GENETICS

Sheet no. 6

Writer:

Doctor 2021

Corrector:

Doctor 2021

Doctor:

Mohammad Al-Shboul

Revision:

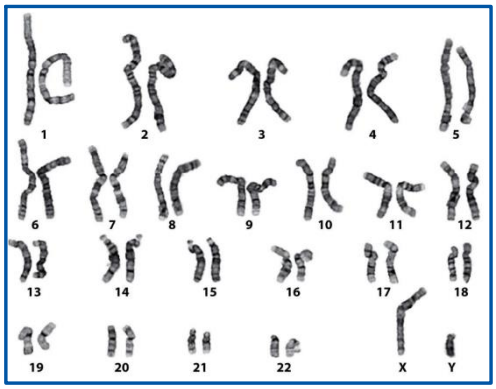
In the previous lecture, we talked about some specific patterns that distinguish between chromosomes and for karyotyping and analysis.

1- We saw that karyotyping requires actively **cultured cells** (mainly in metaphase). We have a wide range of samples, it depends on the patient, the disease, and availability of the samples like: PB, fibroblasts, lymphobalstoid cell lines, Bone marrow, fetal cells.

- **Cell free DNA testing** is a routine test for every newborn, pregnant women, and new married couples, to check for chromosomal abnormalities. Ex; silent carrier (balance) we will talk about it later (and BTW it is common in our society).

2- Banding (G, Q, R):

- G-banding → which is the standard.
- Q-banding → similar pattern to G banding, needs fluorescent microscope (different stain).
- R-banding → light and dark bands are reversed (The opposite of G)
- C-banding → used to identify centromeres/heterochromatin.



- in this figure we have a G banding Karyotype of a male (XY).

3- Special procedures (C-banding, high resolution banding, NOR).

- **High resolution banding** → we use stained cells before metaphase (prophase, prometaphase) to make sure that we have (كمية كبيرة) of genetic material so it would be easier to detect unobvious abnormalities.

4- Molecular cytogenetics (FISH, CGH). They both are same in hybridization and different in resolution **(where CGH has higher resolution than FISH).**

- **In this lecture we will talk about FISH.**

Fluorescent In-Situ Hybridization (FISH)

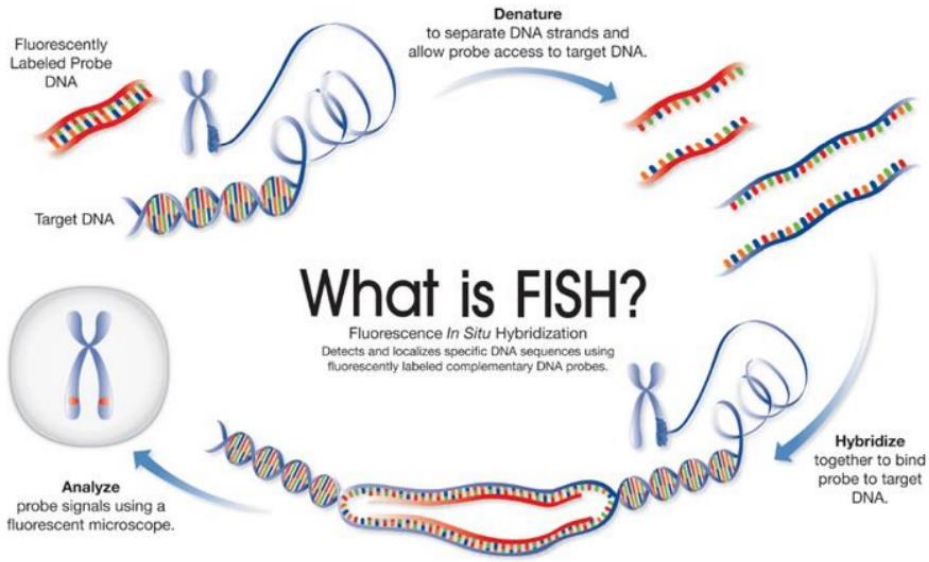
- **“Fluorescent in-situ hybridization”** literally means a technique that uses a fluorescently labeled nucleic acid probes to detect and visualize specific DNA or RNA sequences directly within intact cells or tissues.
- Conventional chromosome banding techniques are not sufficient to detect and identify all chromosomal aberration present in metaphase specifically when it's smaller than (4-5 mega bases).
- So, with the development of microscopes and invention of fluorescent, FISH was introduced to the clinical cytogenetics laboratories in late 1980s.

- Based on the ability of a single strategy DNA (which is a DNA probe that is labeled with a fluorescent dye) to anneal with its complementary target on the chromosome.
  - **DNA probe:** is a single oligonucleotide (100-1000 BP) that is commercially synthesized (it is very specific)
  - Widely used for clinical diagnostics and there are a number of different types of problems like deletion duplication translocation and even number of chromosomal, and all depends on the probe.
  - For example: in some cases, there is a specific probe for specific diseases like trisomy 21, 18, 13 (which there is an extra chromosome) and also can be used to detect common deletion syndromes such as **Prader–Willi syndrome** (microdeletion of 15q11.2) and **Williams syndrome** (microdeletion of 7q11.2)

- Its main limitation that you can't see without a probe.
- In molecular techniques you have to double check by other molecular techniques before the final analysis (عشان ما تخرب بيت المريض)

- In conventional techniques we do it in metaphase, but in FISH it is not necessarily. We can work on **undivided cells, Interphase, and Metaphase.** We don't need culture to do it that's why the results are very fast **(within 24 hours or less)** compared to controversial which takes about months or weeks.

- Metaphase chromosomes, or interphase cells are fixed onto a microscope slide, the DNA is denatured (then we do incubation for a short time) and then hybridized with a probe that can be detected by fluorescence. The chromosomes or hybridized regions are observed microscopically (a fluorescent microscope).

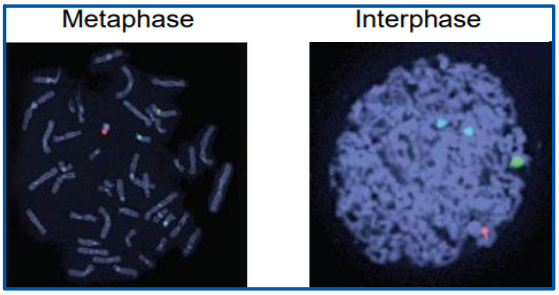


- **<u>How does it works?</u>**
  - Fluorescent probe of a sequence of interest bound to patient DNA.
  - In an unaffected person, a probe hybridizes in two places.
  - If patient DNA segment present, probe binds to the complementary DNA, fluorescent signal present.
  - If patient DNA segment missing, no binding with target probe, no fluorescent signal seen.
- **Interphase cells:**
  - HOW MANY signals are present: we have two copies so it should be two signals any other number or abnormal deletion duplication
  - Usually not where the signal is: because you can't distinguish between chromosomes **yet**.
- **Metaphase cells:**
  - HOW MANY signals are present: you can know the number of chromosomes.
  - WHERE the signal is.
- So, it provides way to see small DNA segments: **Present or absent**, **How many copies**.

- Probes used for clinical purposes are commercially manufactured and sold and must be validated by each laboratory.

❖ Most FISH probes fall into one of three categories:

1- Centromeric probes (CEP) (enumeration): are widely used for determining the number of copies of a particular chromosome. Ex: Prenatal diagnosis (13, 18, 21 and X&Y)

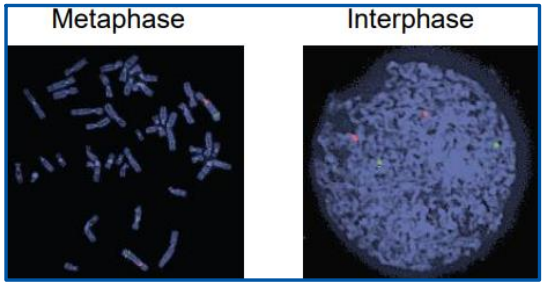


Green (1 copy): chromosome X

Red (1 copy): chromosome Y

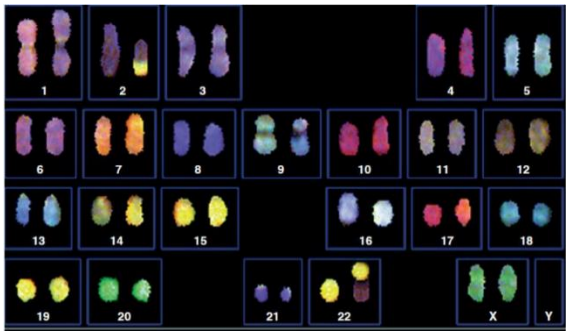
Blue (2 copies): chromosome aqua (18)

2- Locus specific probes: bind to a particular region of a chromosome. Example: the two deletion syndromes that we talked about earlier. if it detected 2 signals: it means it is normal, but it if it was 1 it means he or she has the disease.



On chromosome #4

3- Whole chromosome probes (spectral karyotype): full-color map of the chromosome. We can see if a specific region of the chromosome goes to another part of the same chromosome (not its original place).



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).

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:

1- Patient information (name, age, sex, and any relevant medical history or clinical indications for the test)

2- Name of the doctor

3- protocol number, lab number, hospital number.

4- the karyotype:

- ✓ **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)

Extra: You have to know the number of metaphases in the culture before during the karyotype, why? because let's say you have in the test 20 metaphases, if one of them have the has an abnormality you need to increase the number of the metaphases so you can know if there is a **mosaicism** in it so you increase it to for example 100.

Numerical Chromosomal Abnormalities

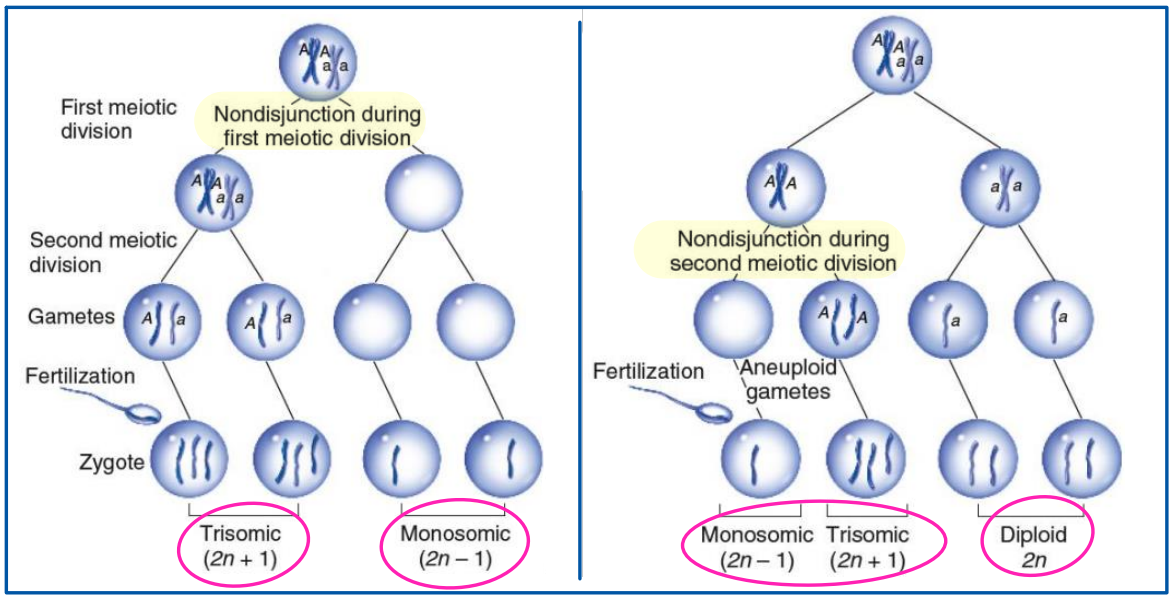
• In nondisjunction, pairs of homologous chromosomes do not separate normally during meiosis (anaphase)

• As a result, one gamete receives two of the same type of chromosome (trisomy), and another gamete receives no copy (monosomy)

- 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.

Meiosis I: one cell with devoid of gametes and one cell has all the three, so we have 100% abnormal cells (50% trisomy and 50% monosomy)

meiosis II: 50% normal and 50% abnormal (25% trisomy and 25% monosomy)



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) **(46-1=45)**

• **Trisomic** – individuals that have one chromosome in addition to the normal diploid number (2n + 1) **(46+1=47)**

• **Tetrasomic** – organisms with four copies of a particular chromosome (2n + 2) **(46+2=48)**

- **Ploidy:** number of basic chromosome sets (a diploid has 2 sets; a hexaploid has 6 sets)

• **Euploid:** organism have varying number of complete chromosomes set

• Most species of animals are diploid

- **Polyploidies** are numerical chromosome abnormalities in which an organism has more than two complete sets of chromosomes (chromosomes 1-21 has an extra copies of each one).

• They are usually incompatible with fetal survival and are extremely rare in liveborns.

EXTRA: PGD (Preimplantation Genetic Diagnosis) Is a procedure used during in vitro fertilization (IVF) to test embryos for genetic abnormalities before they are implanted into the uterus. PGD can help identify embryos that are at risk for certain genetic disorders, chromosomal abnormalities, or specific genetic conditions, allowing healthcare providers to select and transfer embryos that are most likely to result in a successful pregnancy and a healthy baby. This technology can be particularly beneficial for couples with a family history of genetic diseases or those at risk of passing on genetic conditions to their children. By screening embryos before implantation, PGD helps reduce the likelihood of passing on genetic disorders to future generations and increases the chances of a successful pregnancy outcome.

• Polyploidy is common in plants (ex; increasing the size of strawberries) , but not animals.

Triploidy and tetraploidy happen once in human

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	2*n*	AA BB CC	6
Aberrant Euploids			
Monoploid	*n*	A B C	3
Triploid	3*n*	AAA BBB CCC	9
Tetraploid	4*n*	AAAA BBBB CCCC	12
Aneuploids			
Monosomic	2*n* – 1	A BB CC	5
		AA B CC	5
		AA BB C	5
Trisomic	2*n* + 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.

❖ Triploidy:

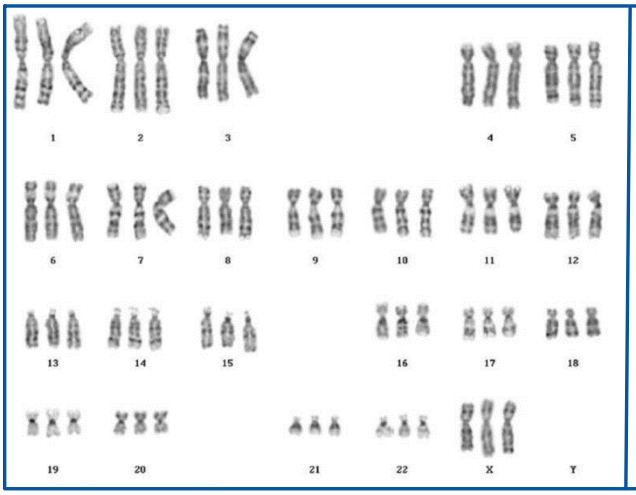
– A chromosomal number that is **three times** the haploid number, having three copies of all autosomes and three sex chromosomes

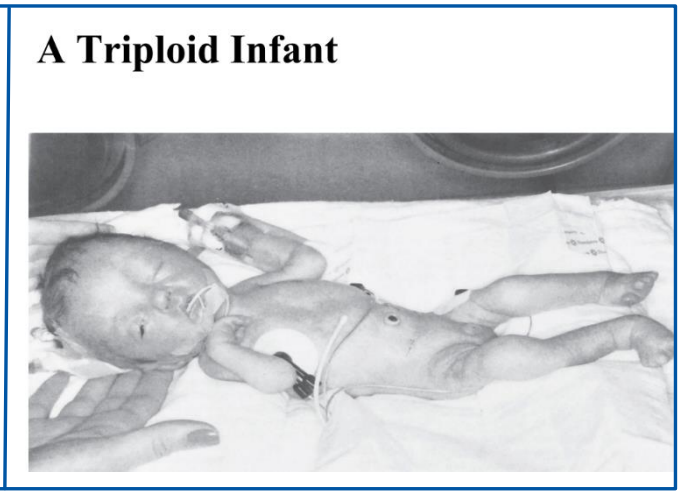
• Found in 15-18% of all miscarriages

• Approximately 75% of all cases of triploidy are 69,XYY in males and 69XXX and females and have two sets of paternal chromosomes.

• Oogenesis: the result will be one Ova and 1 polar bodies which get disintegrated and spermatogenesis 4 sperms. What happens that the Polar body fuse with the ova and the sperm fuse, it will be 3 copies, or if 2 sperms go to one ova.

• 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 (XYYY in males and XXXX in females)

• 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.

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%.

• 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.

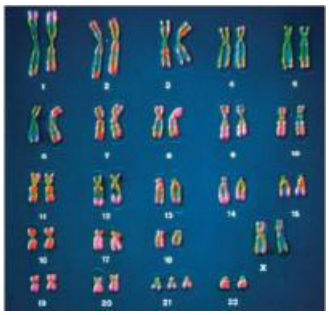
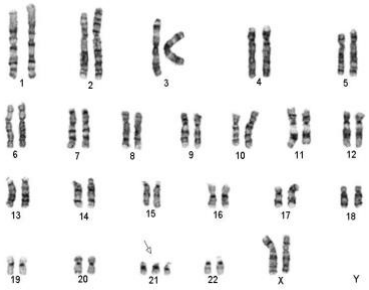
• Trisomies 13, 18, and 21, which involve chromosomes that are “less gene-rich,” are therefore relatively “mild” and fetuses can survive to term. (Even though trisomies 13 and18 are low genes they don't live most of the time (5% rate of survival) because they are gene rich compared to triosomy 21 which is most likely to live)

• Most common type of trisomy in liveborns is trisomy 21: responsible for Down syndrome.

• Aneuploidy is generally caused by chromosome nondisjunction

Anything in a red square is not required.

Triosomy 21

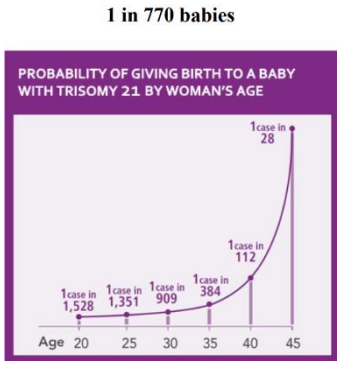



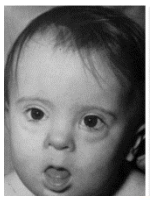
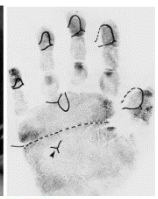

☼ **Down syndrome** is an aneuploid condition that results from three copies of chromosome 21.

• 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.

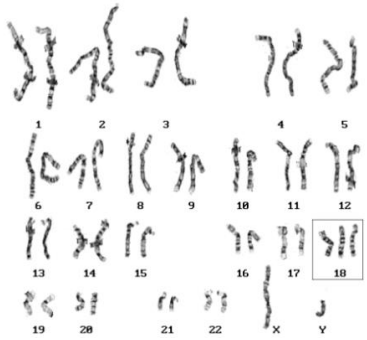



Mental retardation (IQ 25-50), Low nasal bridge (90%), Hypotonia (80%), Up slanting palpebral fissures, Small, low-set ears, congenital heart disease.

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

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

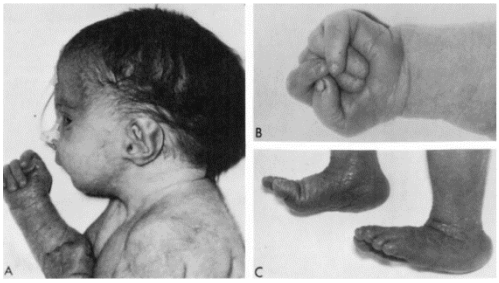
• About 50% of infants with trisomy 18 die within the first several weeks of life, and only about 5% to 8% survive to 12 months of age.

• Marked developmental disabilities

• More than 95% of infants with Edwards syndrome have complete trisomy 18

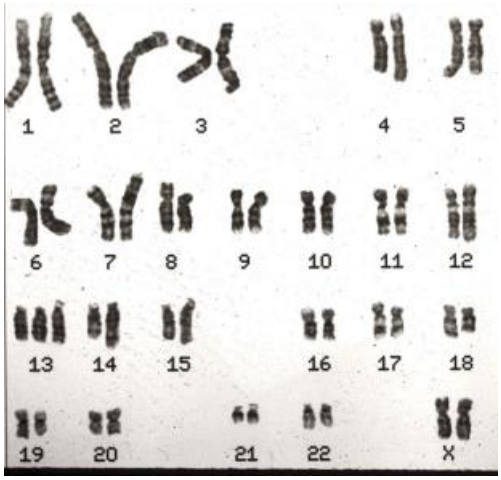
• 90% of trisomy 18 cases are the result of a maternally contributed extra chromosome.

☼ % are not required.



❖ **<u>Findings:</u>** 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



- (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.

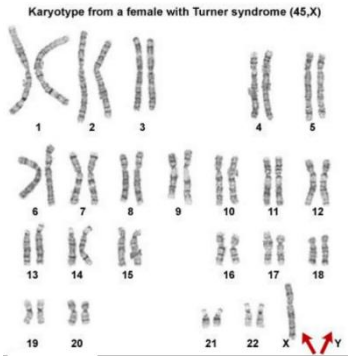
• Trisomies of the 13th and 18th chromosomes are sometimes compatible with survival to term, although 95% or more of affected fetuses are spontaneously aborted.

• 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.

Sex Chromosome Aneuploidy

❖ Monosomy of the X Chromosome (Turner Syndrome):

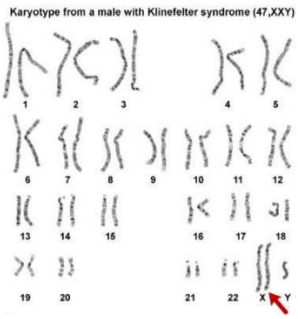


Cytogenetics: The phenotype associated with a single X chromosome **(45, X, FEMALE)** was described by Henry Turner in 1938.

Incidence: 1/5000

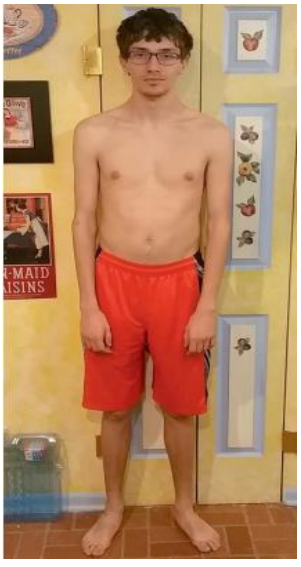
Features: • Short stature • Gonadal dysgenesis • Lymphedema of hands and feet in newborn • Webbing of neck • Renal anomalies and cardiac anomalies.

❖ 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

➢ ***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

AND THAT'S IT !!! ;)

TEST BANK

1- The karyotype where euchromatic regions stain more darkly and the light regions are heterochromatin is:

A) Q-banding

B) C-banding

C) G-banding

D) T-banding

E) R-banding **ANSWER: E**

2- Which one of the following pairs is mismatched?

A) Patau syndrome: 47,XX,+13

B) Edward syndrome: 47, XX, +18

C) Down Syndrome: 47, XX, +18 **ANSWER: C**

3- (للاحتياط) A female with a fattened face, small head, short neck, protruding tongue, small ears, and a poor muscle tone (hypotonia). She probably has a genetic disorder that's caused by _____?

A) Trisomy 21

B) Monosomy X

C) Trisomy X **ANSWER: A**

4- The most common aneuploidy that infants can survive with is _____ (most compatible with life)?

A) Trisomy 18 (Edwards syndrome)

B) Monosomy X (Turner syndrome)

C) Trisomy 21 (Down syndrome) **ANSWER: C**

5- A patent with Klinefelter syndrome can be seen as:

A) A male with 47 XXY

B) A female with 47 XXY

C) A female with 45 OX **ANSWER: A**

6-Which of the following human triploid is possible to be found in adults:

A) 92, XXXY

B) Triploid cannot be found in adult human because it is incompatible with life

C) 23, XY

D) 92, YY

E) 69, XXY **ANSWER: B**

7-Trisomy 47,XYY is a syndrome with signs and symptoms that range from being barely noticeable to learning disabilities, speech delay, low muscle tone. How would you expect this syndrome to have occurred?

A) Dispremy

B) Endomitosis

C) Fertilization by two sperms

D) Chromosomal rescue

E) Nondisjunction of paternal gametes **ANSWER: E**

8- What is the possibility for a couple to have a child with Edwards syndrome if the fathers' homologous chromosomes 18 fail to disjoin during meiosis 1?

A) 25%

B) 0%

C) 50%

D) 100%

E) 75% **ANSWER: C**

9- A child person with clinical features that include: cardiovascular, brain with neurological, renal, gastrointestinal, respiratory, and skeletal malformations, craniofacial abnormalities such as prominent occiput, hand and feet anomalies including clenched hand. This patent is most probably affected with:

A) Trisomy 18

B) Trisomy 21

C) Turner Syndrome

D) Partial Trisomy 21

E) Klinefelter Syndrome **ANSWER: A**

10- 47 XYY occurs due to:

A) Chimeric event

B) Nondisjunction event from both parents

C) Paternal nondisjunction

D) Uniparental disomy (UPD)

E) Maternal nondisjunction **ANSWER: C**

11- Which one of the following karyotypes is most likely to be found in normal human ovarian progenitor cell?

A) 22, Y

B) 46, XY

C) 46, XX

D) 23, X

E) None of the above **ANSWER: C**

12- 45,X/46,XX karyotype was revealed in the peripheral lymphocytes of a Turner syndrome patent. Which of the following is the underlying cause?

A) Reciprocal translocation

B) Nondisjunction in meiosis II

C) Nondisjunction in mitosis

D) Nondisjunction in meiosis |

E) Robesonian translocation **ANSWER: C**

V2

Page 2: **CGH has higher resolution than FISH**