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**Types of genetic diseases:**
Unifactorial abnormalities: the abnormality is in a single gene, the pattern of inheritance of genes can be dominant, recessive, X-linked, etc.
Chromosomal abnormalities: the abnormality is in a larger portion, it includes a whole segment of the chromosome, and it can be abnormal number of chromosomes, or the structure of chromosomes.
Multifactorial abnormalities: it’s not an abnormality in a single gene or a chromosome, it’s the involvement of environment in the abnormalities, so here there is more than one gene involved in addition to the environment, “the genetic involvement may be small while the environmental involvement is big or vice versa”.

**Chromosomal abnormalities**
chromosomes come from fertilization “sperm (23 chromosomes) and ovum (23 chromosome) the result cell will contain (46 chromosomes)”, so chromosomal abnormalities may come from the mother or the father, or it can result after fertilization, due to problems in division.
Normally there are two types of division, meioses and mitoses, all the somatic cells in our body divide by mitoses “which means that one cell will divide into two cells, and both cells will contain “46” chromosomes”, while meioses happens in sperms and ova “the net result will be two cells with “23” chromosomes for both”.
After fertilization, cell division is controlled by many “checkpoints”, which are the phases of cell division “the phases are G0, G1, G2, S, and mitoses”, so when the cell passes from one phase to the other, there will be a “checkpoint” where the cell will be controlled, so if anything wrong happens, the cell will die by apoptosis.

\*chromosome = chromo “means color” + some “means body”
Chromosomes contain proteins in addition to nucleic acids, the basic structure of human chromosome is two strands of nucleotides “2nm”, coiled around nucleosomes “10nm” “which are small beads of proteins present in the nucleus”, the resultant structure will coil also around itself to form chromatin fiber “300nm”, which will also coil to form a loop “700nm” which is a small segment of the chromosome.

Each chromosome consists of two sister chromatids “which are completely identical” connected to each other by a “centromere”, the location of the centromere is important in classification as we will see, in each chromatid above the centromere there is a “short (p) arm”, and under it there is a “long (q) arm”, at the end of each chromatid there is a “telomere”, this telomere is important to keep the integrity of the chromosome, the telomeres contain typical sequence of nucleotides “repetitive TTAGGG”, around the centromere there is another repetitive sequence of nucleotides which is called “microsatellites” (but they differ from the repetitive are of the telomere).

The number of the repetitive areas of the telomere indicates the age of the cell, the more repetitive areas in the telomere, the younger the cell.

The number of chromosomes is the same in any type of nucleated cells, so to study chromosome we choose the easiest cell to manipulate which is lymphocyte “because we can stimulate it to divide using phytohaemagglutinin (PHA)”
\*(PHA) is a non-specific stimulator for lymphocytes, it stimulates it to divide.
To study chromosomes we should get lymphocytes from the patient, so we take blood sample from the patient, then we use anticoagulant “because we need the cells for our study not the serum”, the anticoagulant shouldn’t be toxic to the lymphocytes because we need to culture them “if the anticoagulant is toxic the cells will not divide”, so we use lithium heparin “which is a non-toxic anticoagulant”, then we add the cells to a medium which contains nutrients important for the cell growth, to this medium we add “PHA” to stimulate the lymphocytes to divide, then we incubate the medium for three days.
\*Note cell mitoses have different stages: Interphase, Prophase, Prometaphase, Metaphase, Anaphase, Telophase, Cytokineses
The best phase to study chromosomes in is the Metaphase, “because the chromosomes are long and they start to separate from each other at this stage”, so we need to stop the dividing cells at Metaphase in order to study them, we do that by adding a cytotoxic drug after three days of incubation, examples of these cytotoxic drugs are: Colcemid, Methotrexate. After stopping the cells at Metaphase stage we need to harvest the cells, we do that by putting them in a tube then we put it in a centrifuge. After centrifugation the result will be RBC’s and lymphocytes, so we need to destroy the RBC’s , to do that we add hypotonic solution, to rupture the RBC’s and destroy them, but the hypotonic solution can’t rupture the WBC’s it will just cause it to swell. At this point we add a fixer to the WBC’s “like 3:1 Methanol: Acetic Acid” then we drop the cells on a slide, then we stain the slide with Giemsa stain, then we can study the cells under the microscope.
As mentioned earlier the best cell to study chromosomes from is the lymphocytes, but sometimes we to examine specific tissues in our bodies for abnormalities, so we can use fibroblasts “if we want to examine the chromosomes of a fetus we take fibroblasts from the pregnant mother”, epithelial cells, blood smear, bone marrow, and solid tumor biobsies.

Chromosome number is different in different animals and plants.
There are three ways to examine and study Chromosomes under the microscope:
1- G-banding
2- Fluorescence in situ hybridization (FISH)
3- Molecular techniques

Using Giemsa stain we should look for 3 characteristics to differentiate between chromosomes:
1-the size of the chromosomes.
2-location of the centromere
3-banding patterns: when staining with Giemsa stain the chromosome will contain some dark and some light areas “dark bands and light bands”, the numbers and sizes of these bands make each chromosome unique.

According to the location of the centromere chromosomes can be classified into:
1-metacentric: the centromere is in the middle of the chromosome “p-arm and q-arm are nearly equal”
2-submetacentric: the p-arm here is short while the q- arm is long.
3-acrocentric: here there is no p-arm, instead there are satellites “which are nucleic acid sequences codes for tRNA, but there are no genes in them”


the following photo is important:


when we stain chromosomes with Giemsa stain without treatment the result will be without banding and difficult to study “as in the first picture”, so we should treat them with proteolytic enzymes “like trypsin or papain”, which will act on the nucleosomes and then we should stain them with Giemsa stain, the result will be “chromosome banding (dark and light bands)”, this is called “G-banding”.

\*it’s called G-banding because in this technique the chromosome is divided into 7 groups “A,B,C,D,E,F,G”, “other people say that it’s called G-banding because we use Giemsa stain in it, but this is not the reason because we use Giemsa stain with other techniques like C-banding”.
R-banding: it’s the opposite of G-banding (the areas that where dark in G-banding will be light in R-banding and vice versa)
C-banding: it’s the banding of the centromere “chromosomes are treated with acid and base, then stained with Giesma stain”.
Q-banding: chromosomes stained with a fluorescent dye such as quinacrine.

Light bands: replicated early, contains less condensed chromatin, transcriptionally active, and G-C rich.
Dark bands: replicated late, contain more condensed chromatin, A-T rich.

Functions of centromere: join the chromatids, essential for chromatin segregation and division, contains nearly 100 kilobase in the repetitive area that we mentioned earlier “satellite”.
Telomere: DNA with protein cap, ensure replication, and important for keeping cell integrity.

Generally we use Q-banding in one condition: when a baby is born sometimes we can’t determines if it’s male or female “before Q-banding, doctors used “buccal smears” to determine the gender of the child”, because quinacrine is a very specific dye for the q-arm of “Y Chromosome”.

Sometimes we can’t see the junction between the light and dark bands clearly, especially if there is deletion, translocation or any abnormality in the chromosome.

C-banding: is very important to find the centromere.

After doing normal G-banding, usually on chromosome number 46 we can find about “400-500” bands, but sometimes the abnormality is so small, so we can’t see it using this banding technique, so we should do “high resolution banding”, in this technique we should stop the division of the cell at the prometaphase “because the chromosome in this phase is longer than the metaphase”, using high resolution banding the number of bands increase, for example; for the 46th chromosome the number of the bands will become about “550-600”, by further manipulation I can get even higher resolution and get about “580-800” bands for the 46th chromosome.
In the following photo “q21” band (at normal resolution), became three bands at a better resolution “q21.1, q21.2, q21.3”, while at the high resolution it became five bands “q21.1, q21.21, q21.22, q21.23, q21.3”

\*Each band contains about 5000-10000 kilobase.

Chromosomal analysis used to be manual, by photographing the cell “46 chromosomes” then separate the chromosomes in this photograph and study each one alone to determine if it’s abnormal or not “this took about one week”, nowadays there are fully automated machines which can analyze the chromosomes in 3-4 days “this is called karyotyping, and will determine the banding, the size, and the location of the centromere”.

As mentioned earlier G-banding divides the chromosomes into 7 groups:
Group A: long chromosomes, metacentric centromere “1st– 3rd chromosome”
Group B: long chromosomes, submetacentric centromere “4th-5th chromosome”
Group C: submetcentric and they are large in number “6th- 12th + X chromosome”
Group D: acrocentric “13th-15th chromosome”
Group E: submetacentric “16th-18th chromosome”
Group F: metacentric, but short “19th-20th chromosome”
Group G: acrocentric “21st-22nd + Y chromosome”

Banding help us finding the “address” of a certain abnormality on a certain chromosome.(according to the International System for Human Cytogenetic Nomenclature “ISCN”) If we have an abnormality on chromosome “17”, for example, we should first know if it’s on the “q-arm” or “p-arm”,
then we should know on what region it is “region 1, region 2, …”, then we should know on what band it is “band 1, band 2, … “, then we should know on what sub-band it is “1, 2, 3, …”

then certain location will result “for example 17q11.2”, then we should identify what is the abnormality by putting one of these abbreviations in front of the resultant.
Other notes “according to ISCN”:
“,” is used to separate: chromosome numbers, sex chromosomes, chromosome abnormalities.(used in the example below)
“;” is used to separate: altered chromosomes, break points in structural rearrangements involving more than 1 chromosome. .(used in the example below)

**Example:** 46,XX,t(2;4)(q21;q21)
in the upper example,
“46” indicates the number of chromosomes in the patient.
“XX” indicates that the patient is female “indicates the gender of the patient”.
“t(2;4)(q21;q21)” indicates that the mutation is translocation, and it happened between “q21 region” of chromosome number 2 and “q21 region” chromosome number 4.

**Example:** 46,XX,del(5p)
this indicates that there is a deletion mutation in a part of the p-arm of chromosome number 5 in a female with 46 chromosomes, “not the whole arm is deleted! Just a part of it, but it didn’t specify what part it is in this example, we need to do high resolution banding in order to locate the position of the mutation exactly”.
In most chromosomal analysis, we use the previous technique “banding”, but we can also use Fluorescent insitu hypridization “FISH”.
Hypridization means producing a segment of DNA “using high tech equibment and binding it with another segment.

Usually if we know the sequence of the segment that we want to look for, we can synthesize a complementary segment to it, and this complementary segment should be labeled with fluorescent material, the fluorescent material should be present on a non-reacting area “on the backbone of the synthesized DNA, to prevent it from interfering with the binding between the nucleotides”, the bond between the fluorescent material and the DNA is covalent, so it’s very hard to be separated.
We use it to locate abnormalities, we do that by putting a labeled probe “synthesized strand” with the patient’s DNA inside a tube, then we heat the tube, by heating the double stranded DNA from the patient will be separated from each other, then we cool them to let the complementary DNA strands bind with each other, since the probe has complementary sequence to the DNA strand that I want, it will go and bind to it, so at the end I will get a double stranded DNA with the probe being a part of it I can locate it under the microscope.
For G-banding we need to culture the cell for 3 days to reach the Metaphase “as mentioned earlier”, but in FISH we don’t need to do that, the cell can be even tested at the Interphase “when the cell is at rest”.
In FISH technique also small number of cells are needed “200-500”, so it’s very important in testing whether Bone marrow transplantation was accepted when giving from a male to a female “or vice versa”.
We can assist the acceptance by looking at the cells “when doing bone marrow transplantation from male to female”, if the female didn’t have the “Y chromosome” that came from the male’s bone marrow then she has the disease and her bone marrow has returned, but if the “Y chromosome” is absent then the bone marrow that is working is the one that she has got from the male.

If we want to examine certain chromosome, then we should do a probe for that chromosome and color the probe with certain color to locate the required chromosome.

In G-banding we should look for “the length, location of the centromere, and the size of the bands”, while in FISH we can only use five different colored probe for each nucleotide “for example; green for A, yellow for C, orange for G, …” to stain the chromosome with different colors “spectrum” according to its nucleotides content, this spectrum indicates how many nucleotides inside the chromosome and what they are and their location, and each 2 of the 46 chromosomes will have the same color, so we will have 23 different colors.