Medical Genetics Course

Lecture 4

Dr. Mohammad Al-Shboul
Email: maalshboul@just.edu.Jo

Chromosome Identification

- Culture (PB, fibroblasts, lymphobalstoid cell lines, Bone marrow, fetal cells)
- Banding (G, Q, R)
- Special procedures (C-banding, high resolution banding, NOR)
- Molecular cytogenetics (e.g., FISH, CGH)

Karyotype

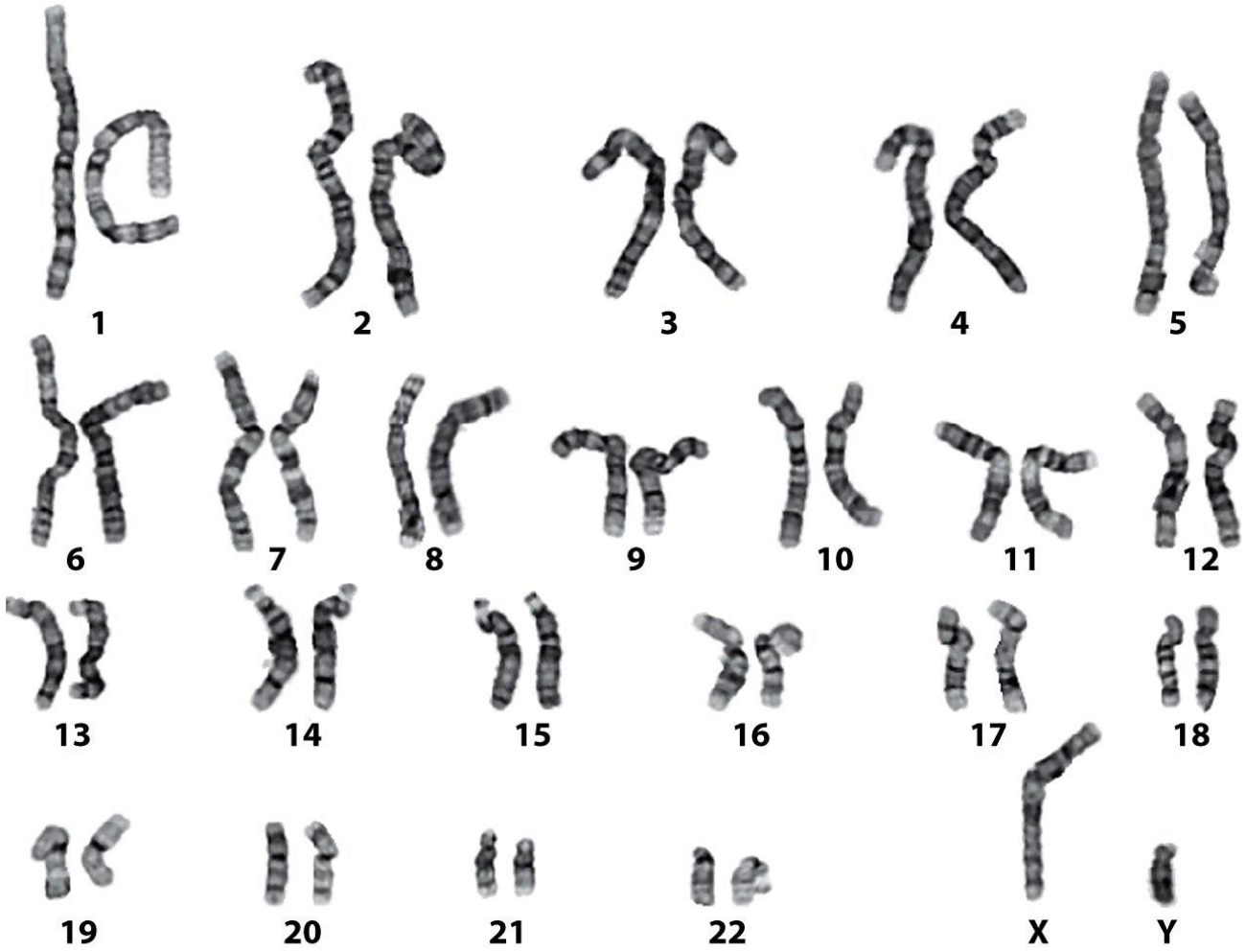


Figure 2.15 Human Molecular Genetics, 4ed. (© Garland Science)

Other Banding Methods

- Q banding
  - Similar pattern to G banding , Needs fluorescent microscope
- R banding
  - Light and dark bands are reversed
- C banding
  - Used to identify centromeres / heterochromatin

Fluorescent In-Situ Hybridization (FISH)

- Was introduced to the clinical cytogenetics laboratories in the late 1980s .
- Based on the ability a single-stranded DNA to anneal with its complementary target
- DNA probe is labeled with a fluorescent dye
- Widely used for clinical diagnostic and there are a number of different types of probes.
  - Deletion, duplications and translocations
- Can be used to detect common deletion syndromes such as Prader–Willi syndrome (microdeletion of 15q11.2) and Williams syndrome (microdeletion of 7q11.2)

MOLECULAR CYTOGENETICS IN DIAGNOSIS OF CHROMOSOME DISORDERS

- FISH allows for the study of genetic aberrations that are too small to visualize by routine cytogenetic studies and too large to detect using standard DNA sequencing.
- Conventional chromosome banding techniques are not sufficient to detect and identify all chromosomal aberrations present in a metaphase (4-5 MB).

Fluorescent In-Situ Hybridization (FISH)

- **Metaphase chromosomes, or interphase cells** are fixed onto a microscope slide, the DNA is denatured and then hybridized with a probe that can be detected by fluorescence. The chromosomes or hybridized regions are observed microscopically (a fluorescent microscope).
- Interphase cells
  - HOW MANY signals are present
  - Usually not where signal is
- Metaphase cells
  - HOW MANY signals are present
  - WHERE

Fluorescent In-Situ Hybridization (FISH)

How does it works?

- Fluorescent probe of a sequence of interest bound to patient DNA.
- In an unaffected person, a probe hybridizes in two places?
- If patient DNA segment present, probe binds to the complementary DNA, fluorescent signal present
- If patient DNA segment missing, no binding with target probe, no fluorescent signal seen.
- So, it provides way to see small DNA segments:
  - Present or absent
  - How many copies

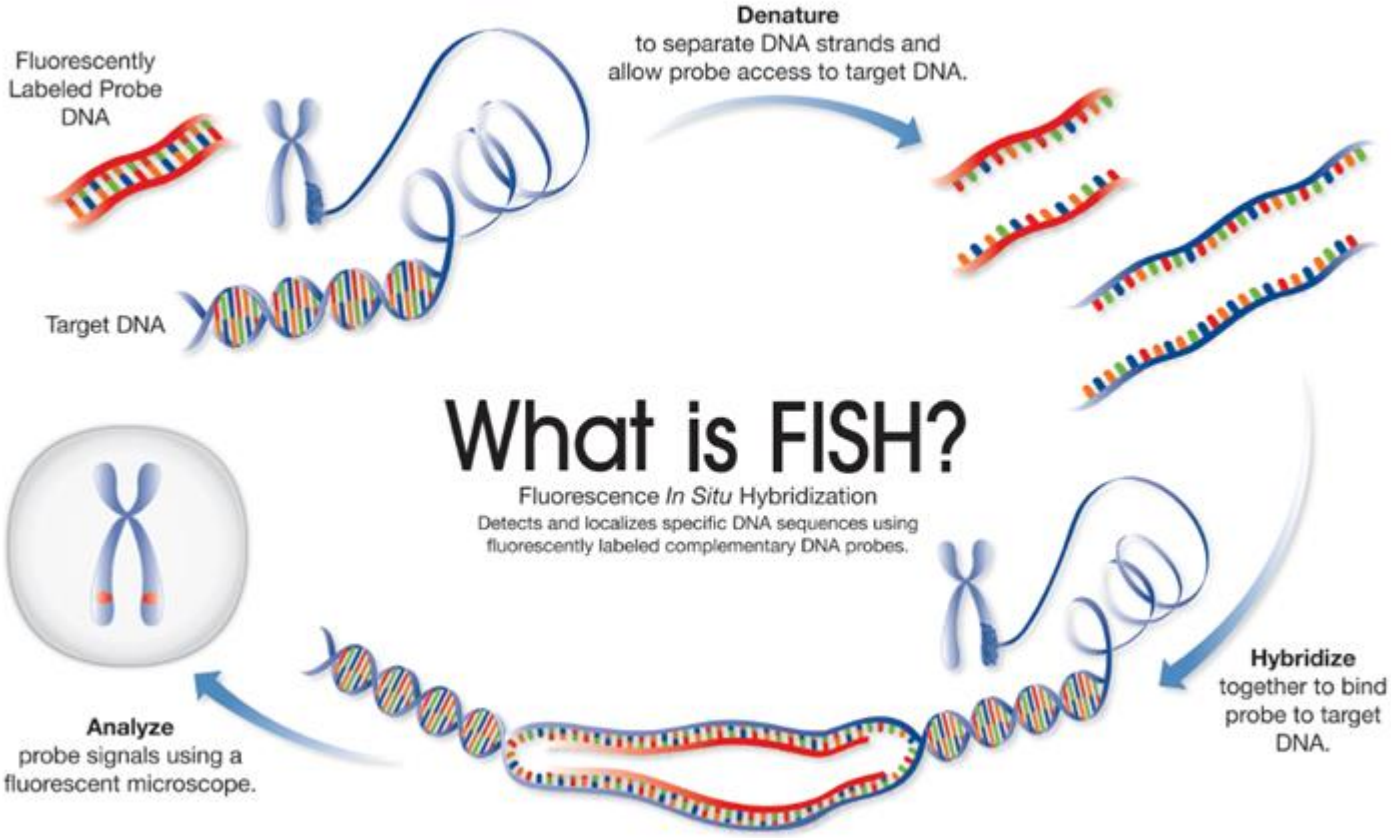


Fig. 17.1 Schematic representation of the basic steps of the FISH procedure. Both the probe and chromosomal target are heat-denatured. Probe sequences hybridize to the complementary target sequences, and nonspecific binding is eliminated via stringent washing. The probe hybridization is detected with fluorescence microscopy

Fluorescent In-Situ Hybridization (FISH)

- Probes used for clinical purposes are commercially manufactured and sold that must be validated by each laboratory.
  - **Most FISH probes fall into one of three categories:**
    - Centromeric probes (CEP) **(**enumeration): are widely used for determining the number of copies of a particular chromosome.
    - Locus specific probes: bind to a particular region of a chromosome.
    - Whole chromosome probes: full-color map of the chromosome is known as a spectral karyotype

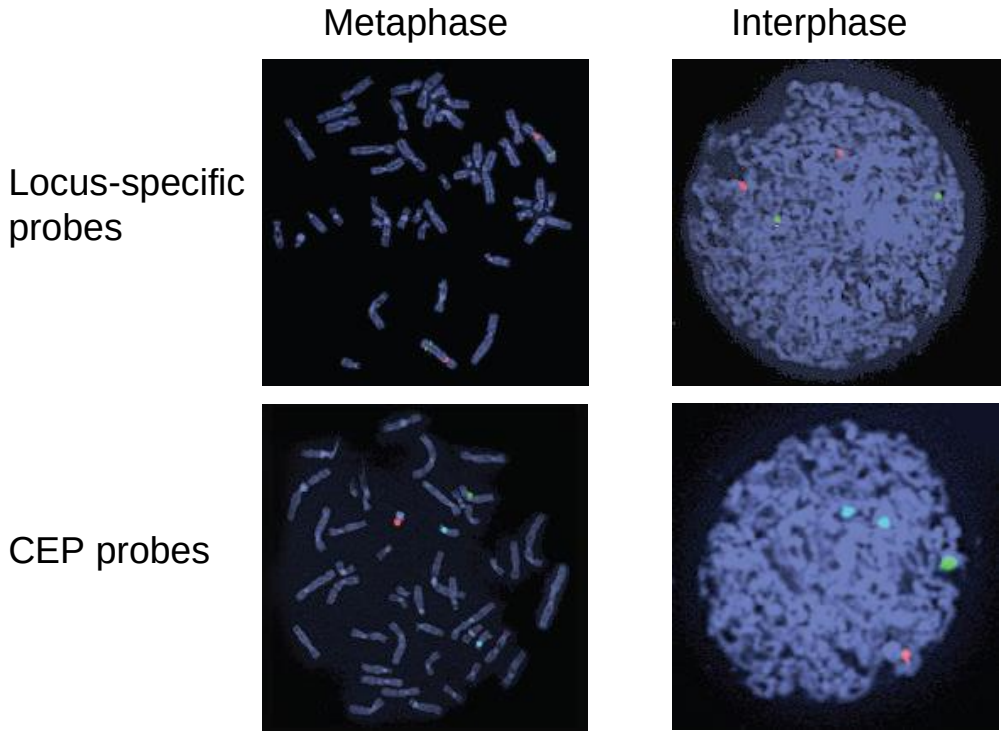
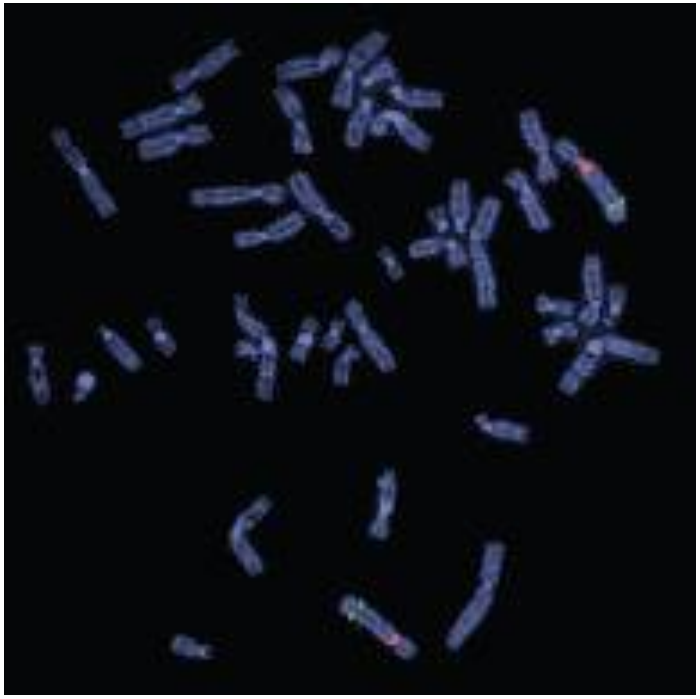
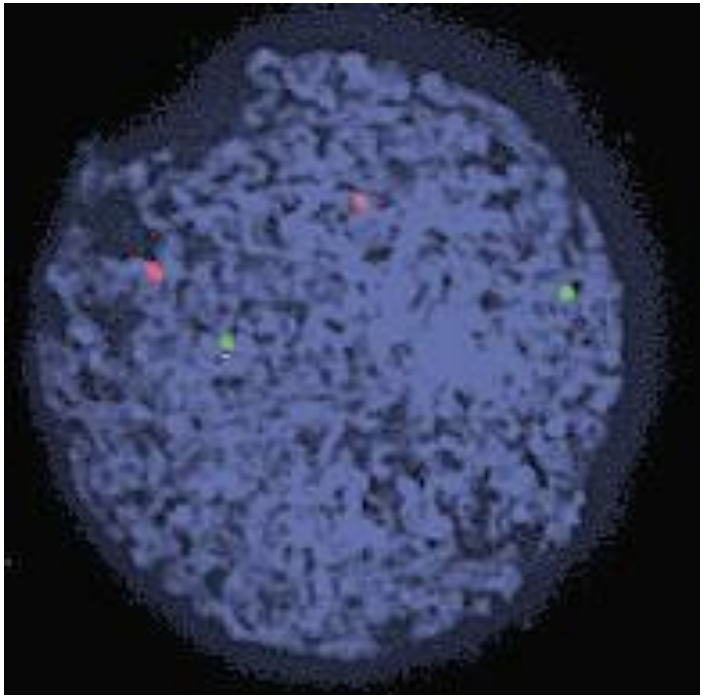
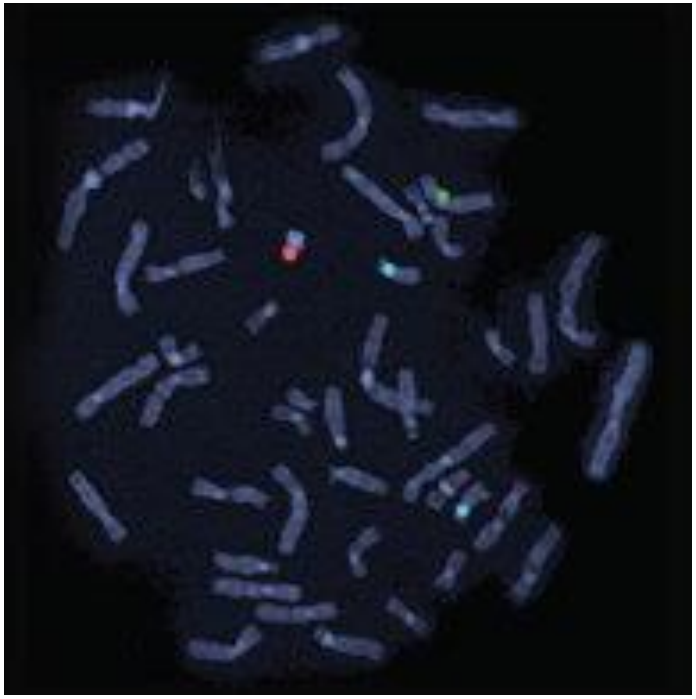
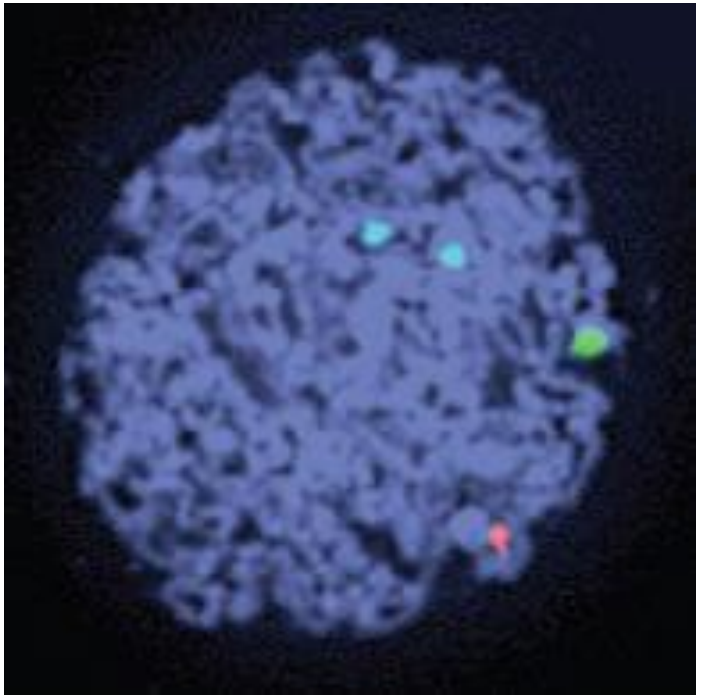


Figure 5-5 **Fluorescence in situ hybridization to human chromosomes at metaphase and interphase, with different types of DNA probe.**
Top, Single-copy DNA probes specific for sequences within bands 4q12 (*red* fluorescence) and 4q31.1 (*green* fluorescence).
Bottom, Repetitive α-satellite DNA probes specific for the centromeres of chromosomes 18 *(aqua),* X *(green),* and Y *(red).*









Spectral karyotyping (SKY)

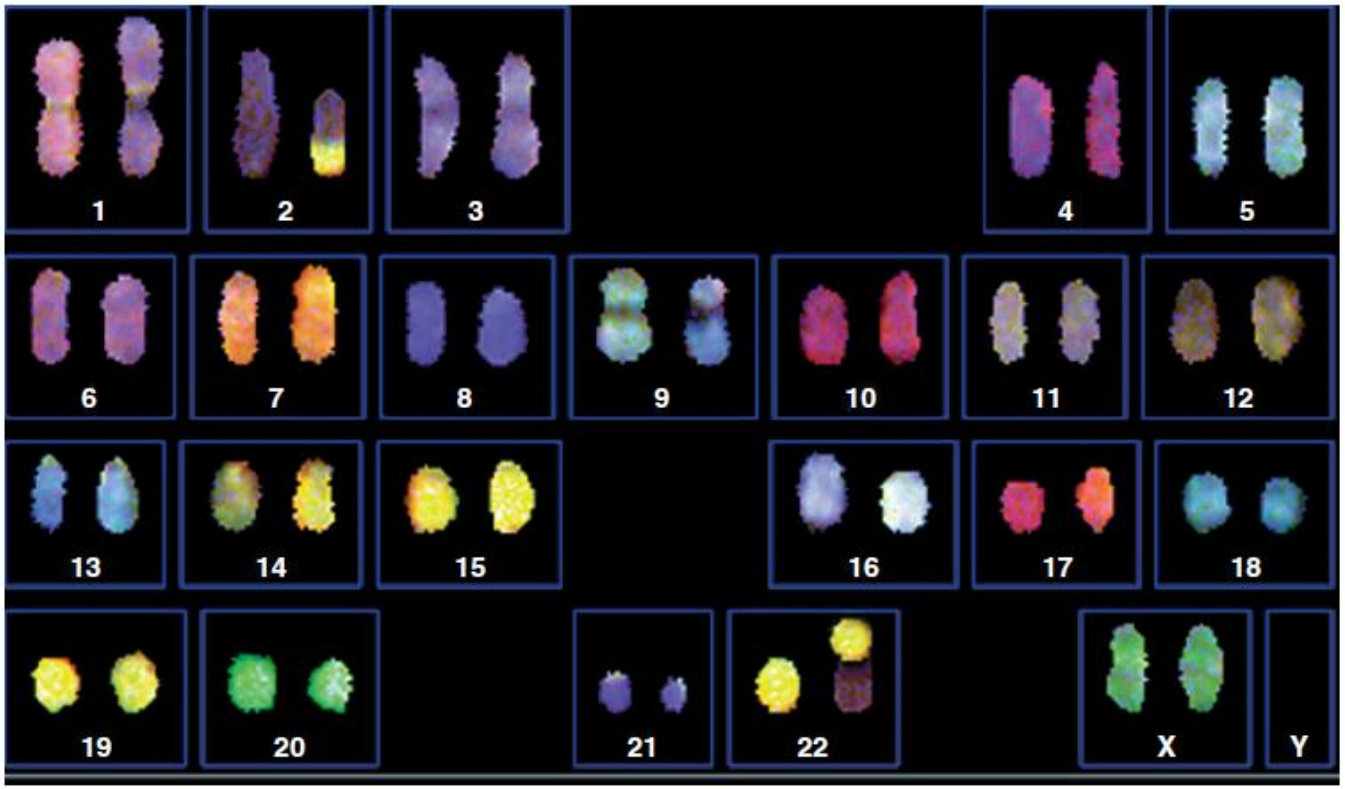


FIG 6-5 Spectral karyotype. An application of spectral karyotyping is demonstrated by the identification of a rearrangement between chromosomes 2 and 22. Note that a portion of chromosome 2 *(purple)* has exchanged places with a portion of chromosome 22 *(yellow).* (Courtesy Dr. Arthur Brothman, University of Utah Health Sciences Center.)

Chromosome Abnormality

1. **Numerical Chromosomal Abnormalities**
2. **Structural Chromosomal Abnormalities**

Numerical

- Aneuploidy
  - Monosomy
  - Trisomy
  - Tetrasomy
- Polyploidy
  - Triploidy
  - Tetraploidy

Structural

- Translocations
  - Reciprocal
  - Robertsonian
- Deletions
- Insertions
- Inversions
  - Paracentric
  - Pericentric
- Rings
- Isochromosomes

- **Different Cell Lines (Mixoploidy)**
- Mosaicism
- Chimerism

Karyotype Report

- **46,XY**
  Normal male
- **46,XX**
  Normal female
- **47,XY,+21**
  Male with extra chromosome no.21 (Trisomy 21) (Down syndrome)
- **47,XX,+13**
  Female with extra chromosome no.13 (Trisomy 13) (Patau syndrome)
- **45,XX, –13**
  Female with missing chromosome no.13 (Monosomy 13)
- **47,XX,+18**
  Female with extra chromosome no.18 (Trisomy 18) (Edward syndrome)

Abnormal Chromosome Number

- In **nondisjunction**, pairs of homologous chromosomes do not separate normally during meiosis
- As a result, one gamete receives two of the same type of chromosome, and another gamete receives no copy

Aneuploidy is caused by nondisjunction

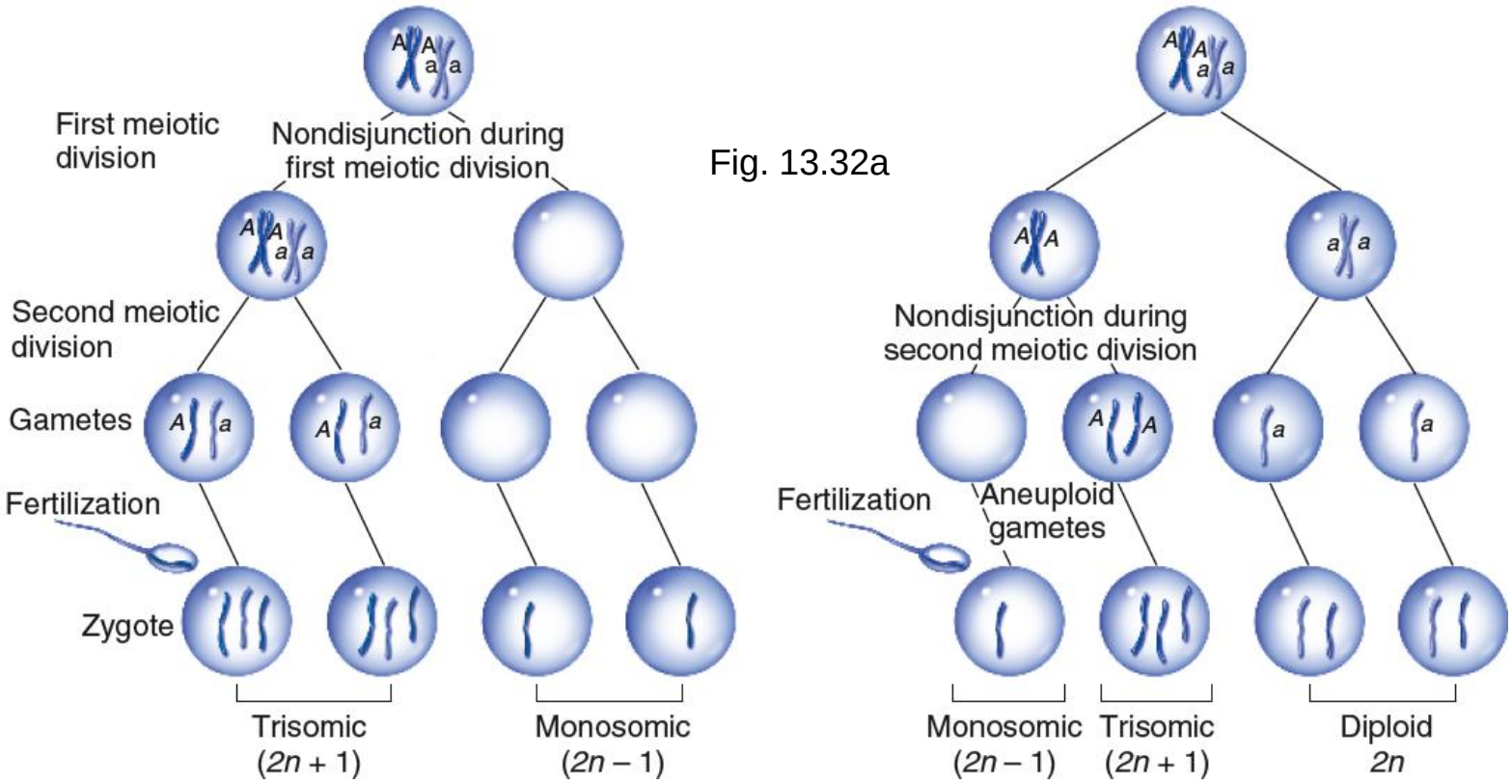


Fig. 13.32a

(a) Nondisjunction of homologous chromosomes in meiosis I

(b) Nondisjunction of sister chromatids in meiosis II

Non-disjunctions usually occur in one of two fashions.

- The first is called **Monosomy**, the second is called **Trisomy**. If an organism has Trisomy 18 it has three chromosomes in the 18th set, Trisomy 21…. Three chromosomes in the 21st set. If an organism has Monosomy 23 it has only one chromosome in the 23rd set.

Aneuploidy is the loss or gain of one or more chromosomes

Aneuploids – individuals whose chromosome number is not an exact multiple of the haploid number (n) for that species

- Monosomic – individuals that lack one chromosome from the normal diploid number (2n – 1)
- Trisomic – individuals that have one chromosome in addition to the normal diploid number (2n + 1)
- Tetrasomic – organisms with four copies of a particular chromosome (2n + 2)

Variation in chromosome number

- **Ploidy**: number of basic chromosome sets (a diploid has 2 sets; a hexaploid has 6 sets)
- **Euploid**: organism have varying number of complete chromosome set
- Most species of animals are **diploid**
- Polyploidies are numerical chromosome abnormalities in which an organism has more than two complete sets of chromosomes
- They are usually incompatible with fetal survival and are extremely rare in liveborns.
- Polyploidy is common in plants, but not animals

TABLE 17-1 Chromosome Constitutions in a Normally Diploid Organism with Three Chromosomes (Identified as A, B, and C) in the Basic Set*

Name	Designation	Constitution	Number of chromosomes
Normal Euploid			
Diploid	$2n$	AA BB CC	6
Aberrant Euploids			
Monoploid	n	A B C	3
Triploid	$3n$	AAA BBB CCC	9
Tetraploid	$4n$	AAAA BBBB CCCC	12
Aneuploids			
Monosomic	$2n-1$	A BB CC	5
		AA B CC	5
		AA BB C	5
Trisomic	$2n+1$	AAA BB CC	7
		AA BBB CC	7
		AA BB CCC	7

*In the case shown, the number of chromosomes in the basic set (the haploid chromosome number) is three.

Polyploidy

- **Triploidy**
  - A chromosomal number that is three times the haploid number, having three copies of all autosomes and three sex chromosomes
    - Found in 15-18% of all miscarriages
    - Approximately 75% of all cases of triploidy are 69,XYY and have two sets of paternal chromosomes
    - Triploid newborns have multiple abnormalities including enlarged head, fused fingers and toes, and malformations of the mouth, eyes, and genitals
- **Tetraploidy**
  - A chromosomal number that is four times the haploid number, having four copies of all autosomes and four sex chromosomes
    - Found in 5% of all miscarriages but is extremely rare in live births
    - Tetraploidy is much rarer than triploidy, both at conception and among live births. It has been recorded in only a few live births, and those infants survived for only a short period.
    - Tetraploidy can be caused by a mitotic failure in the early embryo: all of the duplicated chromosomes migrate to one of the two daughter cells. It can also result from the fusion of two diploid zygotes.

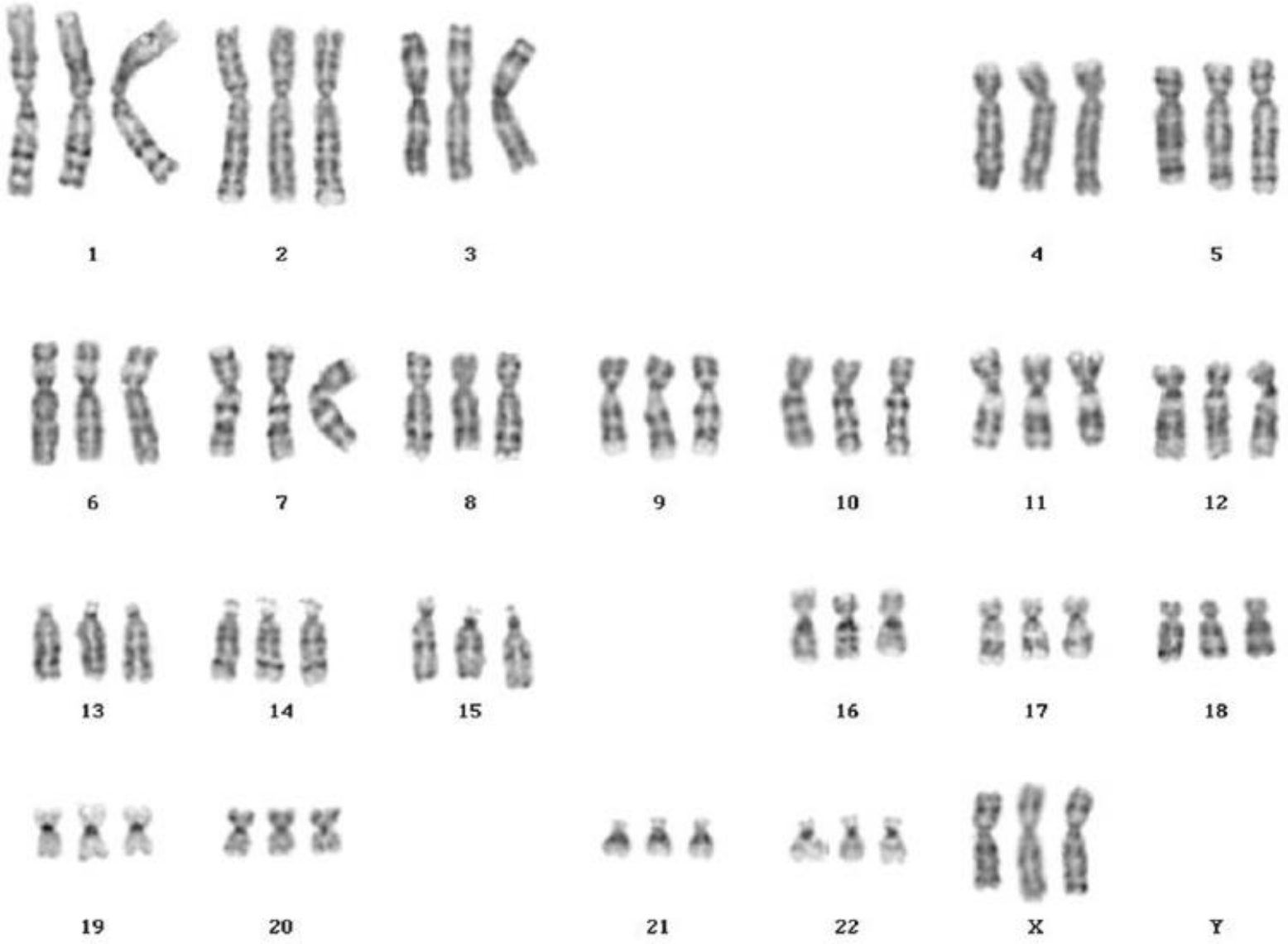
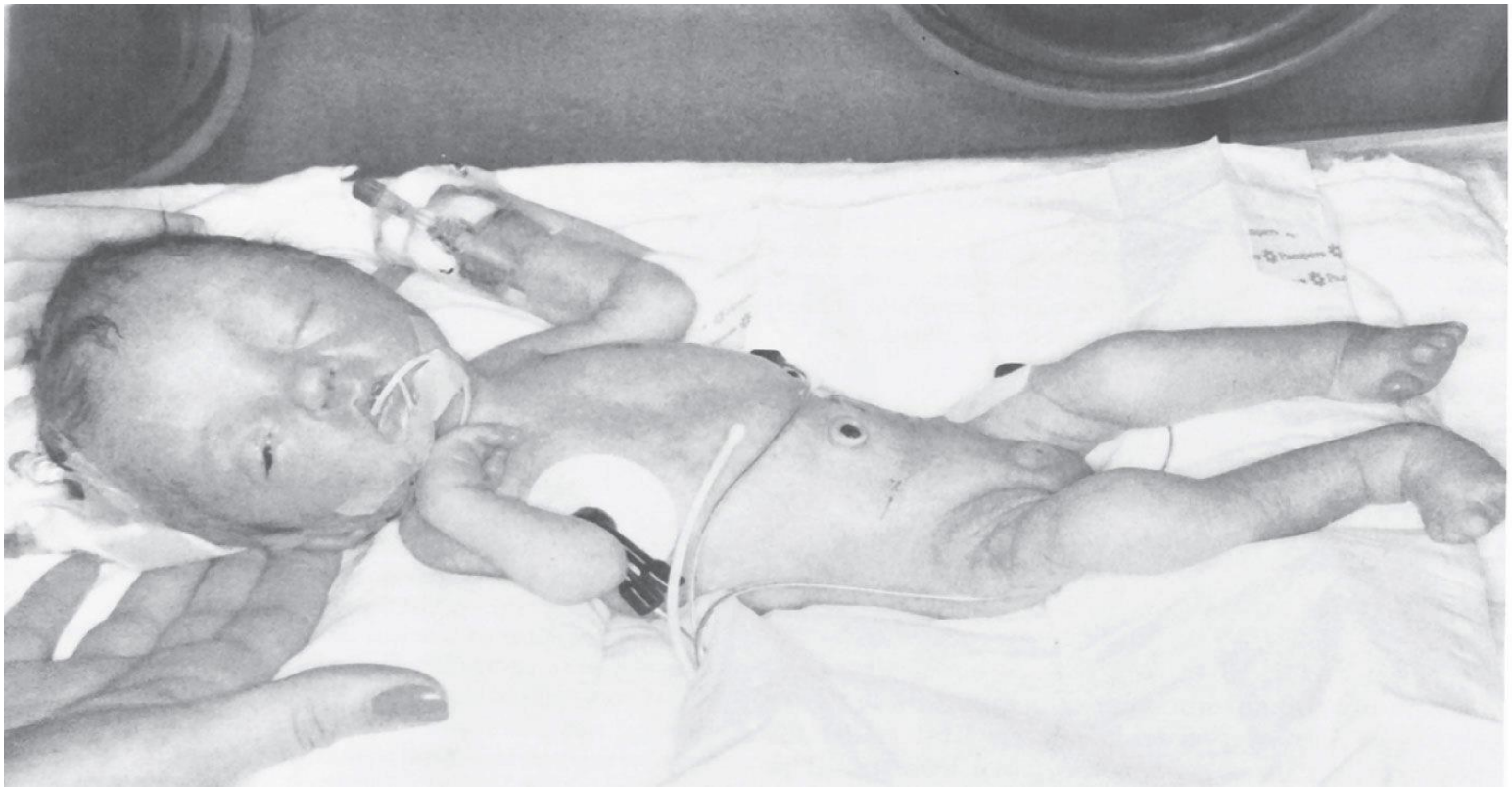


Fig. 8.9 Karyogram of a triploid fetus (69,XXX)

A Triploid Infant



Reproduced by permission of *Pediatrics*, Vol. 74, p. 296 ©1984 Falix et al. *Pediatrics* 74:296–299, 1984, Figure 29.1

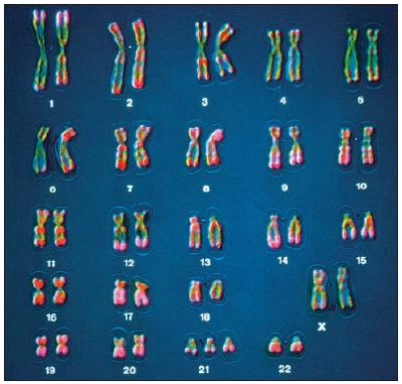
Autosomal aneuploidies

- The term aneuploidy refers to cytogenetic abnormalities in which all or part of one or more chromosomes is duplicated or deleted.
- Autosomal aneuploidy refers to all such abnormalities that do not involve the sex chromosomes.
- These can be either numerical or structural, the vast majority being trisomies, and may be present only in some cells (mosaic aneuploidy) or in all cells (nonmosaic).
- Chromosomal mosaicism is the presence of two or more cell lines with different karyotypes that have arisen from a single fertilized egg.
- Chromosomal mosaics arise from postzygotic events in somatic cells
- The incidence of autosomal aneuploidy in newborns is estimated to be 0.2% .

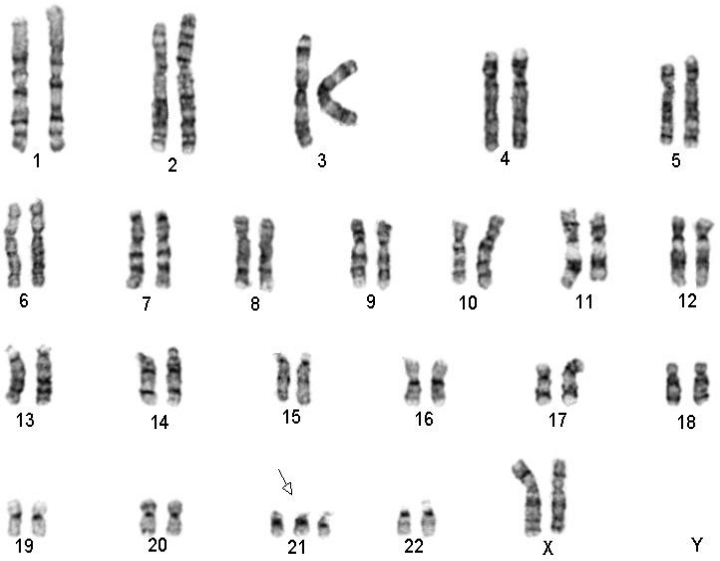
Autosomal aneuploidies

- The lethality of a particular autosomal aneuploidy correlates with the gene content of the chromosome involved. Aneuploidies for “gene-rich” chromosomes are less likely to survive.
- Trisomies 13, 18, and 21, which involve chromosomes that are “less gene-rich,” are therefore relatively “mild” and fetuses can survive to term.
- Most common type of trisomy in liveborns is trisomy 21: responsible for Down syndrome.
- Aneuploidy is generally caused by chromosome nondisjunction

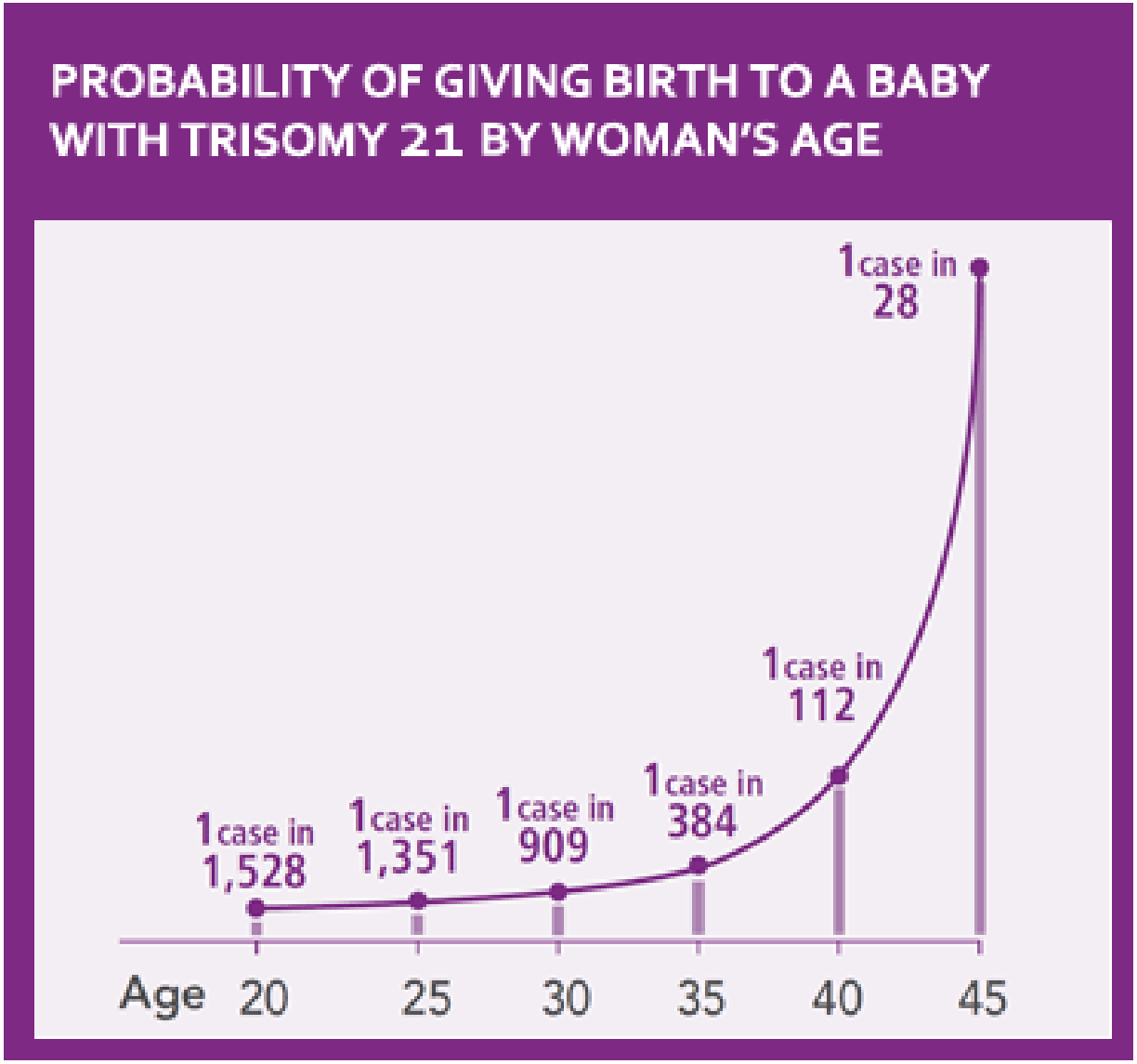
Changes in Chromosome Number



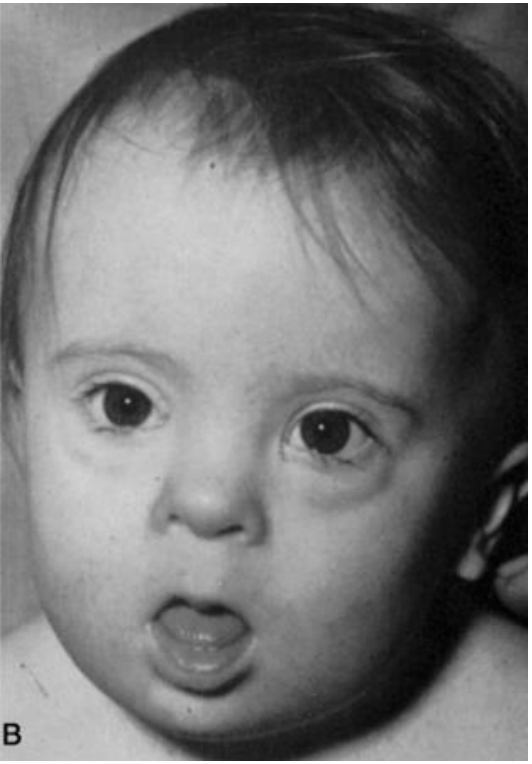
- ***Down syndrome*** is an aneuploid condition that results from three copies of chromosome 21
  - It affects about one out of every 700 children born in the United States
  - children will show some form of mental retardation, and 40% have heart defects.
  - There is an increased probability that a woman over age 35 will conceive an embryo with Down syndrome, yet 80% of trisomic infants are born to younger mothers simply because women ages 18-35 have more babies.

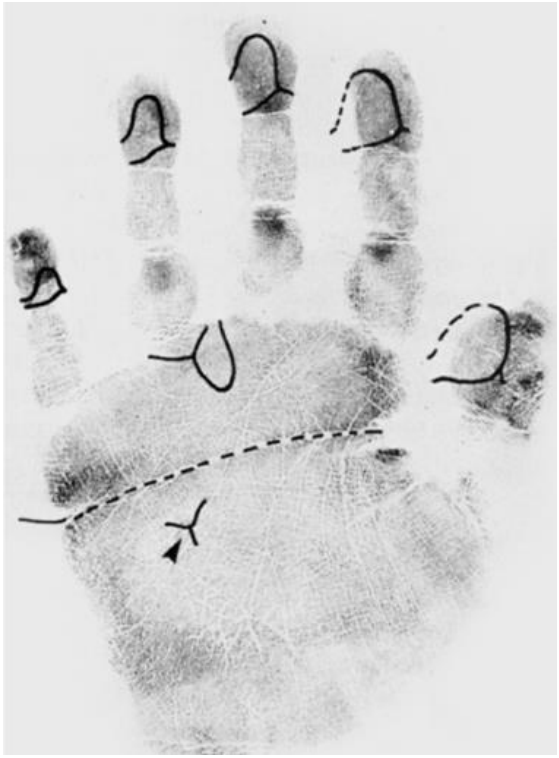


1 in 770 babies



Down Syndrome





Mental retardation (IQ 25-50)
*Low nasal bridge (90%)
*Hypotonia (80%)
*Up slanting palpebral fissures (80%)
Small, low-set ears (60%)
*Congenital heart disease (30%-50%)**

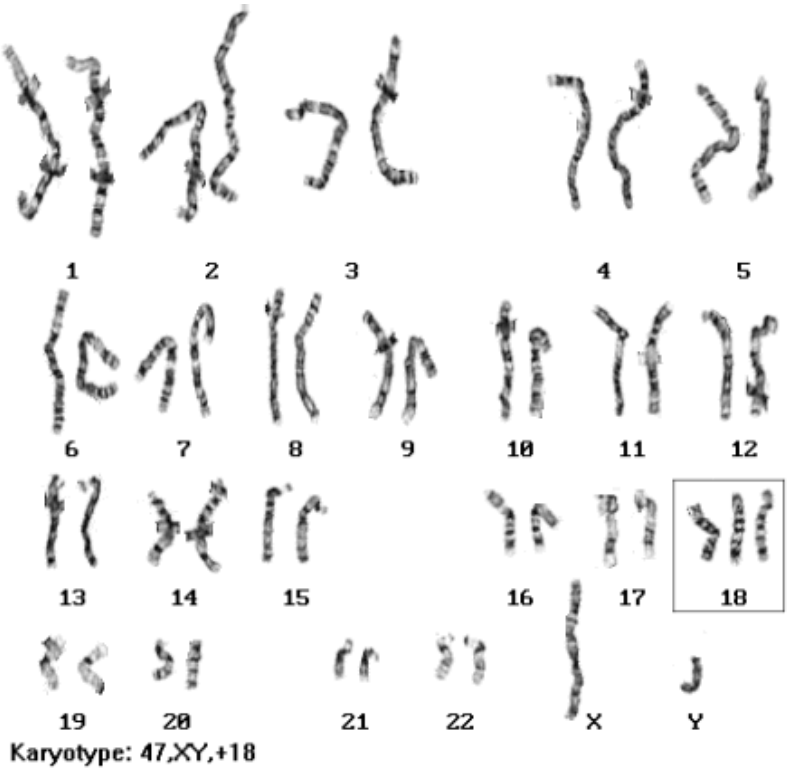
*Epicanthic folds
Protruding tongue
Intestinal problems
Gap between first and second toes
15-fold increase in risk for leukemia
*Simian line (transverse crease) (45%)

These features are easily recognized at birth.

**The congenital heart problems noted in people having Down syndrome include ventricular septal defect (VSD) and arterioventricular defects (AV) canal. Approximately 40% with congenital heart disease die during the first year.

Trisomy 18

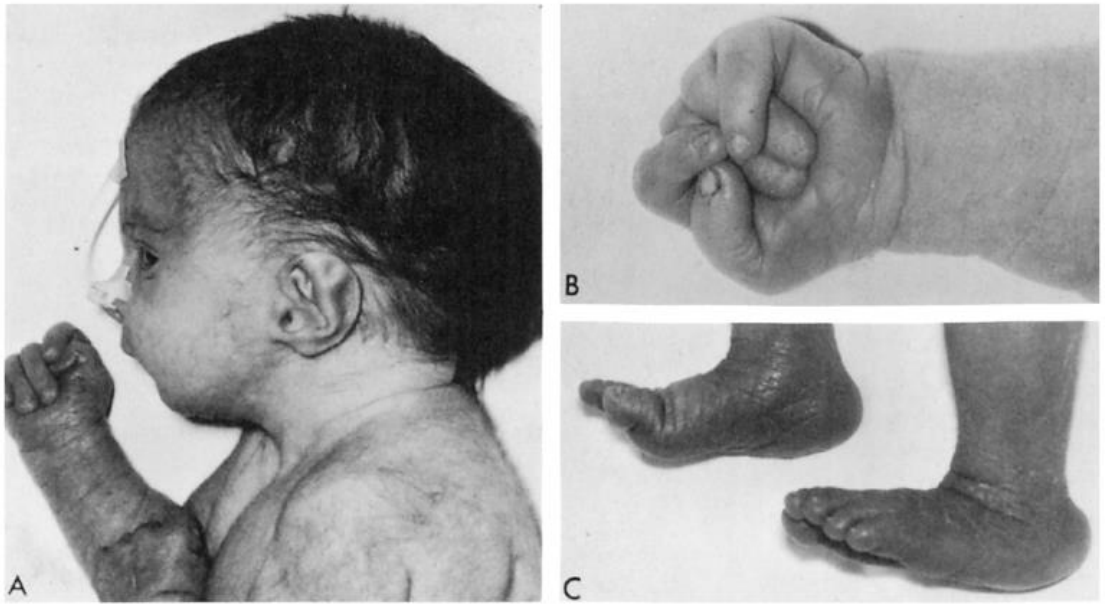
- Trisomy 18 (47,XY,+18 or 47,XX,+18); Edward Syndrome
- is the **second most common autosomal trisomy**, with a prevalence of about **1 per 6,000** live births
- **is the most common chromosome abnormality among stillborns** with congenital malformations



Trisomy 18

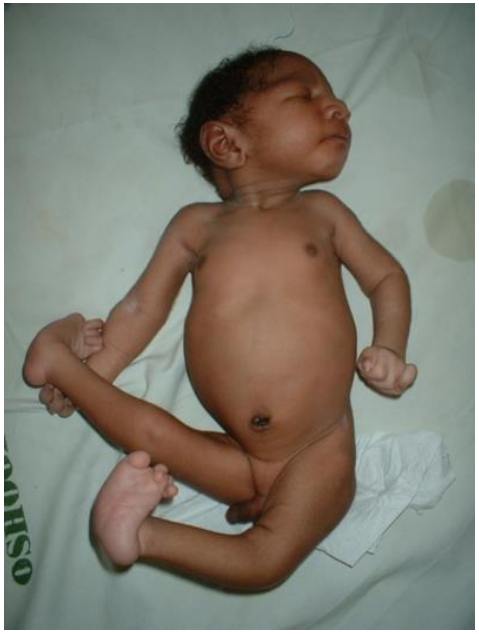
- About 50% of infants with trisomy 18 die within the first several weeks of life, and only about 5% to 8% survive to 12 months of age.
- Marked developmental disabilities
- More than 95% of infants with Edwards syndrome have complete trisomy 18
- 90% of trisomy 18 cases are the result of a maternally contributed extra chromosome.

Trisomy 18 (Edward syndrome)



Findings:

CHD (95%)
Failure to thrive (FTT)
Mental retardation
Growth retardation
Hypertonia
Prominent Occiput



Low-set, malformed ears
Short sternum
Intestinal Abnormalities
Unusual hand position
Rocker bottom feet

Trisomy 13 (Patau syndrome)

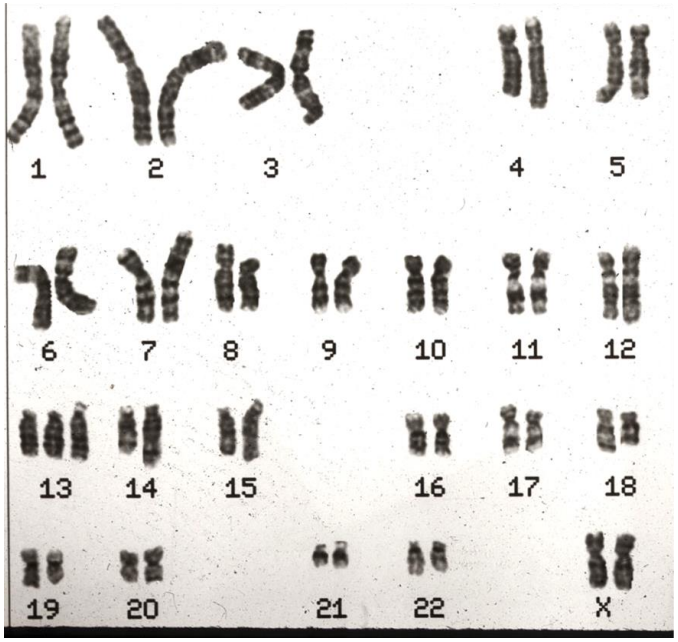
Trisomy 13 (47,XY,+13 or 47,XX,+13); Patau Syndrome

The survival rate is very similar to that of trisomy 18, and about 95% of live-born infants die during the first year of life.

CHD (85%)
Mental retardation
Hyper- or hypotonia
Scalp defects
Microcephaly
Small eyes
Low-set, malformed ears
Cleft lip/palate
Polydactyly and syndactyly
Polycystic kidneys
Rocker-bottom feet

FIG 6-10 An 8-year-old girl with full trisomy 13 showing her small eyes and prominent, wide nose.

- Trisomies of the 13th and 18th chromosomes are sometimes compatible with survival to term, although 95% or more of affected fetuses are spontaneously aborted.
- These trisomies are much less common at birth than is trisomy 21, and they produce more serious disease features, with 90% to 95% mortality during the first year of life.
- As in trisomy 21, there is a maternal age effect, and the mother contributes the extra chromosome in more than 90% of cases.



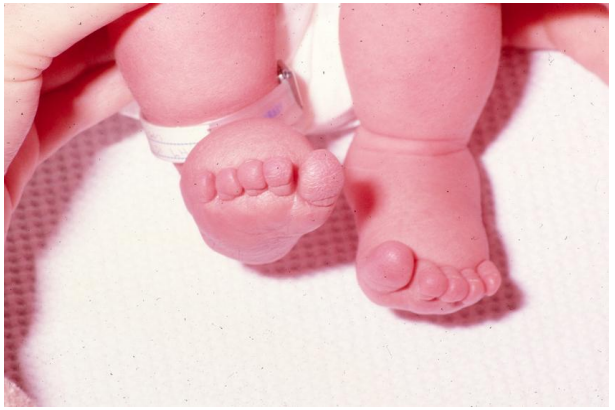
Numerical Chromosomal Abnormalities

Sex Chromosome Aneuploidy

- **46,XY**
  Normal male
- **46,XX**
  Normal female
- **47,XXY**
  Male with extra X chromosome (Klinefelter syndrome)
- **45,X**
  Female with missing X chromosome (Turner syndrome)

Monosomy of the X Chromosome (Turner Syndrome)

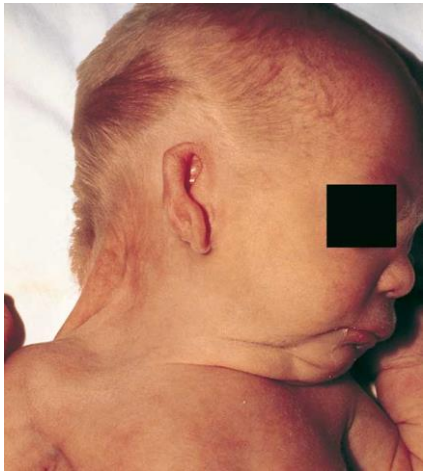
Cytogenetics: The phenotype associated with a single X chromosome (45,X, FEMALE) was described by Henry Turner in 1938.



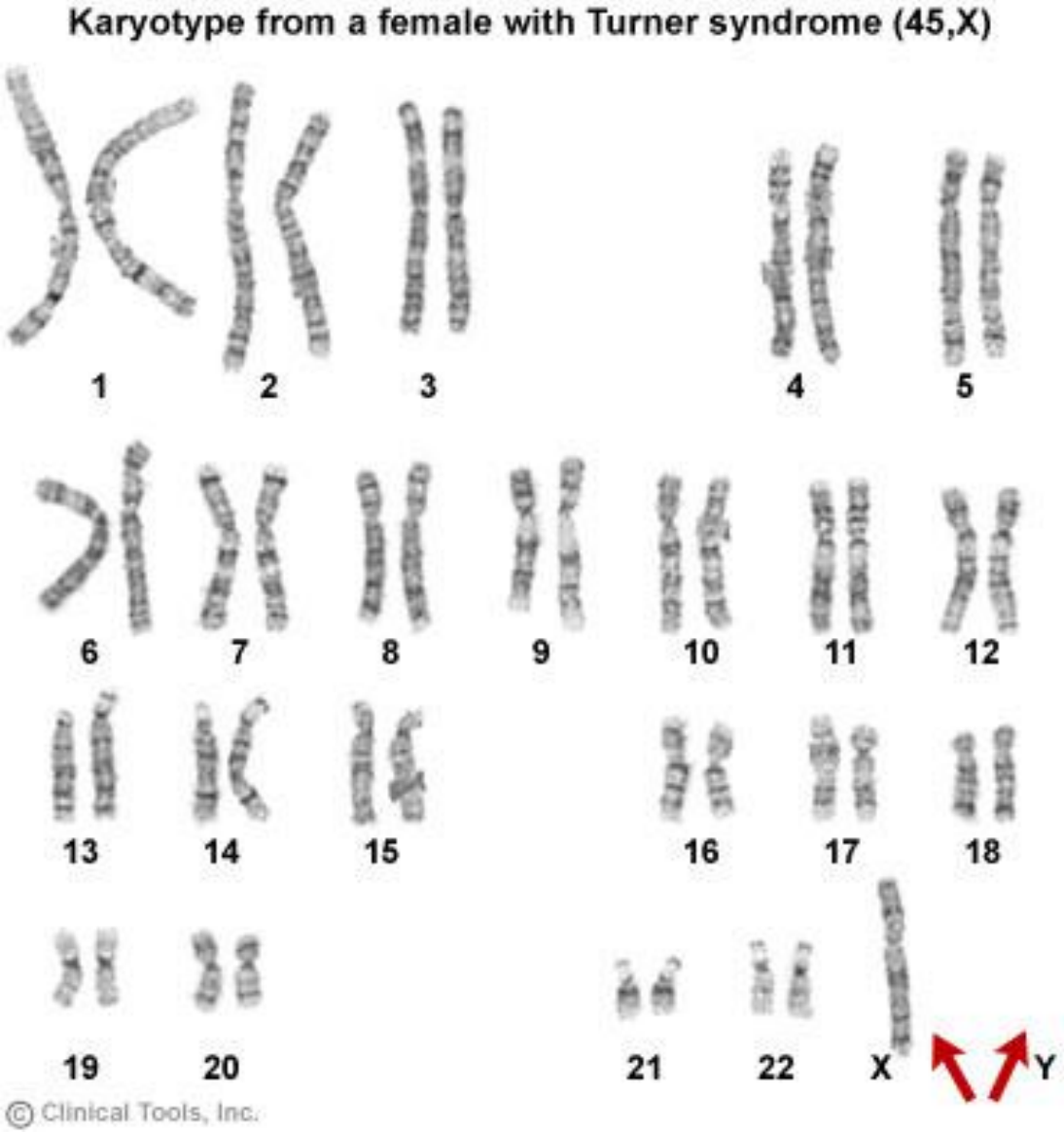
Incidence: 1/5000

Features

- Short stature
- Gonadal dysgenesis
- Lymphedema of hands and feet in newborn
- Webbing of neck
- Renal anomalies and cardiac anomalies



Turner Syndrome



Klinefelter syndrome

Cytogenetics 47,XXY, **MALE**

Incidence: 1/1000

Features

- Hypogonadism with small testes
- Gynecomastia
- Tall stature (tall legs)
- Infertility (most common presentation)
- Low testosterone
- Elevated FSH and LH
- High-pitched voice
- A common but not a serious disease, which may benefit from testosterone therapy

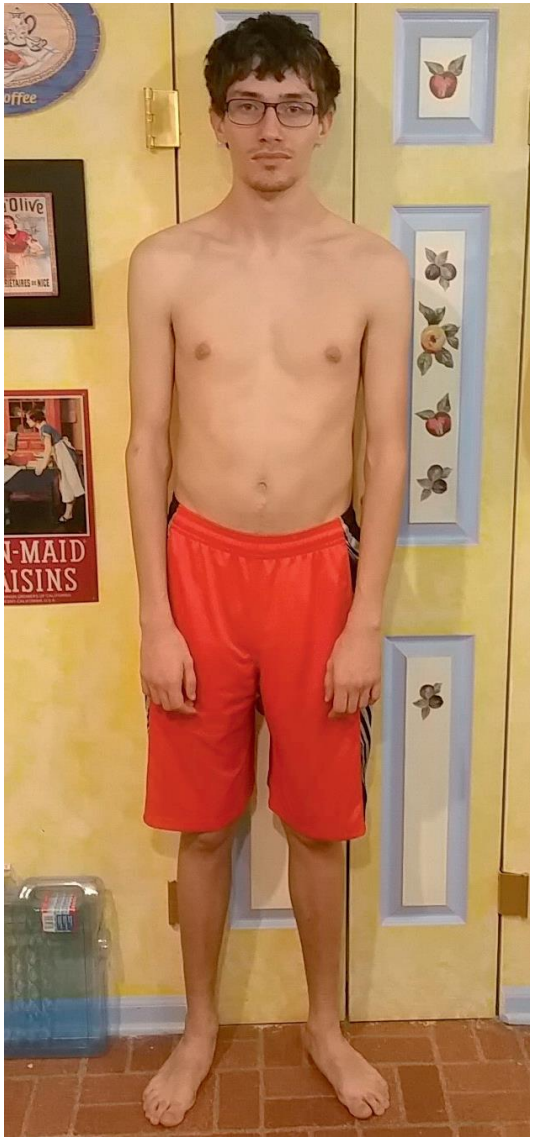
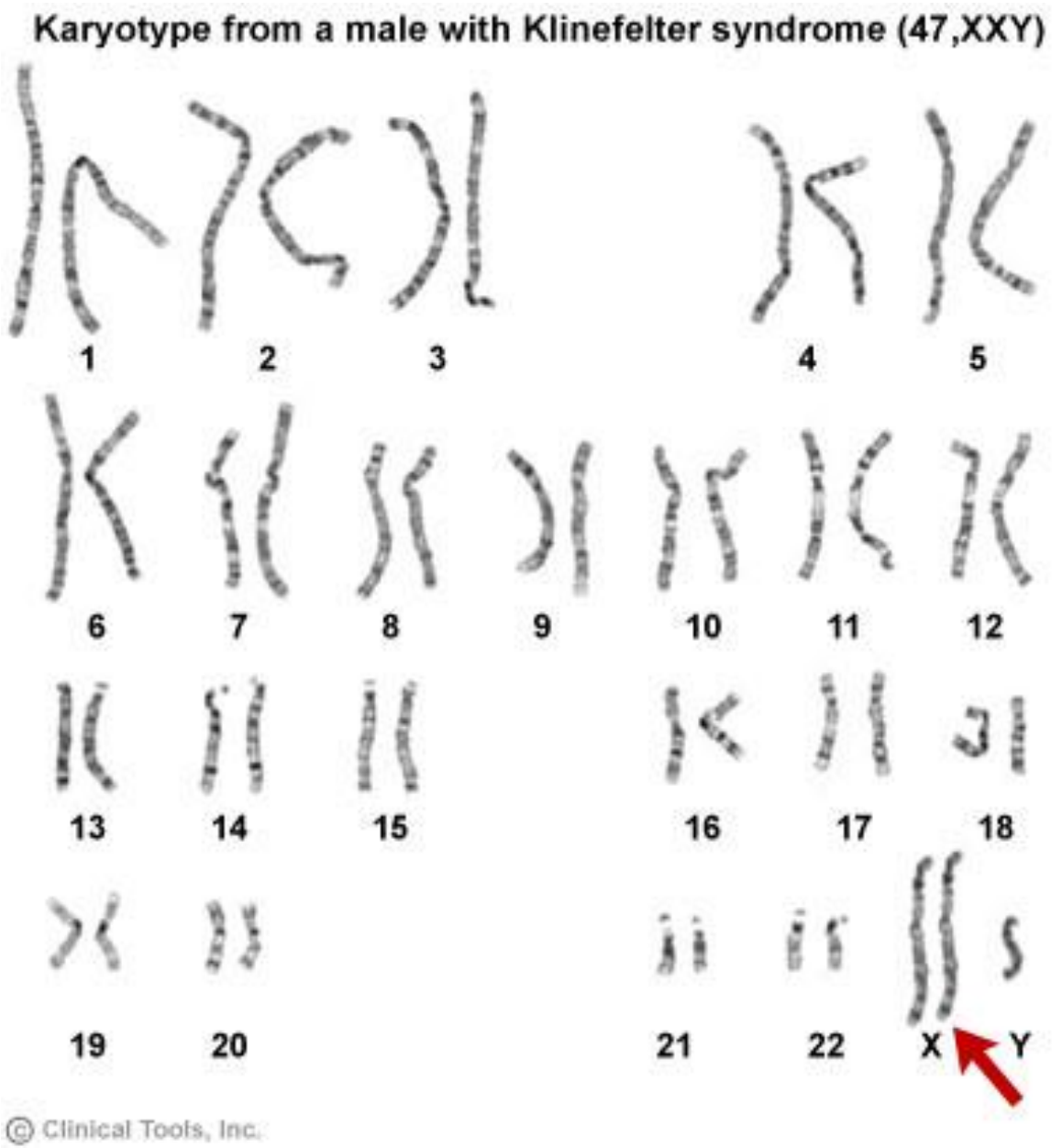


FIG 6-14 A male with Klinefelter syndrome (47,XXY). Stature is increased, gynecomastia may be present, and body shape may be somewhat feminine.



Extra Xs or Ys syndromes

- Males with this karyotype tend to be taller than average, and they have a 10- to 15-point reduction in average IQ.
- increased incidence of minor behavioral disorders, such as hyperactivity, attention deficit disorder, and learning disabilities.
- 48,XXXY and 49,XXXXY: degree of developmental disability and physical abnormality increases with each additional X chromosome.
- 47,XXX and 47,XYY: a slight degree of reduction in IQ but few physical problems