:

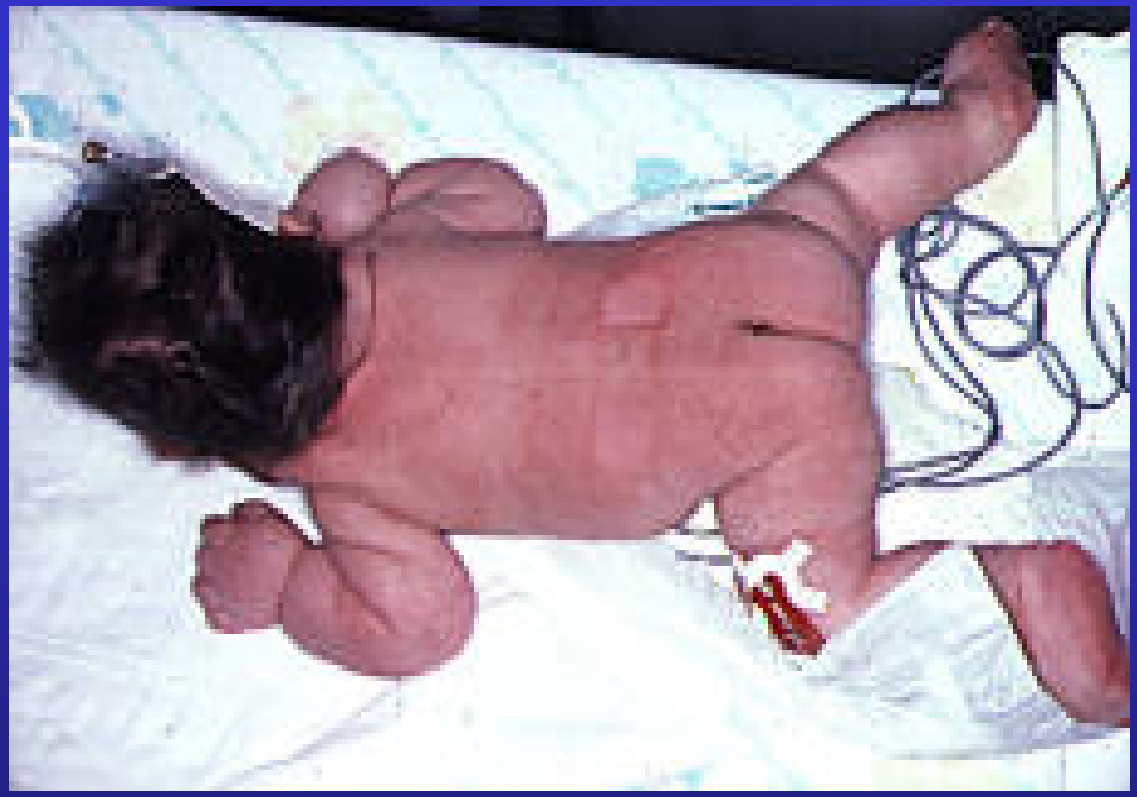
- Classic form (45, X0) ⇒ monosomy X-chromosome
- Turner mosaic: - 45 X0 / 46 X X.
- Deletion of short arm of one X-chromosome.
- with isochromosome 45 X / 46 (X, i)
- with rings 45 X / 46 (X, r)

Incidence: 1 / 5000 (as most cases are aborted)

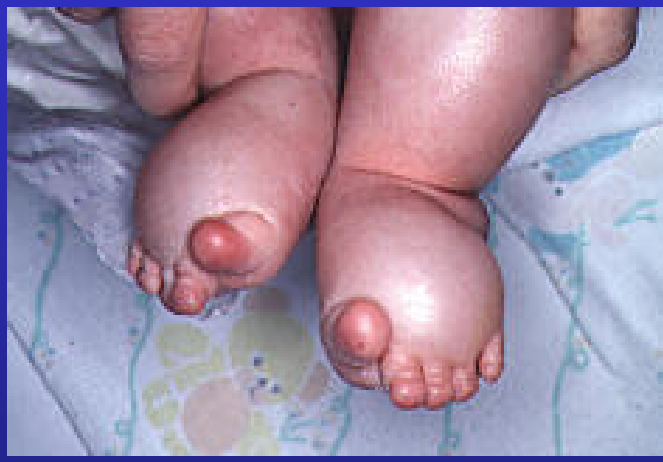
Turner's Syndrome

- 1 in 2500 females
- Lymphoedema of the hands & feet of the neonate
- Short stature
- Shield chest ,Wide carrying angle
- Low hair line,Neck webbing
- Widely spaced nipples
- Streaky Ovary,Infertility
- Normal IQ
- Coarctation of aorta



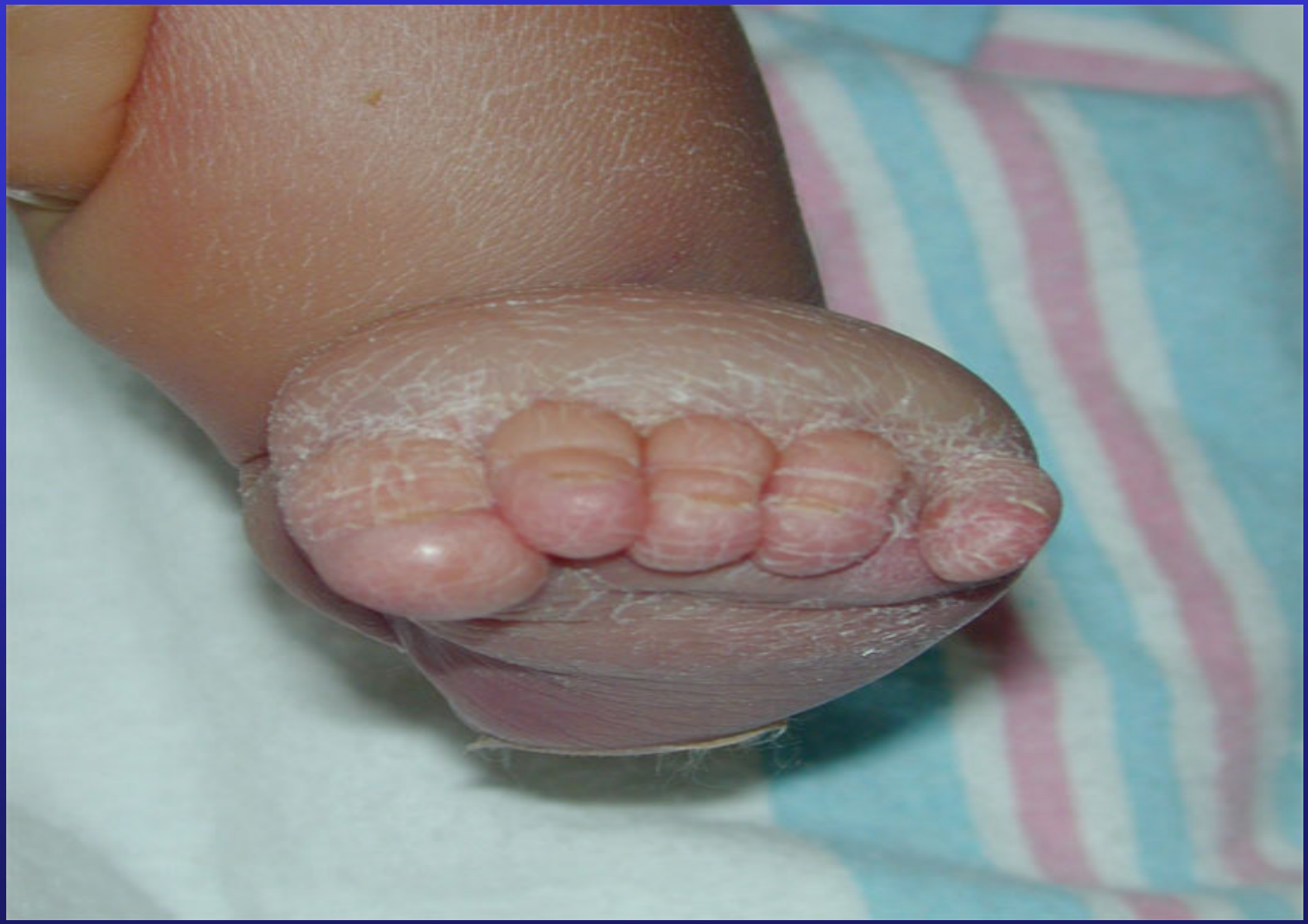


Loose skin at neck nape.



lymphoedema

Turner at birth

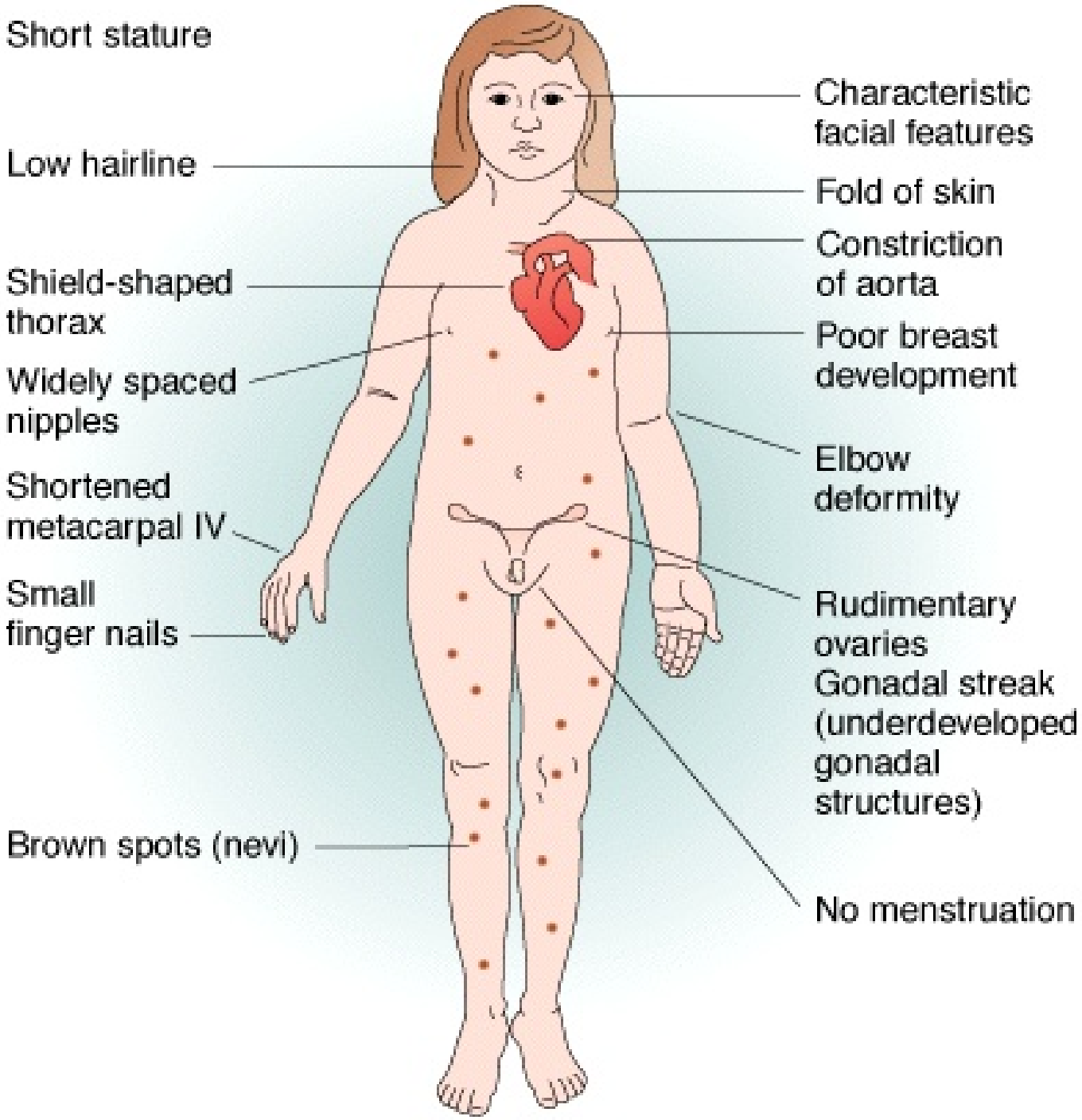


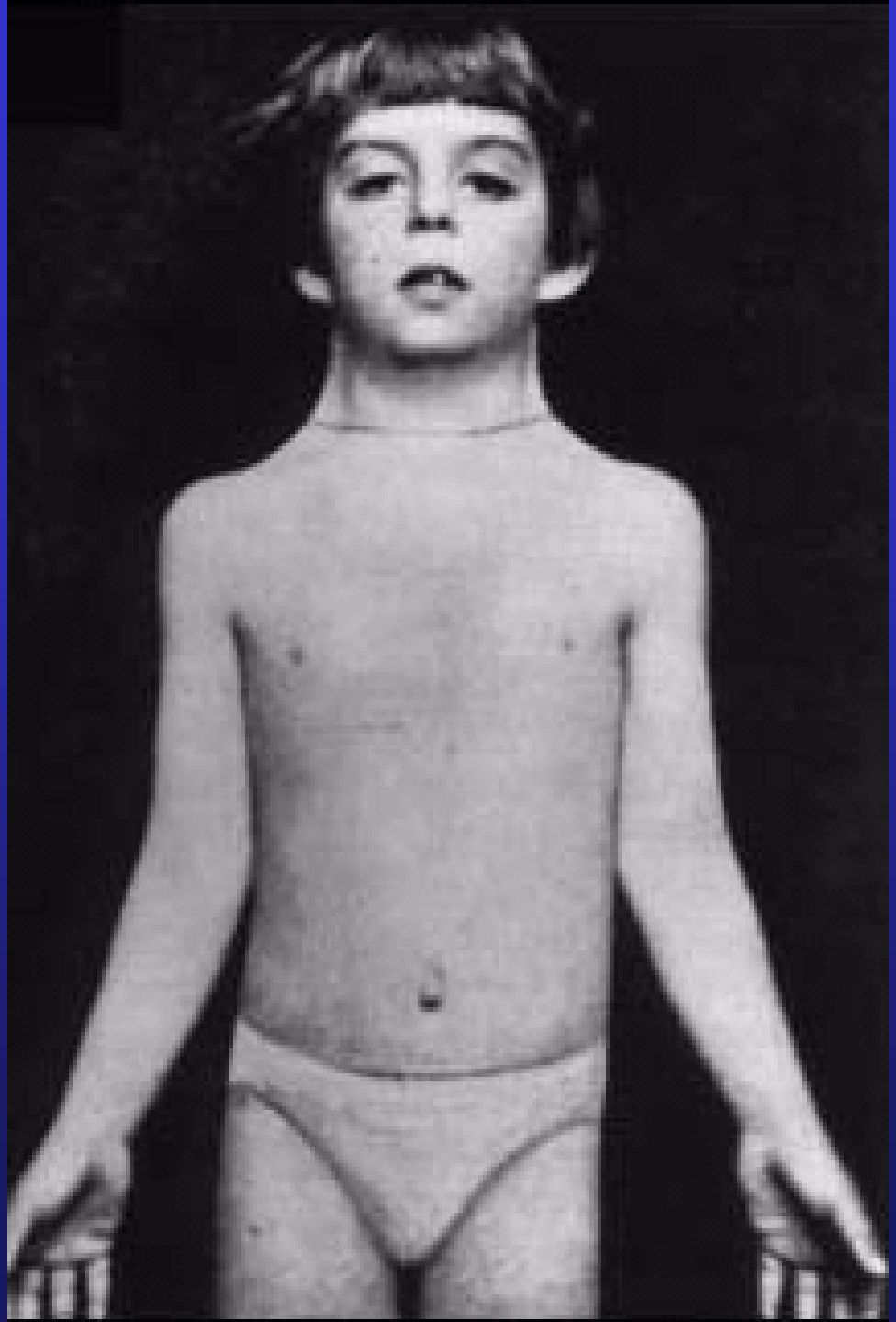
Later on:

- Ugly female.
- Normal mentality
- Low posterior hair line
- Recurrent otitis media
- Webbing of the neck
- Congenital heart disease:

  Aortic coarctation& non stenotic bicusped aortic valve
- Wide spaced nipples
- Cubitus valgus (wide carrying angle)
- Renal anomalies
- Ovarian dysgenesis (streak gonads)
- Short stature; adult height = 140-145 cm

- Buccal smear → chromatin (Barr) body –ve


Short stature
Low hairline
Shield-shaped thorax
Widely spaced nipples
Shortened metacarpal IV
Small finger nails
Brown spots (nevi)
Characteristic facial features
Fold of skin
Constriction of aorta
Poor breast development
Elbow deformity
Rudimentary ovaries
Gonadal streak (underdeveloped gonadal structures)
No menstruation



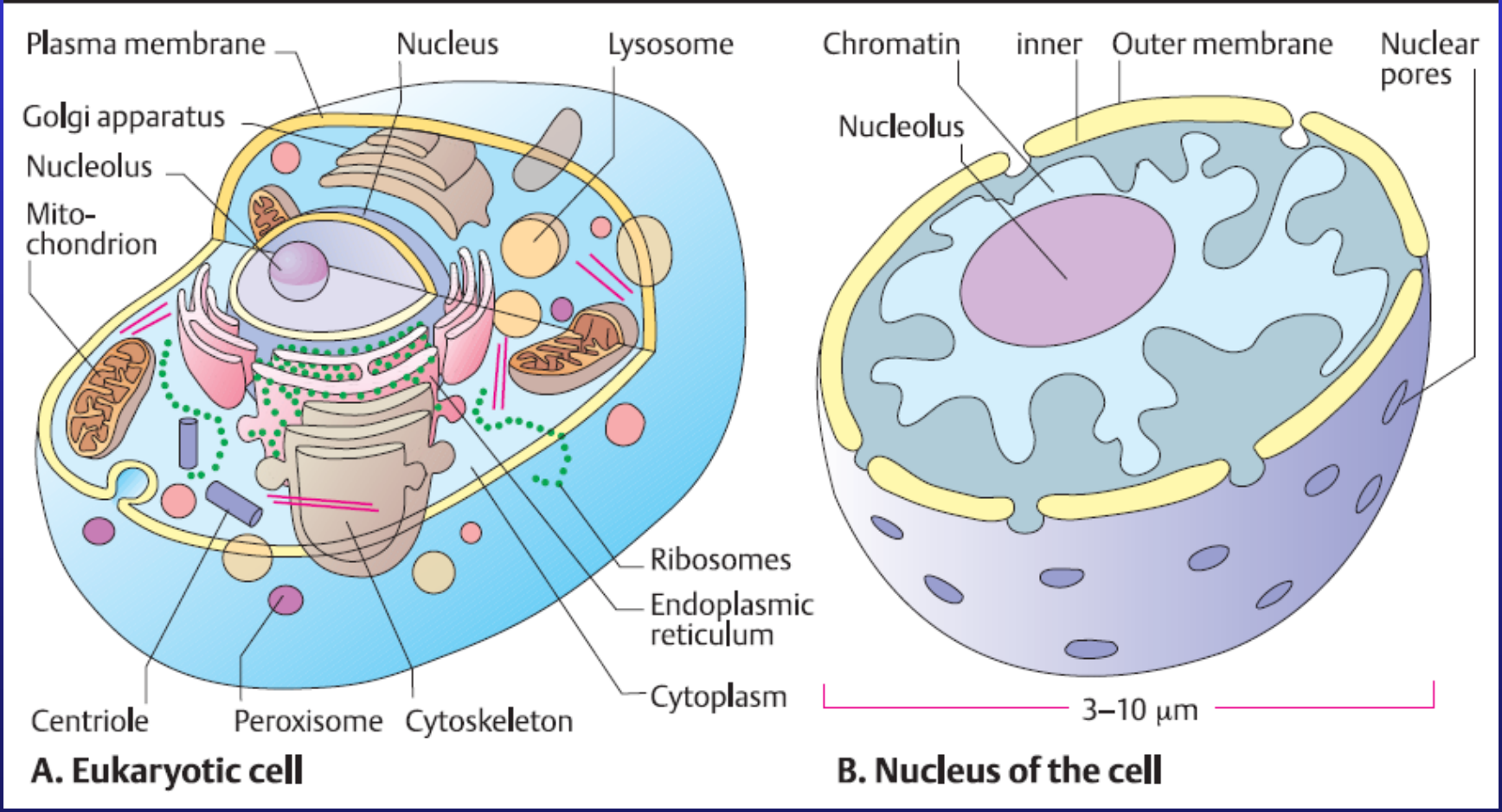
Treatment

- Growth hormone
- Estrogen replacement at 14-15 years

Genetic diagnosis of inherited disorders:

When, why and how to use diagnostic molecular genetic testing methods?

MOLECULAR GENETICS



Mutations

A mutation occurs when an organism develops some strange new characteristic that no other member of the species has had before. For example blue hair

Some mutations are beneficial but most are disastrous.

If a mutation occurs in

a) reproductive cells, the young may develop abnormally or die

b) a body cell the mutant cells may multiply in an uncontrolled way ie cancer

Mutation

- It is the change in the sequence of bases of a gene whether by replacement ,removal or addition of one or more bases
- It is of two types:

1- Non-sense mutation: It is the addition or deletion of nucleotide(a+b):

a- Addition of one base of nucleotid.

b- Deletion of one base of nucleotide

2.Mis-sense mutation:-

c- Subatitution of one base of nucleutide

- Trials for use of chemical agents which may correct this mutation and help in treatment of hereditary disorders.

MUTATION

- Is a faulty DNA or change in the DNA
- Is a change to a gene or several genes
- Is a change in one or more chromosomes
- Starts in the nucleus of one particular cell
- Happens when DNA is not copied properly
- Caused by chemical changes in a gene, or in the DNA , or in a chromosome.

Causes of Mutations

- Mutations can occur naturally
- Nuclear radiation eg alpha beta and gamma radiation (ionising radiation)
- X rays and ultra violet light which are the highest frequency
- Chemicals called mutagens eg carcinogens

The nature of mutation

Coding region:	ACG TGC TAC
Deletion	ACG TAC
Insertion	ACG GGC TGC TAC
Missense	ACG TTC TAC
Nonsense	ACG TGC TAG
Frameshift	ACG TAG C TAC

THE DOG CAN EAT AND RUN

THE DOG CAN TEA TAN DRU N

Applications of genetic testing

- Carrier screening
- Diagnostic genetic testing
- Presymptomatic DNA testing
- Prenatal testing
- Newborn screening

Mutation detection

- Start with PCR

Need:

- designed primers
- dNTP
- MgCl (salt)
- buffer
- DNA polymerase (Taq)
- template DNA

1- PCR steps

Denaturing

Target DNA is separated by heating.

Annealing

A reduction in temperature allows primers to anneal

Extension

The complementary strand is synthesized

PCR pros and cons

Advantages

- Rapidity
- Extreme sensitivity
- Robust
- Specificity
- Inexpensive and simple to perform

Disadvantages

- Contamination
- Need to know sequence in region
- Optimal size of several hundred nt

Detecting point mutations

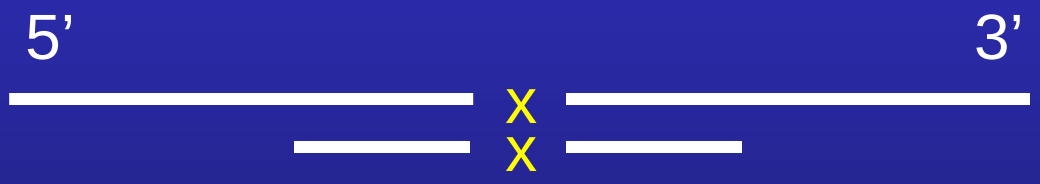
Detection methods: PCR *followed by*:

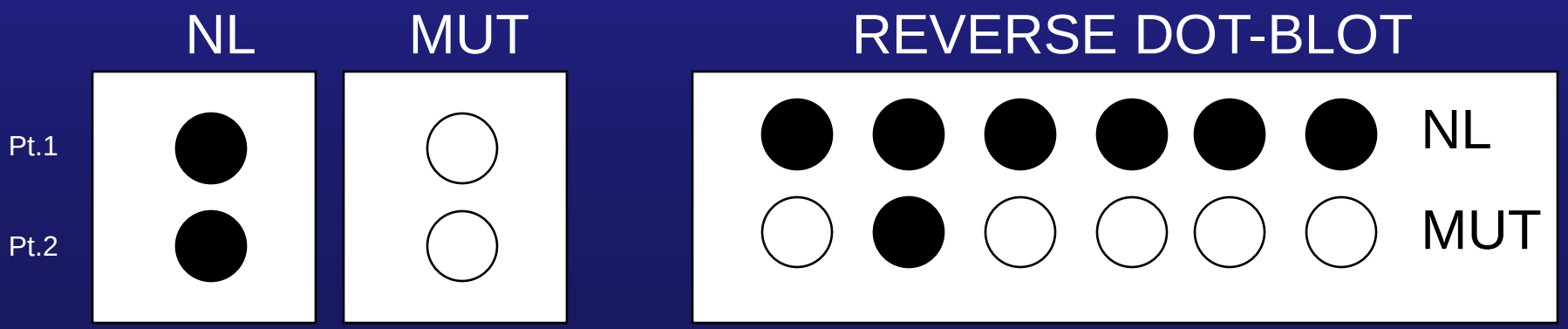
1. ASO (dot blot, reverse dot-blot)
2. OLA (oligonucleotide ligation)
3. Restriction digestion
4. Real-time PCR w/ melting curve analysis
5. Microarray based testing
6. Direct sequencing

1. Principle of ASO

Wild-type sequence with corresponding ASO probe

Mutant sequence with corresponding ASO probe





One patient, many mutations

ASO pros and cons

Advantages

- Multiple mutations at one time
- Sensitive and specific once optimized
- Flexible adaptations

Disadvantages

- One specific sequence detected per probe
- Only suitable to small mutations
- Potential for non-specific results

2. Principle of OLA

- DNA preparation
- Multiplex PCR (16 plex)
- Multiplex OLA with mobility modifiers
- Capillary electrophoretic separation
- Data Analysis

OLA pros and cons

Advantages

- Multiple mutations at one time
- Sensitive and specific
- Detects het and hom
- Suitable for high throughput

Disadvantages

- Expense of sequencer
- Only suitable to small mutations
- Potential for non-specific results

3. Restriction enzyme analysis

- Restriction enzyme analysis requires incubation of a PCR-amplified fragment with an enzyme that is specific for the mutation to be analyzed.
- Specific restriction enzymes recognize unique short sequences within a DNA fragment.
- Enzymes can cleave the DNA strands at that exact site.

Restriction enzyme analysis

- If a mutation either:

  changes the DNA code to a sequence that specifically creates a new restriction enzyme site,

or

  obliterates a normally present recognition sequence,

the mutation can be detected based upon the fragment sizes observed after electrophoresis.

Muenke syndrome

Prominent forehead, wide-set eyes, midfacial flattening, lack of proptosis. Father has macrocephaly.

REA pros and cons

Advantages

- Detects specific mutations
- Can be applied to many samples concurrently, which are then sized

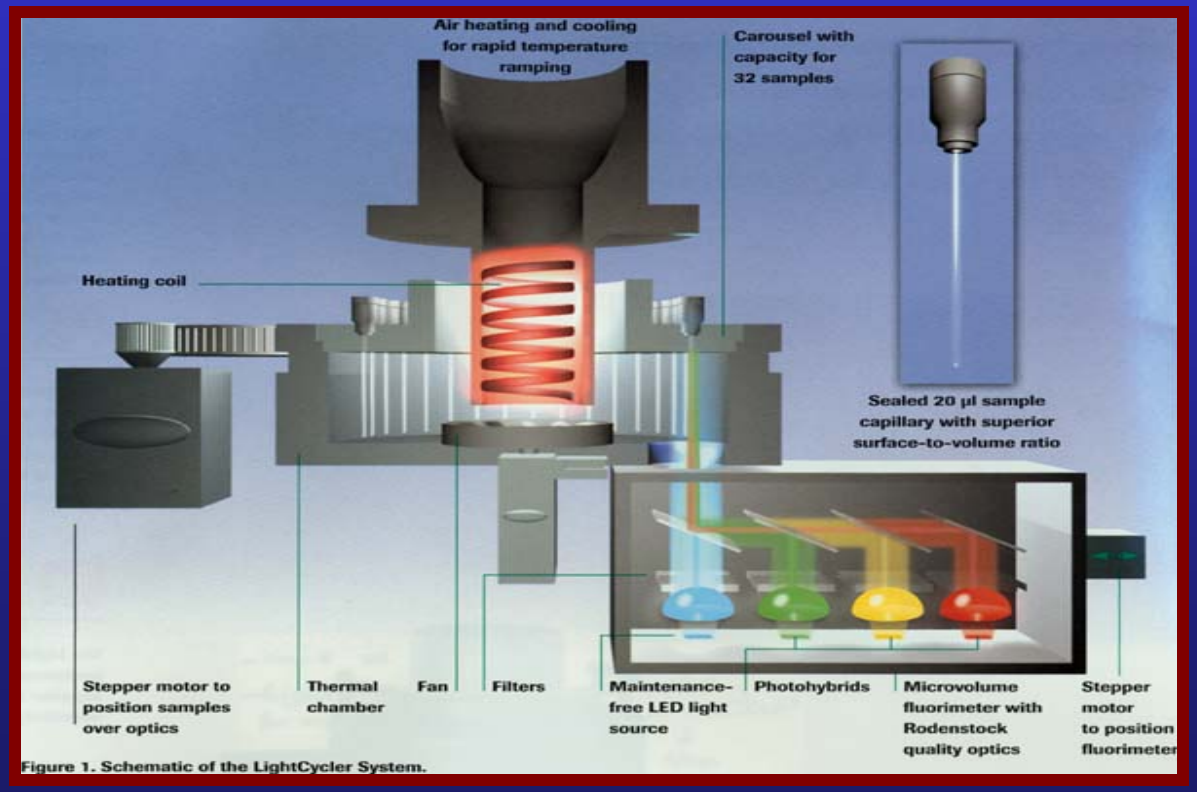
Disadvantages

- Incomplete digestion
- Can be expensive
- Impractical for disorders with multiple mutations
- Lack of natural restriction sites

4. Real-time PCR

- The accumulation of PCR products over time is measured directly, without post-PCR modifications.
- In addition to the two primers that are necessary for PCR, this method applies fluorescent probe(s) that hybridize(s) to the target sequence between the primer pair.
- Due to the exponential nature of the PCR, the fluorescent signal increases proportionally to the amount of generated PCR product.

LightCycler real-time PCR



Real-time PCR

Advantages

- Extreme sensitivity
- Wide dynamic range
- Quantitative
- No post-PCR processing
- High throughput possible

Disadvantages

- Cost of reagents
- Cost of equipment
- Complex to set up

5. Microarrays

- Many different types of arrays are being developed

- Most are currently used in the research setting

Microarray pros and cons

Advantages

- High throughput
- Relatively less hands-on work per sample
- Automated interpretation

Disadvantages

- Expensive
- Not suitable for lower throughput
- Platform issues

5. DNA sequencing

- The most direct approach to mutation detection is sequencing.
- The sequence of interest is amplified in the presence of a primer and dideoxynucleotide chain terminators.
- As the sequence undergoes electrophoresis in an automated sequencer, a laser beam excites the dye(s). The fluorescent signal is computer stored and can be reproduced in the form of an intensity profile.

Sequencing pros and cons

Advantages

- Direct sequencing can precisely characterize the DNA variations present in the target sequence.

Disadvantages

- Expensive
- Quality of the DNA determines quality of sequence
- Detection of a variant does not prove pathogenicity

Mutation screening methods

- Direct DNA sequencing (definitive)
- Heteroduplex analysis (DHPLC)
- Single strand conformation analysis (research)
- Southern blotting

Mutation analysis

- Premutations:

  The size of the trinucleotide repeat can be assessed by PCR or Southern blotting. PCR does not predict methylation status. Southern blotting may miss small premutations.

- Full mutations:

  Very large repeats may be missed by PCR. Southern blotting is preferred.

Summary

- PCR
- ARMS

- ASO
- OLA
- Restriction digestion
- Probe dissociation analysis
- Microarrays

- DNA sequencing
- DHPLC
- Southern blotting

"We would like to be genetically modified to taste like Brussels sprouts."

Good luck

and

THANK YOU