University of Jordan
Faculty of Medicine

GENETICS & Molecular Biology

Number: 32

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Subject: Clinical Cytogenetics

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Topics discussed in the lecture:

- ✓ Definition of Clinical Cytogenetics
- ✓ Definition of a karyotype
- ✓ Classification of chromosomes and chromosome banding
- ✓ Importance of FISH and CGH
- ✓ Numeric chromosomal abnormalities
- ✓ Abnormalities of chromosome structure
- ✓ Chromosome abnormalities and clinical phenotypes
- ✓ Significance of chromosome analysis

Clinical Cytogenetics

Definition

- ❖ The study of abnormal chromosomes which aims to link abnormal chromosomal changes to pathological conditions.

Techniques Used

- ❖ **Karyotype** is a graphical representation of the metaphase chromosomes arranged in decreasing length from the largest to the smallest with the exception of X/Y chromosomes (**sex chromosomes**) that come at the end.
  There are multiple stains. The most commonly used stain is the **Giemsa stain** that produces banding on the chromosomes which helps in the identification of major chromosomal changes (changes in size and number).

- ❖ **FISH** (Fluorescence in situ hybridization): A probe binds to a particular sequence of DNA and lights up that region because of the fluorescent tag.
  Just like karyotypes, FISH cannot detect tiny mutations (**micromutations**).
  FISH only detects existing mutations of known sequences. It cannot detect unknown sequences.

Classification of Chromosomes

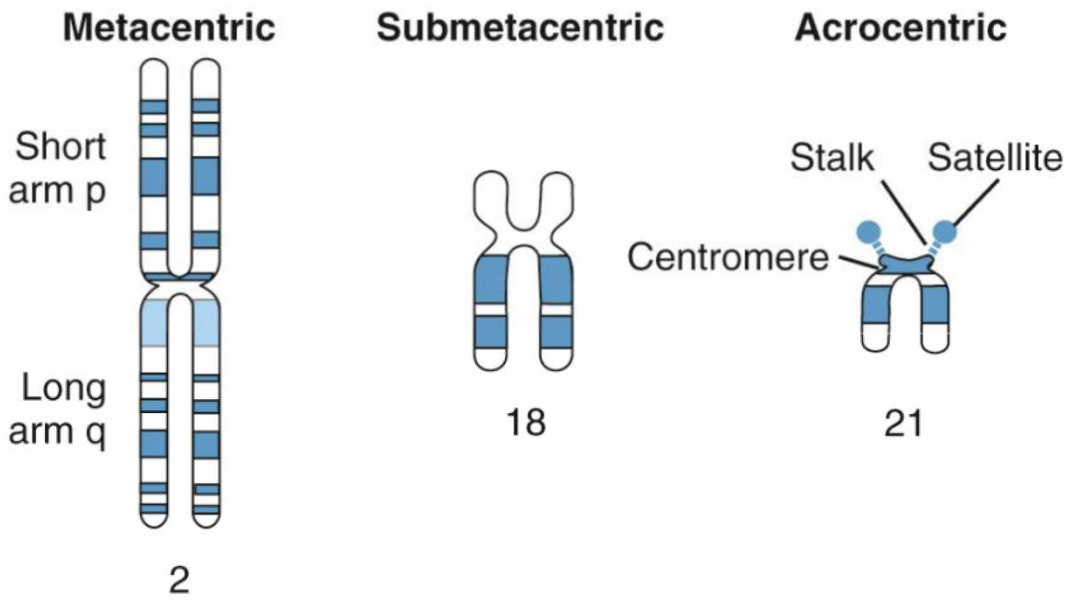
- Remember that human chromosomes can be divided into autosomal chromosomes (first 22 pairs of chromosomes) and sex chromosomes (chromosome pair #23).

- Karyotypes arrange chromosomes in decreasing length starting with the longest. This can be considered a method of classifying chromosomes based on length.

- Moreover, chromosomes can be classified according to the position of the centromere: In **metacentric** chromosomes, the centromere is in the middle. In **submetacentric** chromosomes, the centromere is somewhere between the middle and the tip. The short arm is shorter than that of the metacentric chromosome. In **acrocentric** chromosomes, the centromere is near the tip. The short arm of the acrocentric chromosome is so short that it is referred to as a stalk.

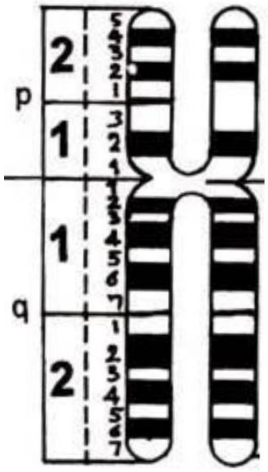
- Some karyotypic abnormalities are more common in the **submetacentric** and the **acrocentric** chromosomes.

- The short arm of the chromosome is labelled *p* (which stands for petit, meaning small in French). The long arm is denoted by the letter *q*. In the alphabet, the letter *q* follows the letter *p* which explains why these two letters were used to denote the arms of the chromosomes.



Karyotypes

- G-banding in a karyotype divides each chromosome into short and long arms.



- Each of the short and long arms is divided into regions. The number of regions depends on the length of the chromosome (there are more regions on longer chromosomes).

- There are bands within the regions and sub-bands within the bands themselves.

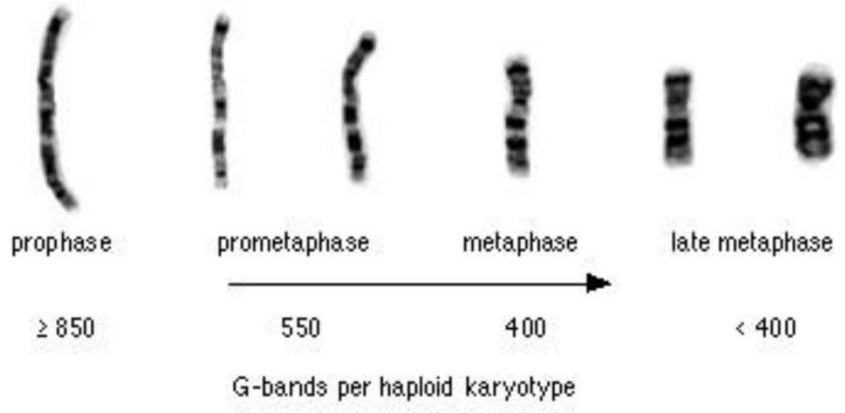
- For example, 22q11.2 means:
  22q = long arm of chromosome 22
  11.2 = region 1, band 1, Sub-band 2

- This notation is used to indicate the site of deletion or mutation in a chromosome causing chromosomal abnormality.

- Note that there is no need to memorize the notation of each chromosomal abnormality. They are not required in the exam. The notation of each chromosomal abnormality can be found in online databases.

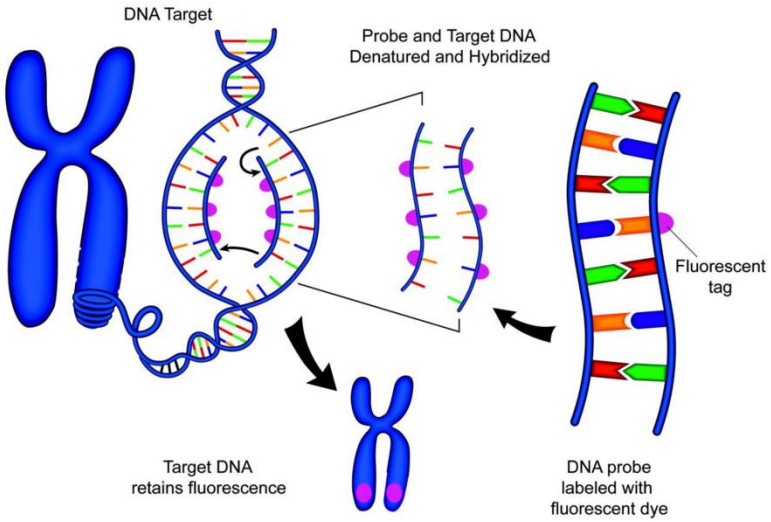
- In most karyotypes, the chromosomes are in late metaphase stage. Therefore, there are about 400 bands.

- **High-resolution banding**, or high definition banding, involves staining chromosomes during prophase or early metaphase before they reach maximal condensation. Using this technique, the number of bands observable for all chromosomes increases to as many as 800.

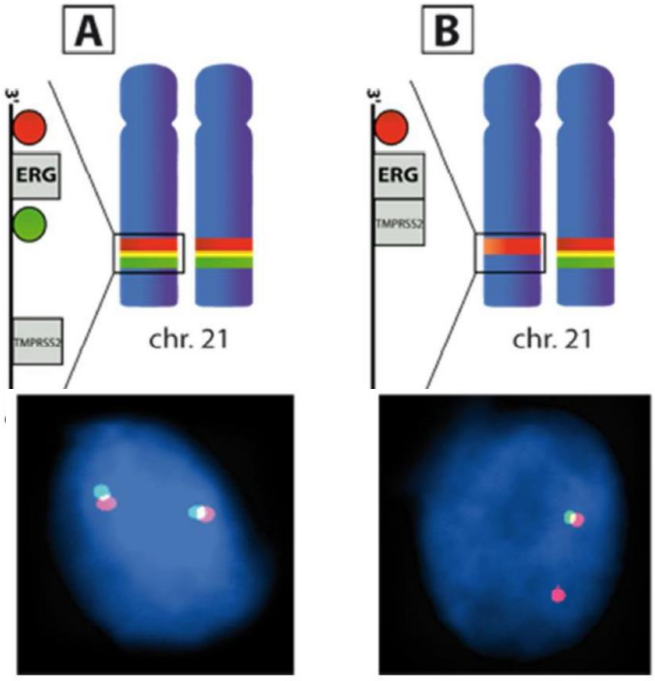


FISH (Fluorescence in situ Hybridization)

- In this procedure, there is a probe and a certain known DNA sequence.



- A probe binds to a particular sequence of DNA and lights up that region because of a fluorescent tag.
- The cell does not have to go through mitosis to do FISH. FISH can be performed when the cell is in interphase.
- FISH detects insertions, deletions and translocations.



- If one probe binds to one chromosome in metaphase and the other probe binds to something that looks completely different, then translocation has occurred.
- For interphase chromosomes, two probes should be used for each chromosome. These two probes should be fairly close to each other as they are on the same chromosome. If they hybridize far away from each other, then translocation has occurred.
- Remember that FISH was used to detect prostate cancer. In the case of prostate cancer, TMPRSS region which is androgen sensitive goes right next to the ETS transcription factor causing the cell to proliferate rapidly due to a deletion in that region (as shown in the figure above).

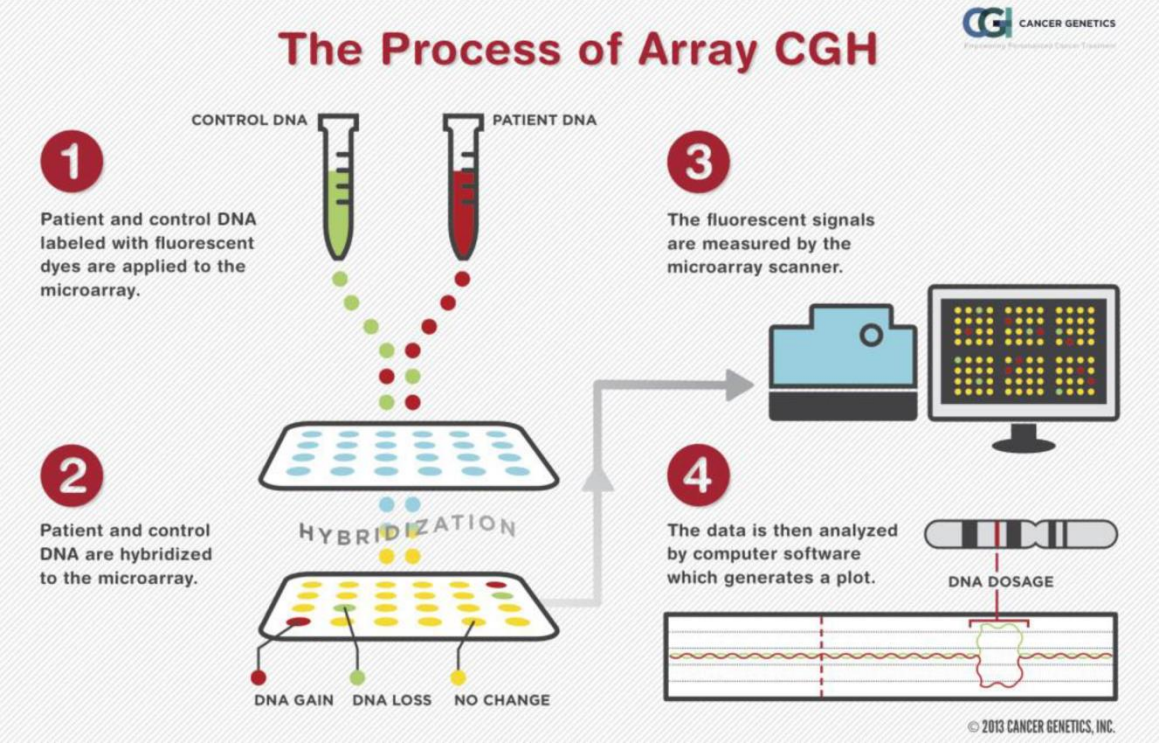
Array Comparative Genomic Hybridization

- Array comparative genomic hybridization (aCGH) is more accurate than normal comparative genomic hybridization (CGH) because unlike the conventional comparative genomic hybridization which detects changes in 10Mb (Mega bases), it can detect changes in kilo bases of DNA.

- A normal patient's DNA and a diseased patient's DNA are chopped up separately. Probes are added to each sample and then they are annealed on a microarray chip. A machine reads this chip. Should the probes come together, yellow colour will appear.

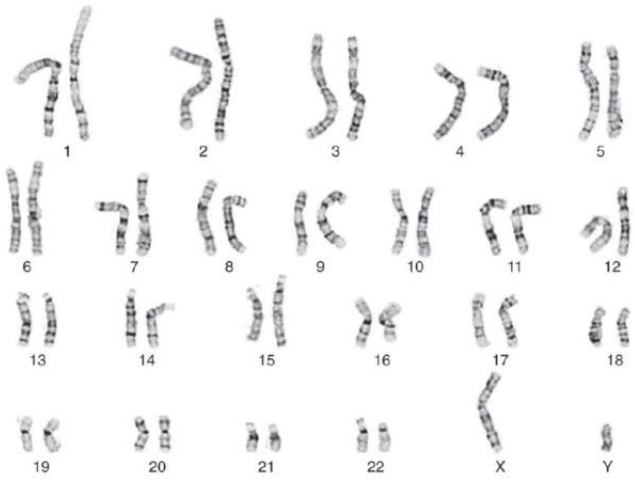
- Normal patient's DNA is labelled green and the diseased patient's DNA is labelled red.

- Green colour means deletion. Red colour means amplification or duplication of DNA (it does not mean overexpression because this is not RNA).



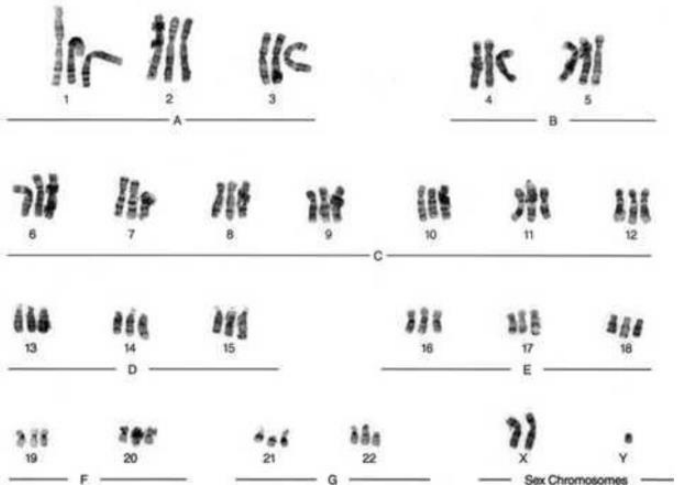
Numeric Chromosomal Abnormalities

- **Euploidy** means <u>multiples of the haploid</u> number of chromosomes. In human cells, any multiple of 23 chromosomes is euploid.



Euploidy

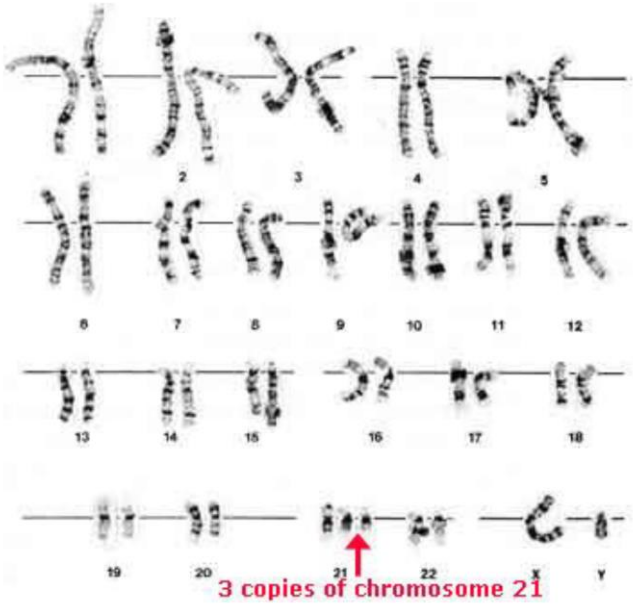
- **Polyploidy** is the presence of a complete set of extra chromosomes in a cell.



Polyploidy

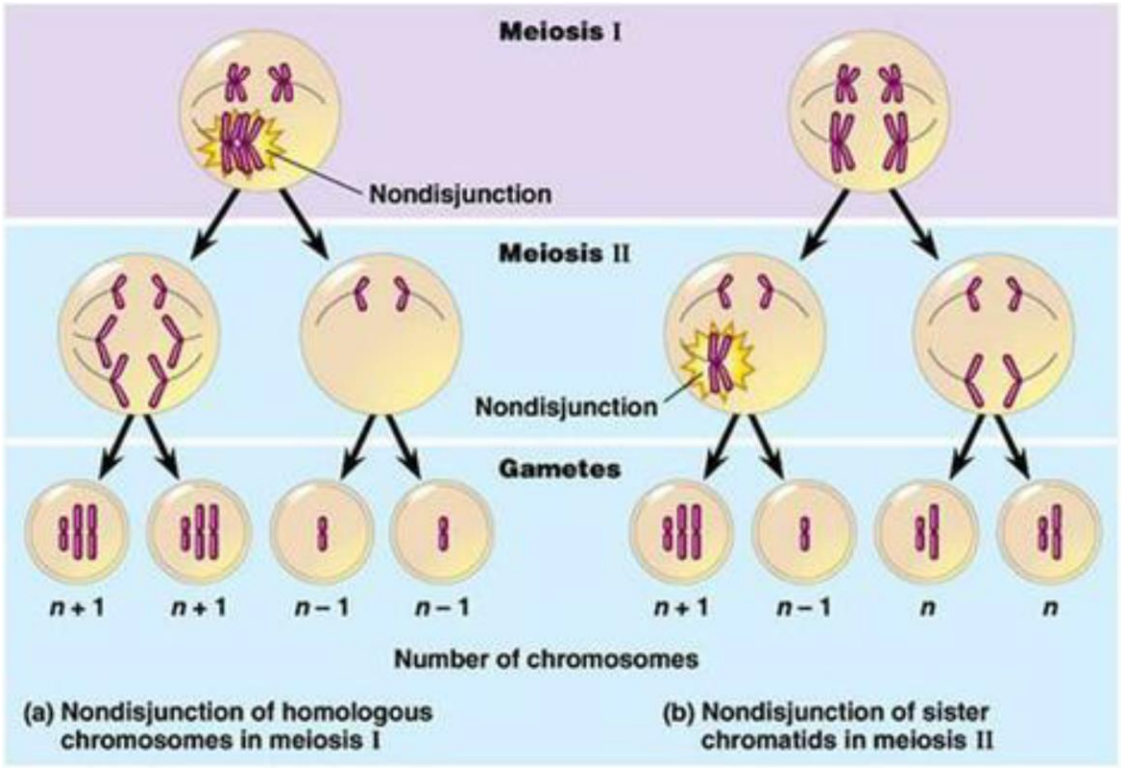
- **Aneuploidy** means <u>missing or additional individual chromosomes</u>. For example, an extra copy of chromosome #21 causes trisomy 21, which is otherwise known as Down syndrome.

- Numeric chromosomal abnormalities can occur due to non-disjunction in either meiosis I or meiosis II. Normally, the chromosomes separate during meiosis and are divided equally among daughter cells (gametes). However, due to non-disjunction, chromosomes do not separate. Therefore, one daughter cell will have chromosomes and the other daughter cell will have none.



Aneuploidy

- If non-disjunction occurs in meiosis I, all four of the gametes produced are going to be abnormal. If non-disjunction occurs in one of the daughter cells in meiosis II, 2 out of 4 daughter cells will have chromosomal abnormalities.



Trisomy 21: Down Syndrome

- Patients have a karyotype of 47,XY,+21 or 47,XX,+21.
- It can also occur due to translocations and mosaicisms.
- A mosaic karyotype would be 47,XY,+21[10] or 46,XY,+21[10]. This means that 10 cells had trisomy.
- In the case of mosaicism, the zygote already had trisomy 21, but during embryogenesis, one of the cells in the embryo lost this extra chromosome and became mosaic.
- Mosaic patients will have less severe symptoms.
- Down syndrome can also be the result of translocations. If, for example, a chunk of chromosome 21 was put on chromosome 3 and vice versa, then during the production of gametes, the section of

chromosome 21 (which is originally from chromosome 3) might also segregate with the chromosomes by chance. Therefore, that particular gamete will have a full chromosome 21 and a small chromosome 21. Nonetheless, these translocations cause a less severe type of Down syndrome.

- Down syndrome patients are very **mild-mannered**, **gentle** patients. They have very characteristic facial features. They have a single **palmer crease** (used to be called simian crease but not anymore) and epicanthic folds (which is a layer of skin covering the edge of the eye; this trait is normal among people of Asian descent).

- Patients frequently have a much lower IQ than the general population.

- The most important symptoms to watch out for are: congenital heart defects, predisposition to leukaemia, intestinal stenosis and umbilical hernia.

- If the abovementioned life threatening effects are taken care of, patients can reach their 50s.

- Once patients reach their 40s, they start showing signs and symptoms of Alzheimer's disease. This is due to the fact that translocations on chromosome 21, causing Down syndrome, increase synthesis of the product of APP gene (β-amyloid precursor protein) because all 3 chromosomes #21 have an APP gene.

- All trisomies, including Down syndrome, are positively correlated with maternal age, but not paternal age (because males continuously generate new sperms). As maternal age increases, the risk of bearing a child with a trisomy increases. This is owing to the fact that a female is born with a certain amount of oocytes, and some of these oocytes are suspended in prophase I. They continue meiosis at the time of ovulation. Increasing maternal age increases the chance of meiotic non-disjunction.

Trisomy 18: Edwards Syndrome

- The karyotype is 47,XY,+18 or 47,XX,+18

- The mosaic type is 46,XX/47,XX ,+18

- Patients have distinctive facial features. They show other symptoms such as: prominent occiput, intellectual disability, micrognathia (small mouth), low set ears, short neck, overlapping fingers (distinct feature of this syndrome), limited hip abduction, and rocker-bottom feet.

- Some of the life threatening conditions include: congenital heart defects and renal malformations.

- Unfortunately, the vast majority of these patients die within their first year.

- There is no health algorithm or regime to detect and treat these patients as effectively as in Down syndrome patients.

Trisomy 13: Patau Syndrome

- The karyotype is 47,XX,+13 or 47,XY,+13

- May occur due to translocations duplicating the long arm of chromosome 13.

- Patients have the following symptoms: microcephaly, intellectual disability, micropthalmia (small eyes), cleft lip, cleft palate, rocker-bottom feet and polyadactyly.

- Life threatening symptoms of this syndrome include: congenital heart defects, renal defects, and umbilical hernia.

- Most of the patients don't survive after their first year due to congenital heart defects. Luckily, a very small percentage of patients live beyond their first birthday.

Why are the numeric chromosomal abnormalities mentioned so far related to an increase in chromosome number, but **not a decrease**?

Cells have the ability to withstand an increase in genetic material rather than the loss of genetic material with the exception of sex chromosomes as in Turner syndrome.

In females, one of the X-chromosomes is inactivated, but 15% of that chromosome can be active. This 15% is referred to as the pseudo-autosomal region. The alleles in that region are not hemizygous (like the rest of the alleles on the active X-chromosome); they're either homozygous or heterozygous. Turner syndrome is the partial or complete monosomy of the short arm of the X chromosome.

Turner Syndrome

- It is the partial or complete monosomy of the short arm of the X-chromosome.
- The pseudo-autosomal region mentioned above is on the short arm of the X-chromosome.
- Symptoms of this syndrome include low posterior hairline, webbing of the neck, short stature, broad chest (triangular shield like chests), widely spaced nipples, cubitis valgus (carrying angle or the *deviation of the forearm away from the body*; males have a wider carrying angle than females), streak ovaries, infertility and primary amenorrhea.
- Coarctation of aorta is a life-threatening symptom of this syndrome.
- These patients can be given hormone replacement therapy (estrogen for secondary sexual characteristics and growth hormones for a longer stature). Cardiac problems can also be treated to allow these patients to live a fulfilling life.
- Females with this condition do not have intellectual disability. If they did, it wouldn't be as severe as the abovementioned trisomies.

Klinefelter Syndrome

- Most common genetic cause of hypogonadism in males
- They are very hard to detect.
- Males with this condition are taller than average, have reduced body and facial hair, long upper extremities, feminine fat distribution and microorchidism (small testes).
- Patients may suffer from osteoporosis. They also have a relatively higher risk of getting breast cancer than other males who do not have this syndrome due to breast development (gynaecomastia).

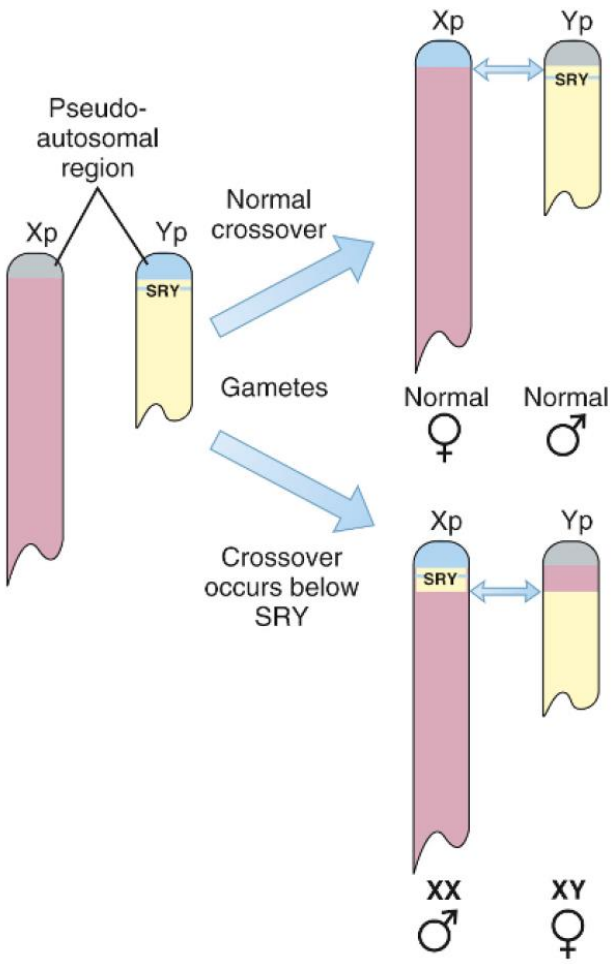
- Unfortunately, these males don't show up to clinic until they are married and can't have any children (due to infertility). If Klinefelter syndrome patients are recognized early on, testosterone can be given.

- Some studies show that giving testosterone to these patients improves their psychological well-being.

- Some mosaics can be fertile with a milder clinical condition.

- The figure on the right shows pseudo-autosomal regions on the short arms of the X and Y chromosomes.

- A normal crossover in homologous recombination would occur above and away from the sex-determining region on the Y-chromosome.



- The SRY gene in the sex determining region should stay on the Y-chromosome because this gene activates another gene called SOX9 which is a transcription factor that activates many pathways to form male characteristics.

- If the SRY or SOX9 is lost, an XY male will become phenotypically female.

- If crossover occurs below SRY and an XX zygote may receives the SRY region, the XX female will become phenotypically male.

- This extensive crossover actually happens in 1:20,000 Klinefelter patients.

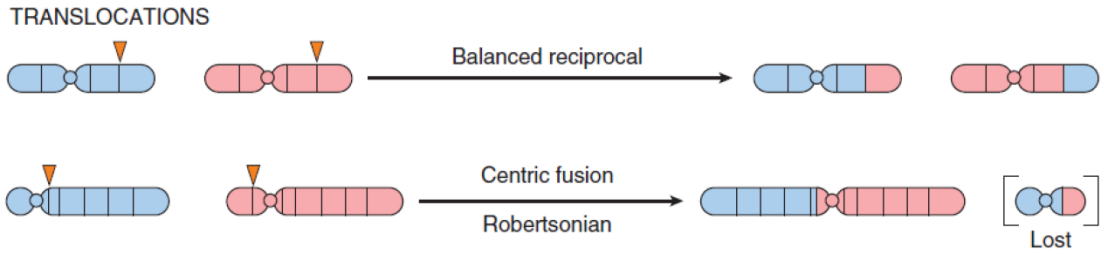
Structural Abnormalities

- A **translocation** is an exchange of genetic material between non-homologous chromosomes. Exchanges of genetic material between homologous chromosomes is called **crossover**.

- If the translocation does not result in increased or reduced genetic material, then this is referred to as a **balanced translocation**.

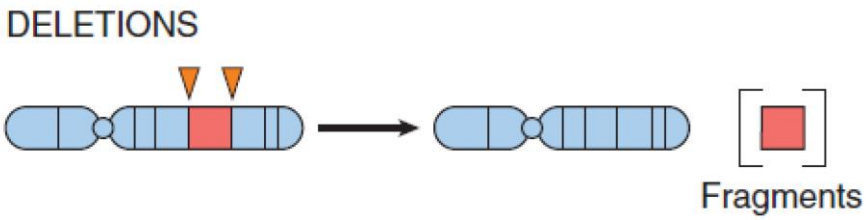
- **Reciprocal translocation** occurs in both directions.

- If the translocation results in fusion of centromeres, then the result will be one long chromosome (which is actually two chromosomes). The short arms are lost and the long arms of two chromosomes fuse together at the centromeres. If the short arms don't contain many genes that are active in most of the somatic cells, then that person will be phenotypically normal (shows no symptoms). The problem is when that person produces gametes.



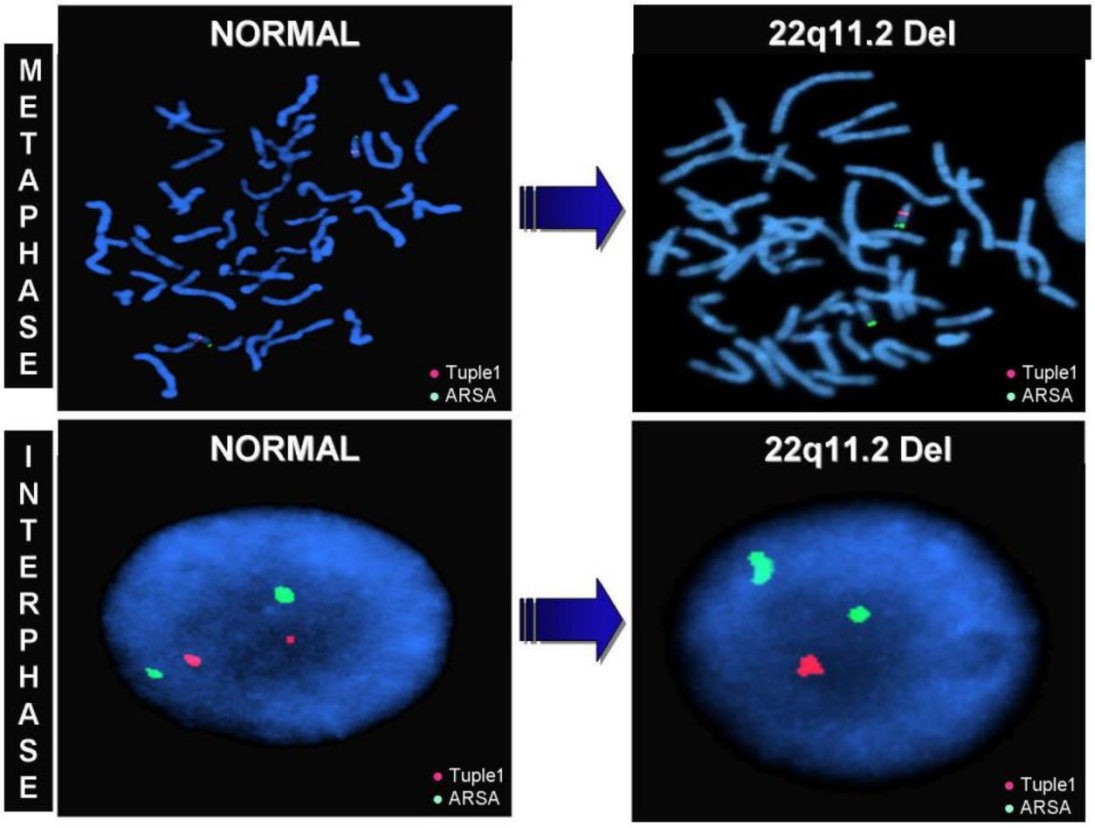
- **Deletions** are the removal of chunks or regions of DNA either from the ends (**terminal deletion**) or between two cuts (**interstitial deletion**).

- If the region that was deleted is a condensed region that is not transcribed, the person is phenotypically normal (unless it's an embryo). Again, the problem is when that person produces gametes.



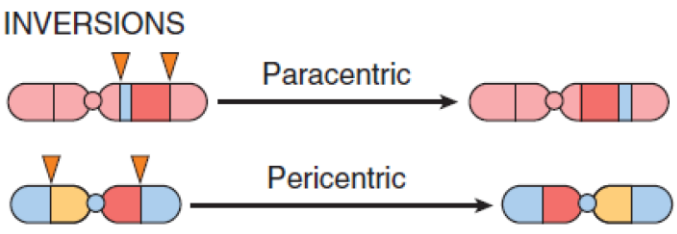
- 22q11.2 Deletion Syndrome (previously known as DiGeorge or Velocardiofacial syndrome) is variable between patients. The symptoms that occur are a continuum of the disease. Traditionally, thymus problems, T-cell problems, parathyroid problems and

hypocalcemia are the symptoms of the DiGeorge syndrome. Congenital heart disease, palate abnormalities, facial dysmorphism, and developmental delay are symptoms of the velocardiofacial syndrome. These names are not used anymore because the symptoms are mixed. There is not a clear cut between the two syndromes.



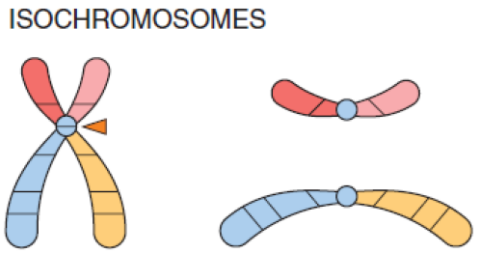
- **Ring chromosomes** are the result of DNA repair mechanisms trying to fix the loss of genetic material from both ends. This usually doesn't cause any problems if the lost genetic material is non-coding. However, this chromosome is very large and circular so it is very difficult to replicate it. Problems in replicating the circular chromosomes often cause insertions or deletions. The only circular DNA that the cell machinery can replicate is the mitochondrial DNA.

- **Inversions** are essentially interstitial deletions that have been aborted unsuccessfully.



The two breaks have occurred, the deletion is about to go away, and the DNA repair machinery recognizes this break and puts it in again backwards. Unless a person has interrupted genetic material there are no symptoms. One example of interrupted genetic material is seen in haemophiliac type A. An inversion can cause haemophilia type A if the original break of that inversion cuts the region of factor VIII gene. If a person has uninterrupted genetic material, there are no symptoms. However, there will be problems when that person produces gametes. During homologous recombination, the two chromosomes start to produce loop structures to get themselves lined up for exchange of genetic material. This frequently causes insertions and deletions.

- **Isochromosomes** are chromosomes where the centromere is divided horizontally instead of vertically. This leaves the long arms together at one end and the short arms in another. Duplication of an isochromosome can cause any trisomy such as trisomy 13 (Patau syndrome).



Significance of Chromosome Analysis

- A physician should order a chromosome analysis when there is a suspected chromosomal syndrome.

- General features of chromosomal syndrome that physicians should look for include:
    1. Developmental delay
    2. Intellectual disability
    3. Characteristic facial and limb features (polyadactyly or overlapping fingers)
    4. Growth delay
    5. Congenital malformations (especially when there are 2 or more malformations)

- Indications for chromosomal analysis include:
    1. Ambiguous genitalia is a rare condition in which an infant's external genitals don't appear to be clearly either male or female.
    2. Stillborn with either malformations or no recognizable reason for death.

3. Males with small testes and/or gynecomastia means that they have Klinefelter syndrome.
4. Short females with primary amenorrhea means that they have Turner syndrome.

Good Luck!!