Medical Genetics

Sheet: 2

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QUICK RECAP:

- ✓ Both terms "**DNA** and **DNA molecule**" generally refer to "double stranded DNA" unless stated otherwise.
- ✓ **DNA** is wrapped around proteins called **histones**, forming a **DNA-protein complex** known as a **nucleosome**. Nucleosomes are packed together forming coils of **chromatin**. Chromatin is anchored onto what is called a scaffold, forming a **chromatid**.
- ✓ A **chromosome** is defined as condensed chromatin, and depending on the cell cycle phase (before S-phase or after S-phase) the chromosomes of the cell may be either in the form of single chromatids or sister chromatids.
- ✓ Before S phase (=before DNA replication): A chromosome consists of one chromatid (=one double stranded DNA molecule).
- ✓ At the end of S phase: a chromosome consists of two sister chromatids.
- ✓ Most of the cell cycle is taken by interphase which has 3 phases (G1,S,G2)
- ✓ During interphase , the DNA must be accessible and that's why it is found in the diffuse form which is called chromatin, while during M phase (specifically metaphase) the DNA is most condensed (forming chromosomes)
- ✓ Mitosis ; the division of the cell to produce daughter cells that are almost identical to the parent cell in terms of their genetic information and DNA sequences.
- ✓ Meiosis ; the daughter cells are gametes (sperms , eggs) that only have half the number of the chromosomes.
- ✓ each chromosome has a short (p) arm and long (q) arm (later in the sheet , we'll talk about the centromeres positions again)

•• Gregor Mendel's Laws:

1.Law of Segregation: Each homologous chromosome will separate and appear in different daughter cells, such that each gamete receives one allele for a given trait

REMEMBER that homologous chromosomes carry the same genes but not necessarily "identical" genes.

>> **Explanation**: Let's say that chromosome number 2 has the gene for eye color. The paternal copy of this chromosome may have the green eye allele while the maternal copy may have the brown eye allele. During meiosis one daughter cell will have the green eye allele and the other one will have the brown eye allele, and that's why we always say the cells that result from meiosis are **NOT identical.**

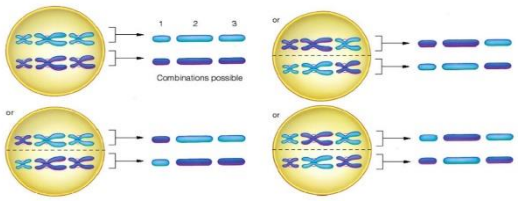
>> **Genes vs alleles** !!!?
Each chromosome has genes which are segments of DNA that carry genetic information for a specific trait. The different versions of the gene are called alleles. In other words, somatic cells have 2 copies of each chromosome (homologous chromosomes) >>> two copies of each gene and each copy is called allele.

2. Law of Independent Assortment: The arrangement of the paired chromosomes with respect to the poles of the spindle apparatus is random along the metaphase plate. the number of possible arrangements is $\mathbf{2^n}$ where n represents the number of chromosomal pairs (in humans n=23). So, the number of possible combinations is over 8 million.

••**In conclusion**: Meiosis generates genetic diversity through:

- The random alignment of maternal and paternal chromosomes in meiosis 1 (**independent assortment**).
- The exchange of genetic material between homologous chromosomes during meiosis 1 (**crossing over**).

Chromosome combinations: independent assortment



••Why do we study CHROMOSOMES?

Knowledge of chromosomes is important in many areas of clinical medicine and research, Many clinical conditions are linked to chromosomal abnormalities and approximately 0.6-1% of all newborn humans have a chromosomal abnormality.

Chromosomal aberrations are noted in:

(1) 20%-27% of individuals having sex reversal or pubertal anomalies.

(2) 33% to 67% of spontaneous miscarriages (which means up to 70% of the fetuses who die through miscarriages, they die due to chromosomal abnormalities).

(3) 2% to 5% of couples having a history of multiple miscarriages.

(4) the majority of cells from leukemia samples or solid tumors.

Why Study Human Chromosomes?

Morbidity/Mortality	Estimate of Cases with Cytogenetic Abnormality
Early embryonic death in unrecognized pregnancies	?? 33-67%
Recognized embryonic and fetal deaths (≥ 5 weeks)	About 30% total; rate varies from 50% at 8-11 weeks to 5% in stillbirths (≥ 28 weeks)
Infant and childhood deaths	5-7%
Birth defects	4-8%
Congenital heart defects	13%
Sex reversal/pubertal anomalies	20-27%
Multiple miscarriages in couples	2-5%
Neoplasms	20-80+%

These numbers aren't for memorizing. Just appreciate that cytogenetic abnormalities have clinical effects

Research Uses for Cytogenetic Evaluation

- **Localization of DNA onto a chromosome(s)**
- **Determination of genomic complement**
- **Characterization of genetic change(s)**
- **Recognition of chromosomal changes following treatment(s) or *in vitro* culturing**

••How to study chromosomes?

First of all, we need to determine from where we should extract the chromosomes. The extraction of the chromosomes from certain tissues depends on the type of the disease and the purpose of the study. For example:

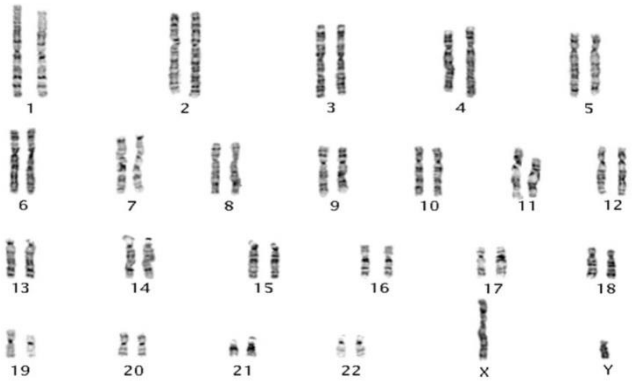
1-Amniotic fluid cells are used to study embryonic genes. Let's say that we have a pregnant woman and through ultrasound it is thought that her fetus has some abnormal features. To confirm that we perform a genetic test by inserting a needle intraperitoneally or intravaginally guided by ultrasound. Then, we take a sample of amniotic fluid cells or chorionic villi cells to use for genetic studies.

2- Bone marrow is used to study chromosomes for individuals having diseases in blood like leukemia since the bone marrow is where hematopoiesis takes place.

3-Peripheral blood (lymphocytes).

4-Skin or organ biopsy.

•• Karyogram (also called Karyotype) :

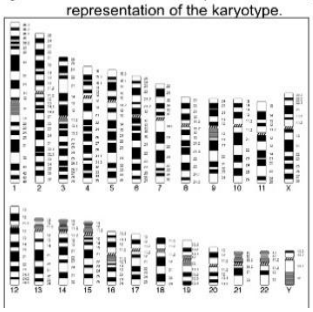


Karyotype: Is a photograph or a diagram of an ordered arrangement of chromosomes from a cell… the chromosomes are placed in a standard order (generally by length, chromosome 1 is the longest and 22 is the shortest followed by the sex chromosomes).

- ✓ The computer image of the chromosomes is obtained from a **dividing cell** (in M: mitosis phase) specifically in **metaphase** because that's when the chromosomes are most condensed. The chromosomes are then arranged as homologous pairs .

- ✓ Each homologous pair of chromosomes consists of one maternally and one paternally inherited chromosome, and the normal diploid chromosome number for humans is 46.

The ideogram of a chromosomal complement is a diagrammatic representation of the karyotype.



•• Ideogram:

An ideogram is a diagrammatic representation of the karyotype.
✓They are used to show the relative size of the chromosomes and their **characteristic banding patterns.**

✓ Notice in the picture there are 24 different chromosomes (22 autosomal and the 2 sex chromosomes). The ideogram is a representation of the different banding patterns of the chromosomes which we use to distinguish the chromosomes to make the karyotype.

✓ Each chromosome has different banding patterns (dark and light bands), and the location of the dark or light bands for the **same chromosome** is different among different types of banding methods.

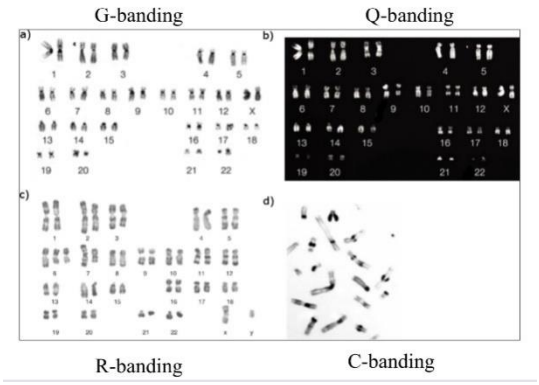
✓ There are (G,R,C,Q,T banding techniques). We're required to know **G** and **R** banding only.

EXTRA (this clarification just for you to understand the technique story and its not mentioned by the doctor):
In chromosome banding, we treat chromosomes with chemicals to stain them and learn about it based on how it stains, just like how we stain cells and their components in order to visualize them. Here we want to visualize the different regions of the chromosome and this technique is done during metaphase (in the M phase) where the chromosomes are in their highly condensed form, thus being easily stained and visualized .There are several different types of stains we can use.

✓ G-banding uses a stain called Giemsa stain. G-banding gives you a series of light and dark stripes along the length of the chromosome

✓ Q-banding uses a stain called quinacrine. Q-banding yields a fluorescent pattern.

✓ C-banding only stains the centromeres. And so on ..



•Now how we do that?

Going back to the pregnant woman and her fetus, after determining which tissue we must use, now it is time to show the chromosomes and study them:
>>> **Remember**, most of the cell cycle is in the **interphase** where the chromosomes are loosely packed (diffused) as chromatin. So, we perform the banding techniques during metaphase where the chromosomes are highly condensed and easily visualized. So, cells must be **induced** to enter the cell cycle and reach the M phase and then stopped there.

>>> Keep in mind that the majority of cells that are obtained from the tissue are **either at rest (G0)** (if the cell can't divide or regenerate it will be arrested at G0 phase like the neurons) , **or in the interphase**. In both cases the chromosomes are diffused so they're very hard to study, so what we can do?

1 – Blood is taken and placed in a petri dish or flask with media (sterile water with nutrients [sugars, proteins, amino acids] in order to keep the sample alive). The media also contains a **mitogen** (it is a chemical that induces the cell to enter the cell cycle). An example of a mitogen is phytohemaglutenin.

2 – After that another chemical compound is added, usually colchicine, which is a **mitotic inhibitor**, to stop the cells at the M phase, specifically at metaphase.
(If this isn't added then the cell cycle will continue and you don't want this to happen , you want the cell to be arrested at M phase and that's why you induce the cell to enter the M phase by a mitogen then you inhibit mitosis by mitotic inhibitors).

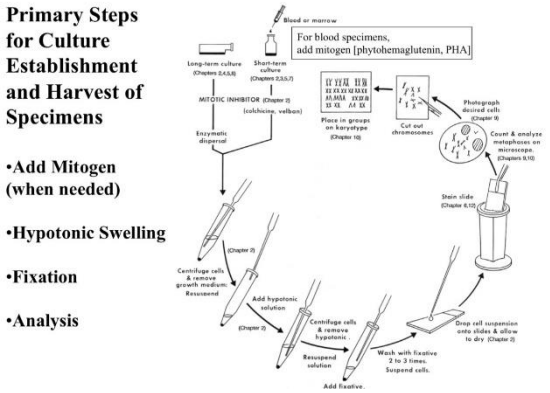
•Note: Colchicine prevents the assembly and polymerization of microtubule filaments of the centrosome, and thereby disrupts their function. Thus, the chromosome will be stuck at metaphase and won't proceed to the next stages.

3 – The cells are centrifuged at low speed to remove the media.

4 – A hypotonic solution is added. This solution enters the cells and make them swollen and fragile.

5 – Suspended cells are dropped onto slides. Cells burst and chromosomes will be scattered on the slide.

6 – Giemsa stain is added to show the chromosomes (it is the standard and most commonly used stain).



••G-banding (GTG) : (the standard banding technique)

This explanation of the technique seems to be more logical than the one mentioned by the doctor, so please read it and if you want what the doctor said refer to the video [11:00 - 12:30] .

✓ In the DNA molecule adenine binds with thymine through 2 hydrogen bonds and Guanine binds with cytosine through 3 hydrogen bonds.

✓ Always keep in mind that gene rich regions are in the **euchromatic areas** of the chromosomes (=less condensed areas) in order to be easily accessible by the transcription enzymes and thus can be transcribed and then translated, while gene poor regions are in the **heterochromatic areas.**

✓ It was found that AT-rich DNA is mostly associated with heterochromatic areas while GC-rich DNA is mostly associated with euchromatic areas.

>> A good explanation for the previous point is that: **AT-rich** areas have genes that are **"tissue specific"** (which are silenced in most of the tissues as they don't need to be expressed, and that's why these areas are mostly condensed). **GC-rich** areas have the **housekeeping** genes which are expressed in all tissues (that's why these areas are mostly euchromatic).

✓✓ Now let's go back to the G banding method: G stands for the **Giemsa stain** that's used for staining the chromosomes.

✓ Staining of the chromosomes involves the interaction between giemsa and certain part of the DNA molecule (by forming hydrophobic interaction with that part facilitated by hydrogen bonding that's found between the base pairs) and this part that's favor the interaction is found more in AT rich than GC rich

✓ Before staining, the chromosomes are first treated briefly with **trypsin**.

>> trypsin : is a digestive enzyme that degrades peptide bonds between the amino acids of different proteins ,,, Trypsin partially digests some of the chromosomal proteins, thereby relaxing the chromatin structure and allowing the Giemsa dye to access the DNA

✓ And As we said the interactive molecules are found more in the AT-rich areas than GC , thus after adding giemsa , it can interact and get stained with its interactive part strongly giving a dark band in the AT areas, while in GC-rich areas, giemsa will bind weakly therefor this area will have light color.

>> This method normally produces 300-400 bands among the 23 pairs of human chromosomes. Each band represents several million to 10 million nucleotides .

••R-banding : also here the written explanation seems more logical if you want the doctor said refer to the video [12:30-13:22] .

It is the reverse of G-banding (the R stands for reverse). The dark regions are euchromatic (GC rich) and the light regions are heterochromatic (AT rich)

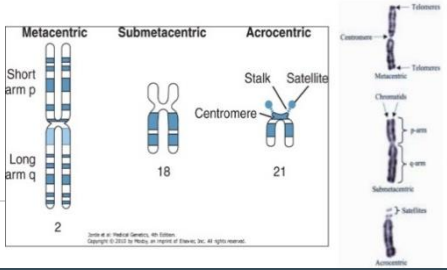
>> This banding pattern is produced by heating the chromosomes before Giemsa stain is applied.

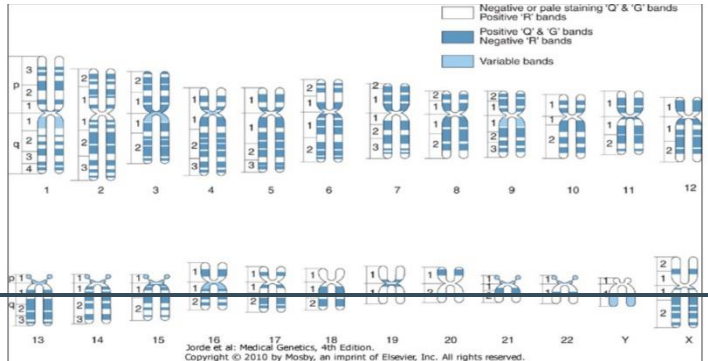
>> Remember that heat has the ability to break down the hydrogen bonds , since AT has 2 hydrogen bonds so heat treatment is thought to preferentially melt the DNA helix in the AT-rich regions (destroy the hydrogen bonds) that usually facilitate the interaction Giemsa, thus it will not bind the giemsa as in the previous technique (G banding) , leaving only the comparatively GC-rich regions to take up the stain (since heat only weakens the hydrogen bonds here) so only GC will take the stain resulting in dark bands for GC-rich areas and light bands for AT-rich areas. And we use fluorochromes to sharpen the color to be more enhanced and visualized. **When to use it**: Used with G-banding to determine whether there are deletions of some regions or not. Also, in some cases R-banding helps confirm the findings of G-banding when there is some bands that aren't clear 100% so flipping the colors might help a bit.

•• Chromosome shape:

*Metacentric: the centromere is located in the center of the chromosome .
*Submetacentric: centromere is displaced from the center. *Acrocentric: centromere is placed near the end. , so there is barely a p arm

- ✓ From the following pictures you can notice that : each chromosome has a (p) arm and a (q) arm and each arm is divided into regions having numbers 1,2,…
- ✓ Numbering starts from the centromere (center) toward the telomere (end).





>> The acrocentric chromosomes are 13,14,15,21,22 (for memorization "**very important**").
>> The "P arm" of **ALL** acrocentric chromosomes contains two distinct regions:

1 – A heterochromatic region that is darkly stained and doesn't code for genes and therefore doesn't codes for proteins. It is called the **satellite** (only a repetitive sequence of base pairs)

2 – A euchromatic region that is lightly stained and codes for rRNA. It is called the **stalk**. (DNA regions that can be transcribed into rRNA)

→ **So where does rRNA originate from? From the stalk of the p arm of ALL acrocentric chromosomes.**

Question: If you have a patient with abnormal clinical manifestations and by doing chromosomal studies, it's shown that there is a deletion in the P arm of chromosome 14, do you think this deletion is the cause for these abnormal features ???

Always keep in mind: **The P arm of an Acrocentric chromosome has no clinical significance** even if the deletion affects the rRNA producing region (stalk) because all five acrocentric chromosomes have the same DNA sequence in their p arm. Thus, the other chromosomes can compensate for the production of rRNA. (So there must be another abnormality that resulted in the patient's clinical manifestations.)

•• High resolution banding:

>> G and R banding produce about 300-400 bands among the 23 pairs of human chromosomes. Each band represents several million to 10 million nucleotides.

>> Normally, damage (mutation, deletion, duplication, inversion and so on) to about 3 million nucleotides or more can be detected in the band by G or R techniques but damage to a lesser number like 200 nucleotides cannot be detected. To detect that we need to visualize the chromosomes in the less condensed form to easily determine the location of the abnormalities.

Here comes the high-resolution banding. This method involves the staining of chromosomes during prophase or pro-metaphase, before they reach maximal condensation. Because chromosomes in these two phases are more extended and less condensed, the number of bands observable for all chromosomes increases from about 300 to 450 to as many as 800 per haploid set. This allows the detection of less obvious abnormalities usually not seen with conventional banding.

>>The picture on the right shows chromosome 4 .

- By using high resolution banding we can visualize the subdivision of the band 21 into sub-bands and sub-subbands as the resolution increases.

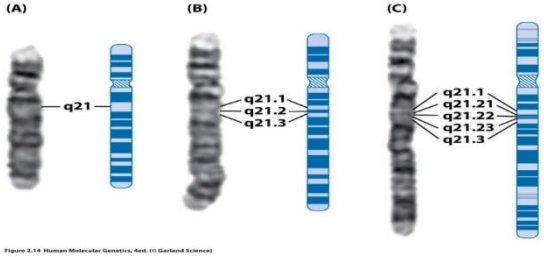


Figure 2.14 Different chromosome banding resolutions can resolve bands, sub-bands, and sub-sub-bands.

G-banding patterns for human chromosome 4 (with accompanying ideogram at the right) are shown at increasing levels of resolution. The levels correspond approximately to (A) 400, (B) 550, and (C) 850 bands per haploid set, allowing the visual subdivision of bands into sub-bands and sub-subbands as the resolution increases. [Adapted from Cross & Wolstenholme (2001). Human Cytogenetics: Constitutional Analysis, 3rd ed. (DE Rooney, ed.). With permission of Oxford University Press.]

•• Structures of chromosomes :

Centromeres :

- ✓ The genetic locus required for chromosome segregation.
- ✓ Contains DNA and proteins. It doesn't have genes but rather it's structural DNA that plays a role during cell division in chromosomal segregation (spindle fibers attach to it, therefore it's a **heterochromatic** region.
- ✓ If we sequence this region, we will find that it contains a **tandem repeat** of 171 base pairs and proteins (The same 171 base pairs repeat) (this region is called the **alpha satellite** and all chromosomes have the same alpha satellite in humans).

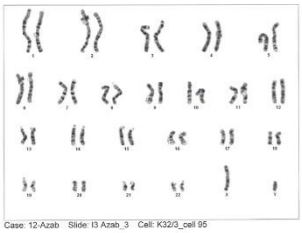
Telomeres:

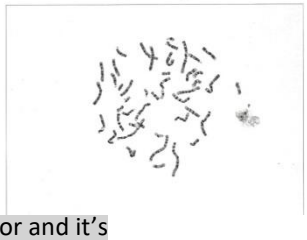
- ✓ It is a specialized structure at the ends of eukaryotic chromosomes. This region represents a **TTAGGG** sequence tandemly repeated thousands of times. (The whole telomere has the same sequence.)
- ✓ It does not code for genes. It's a structural region that maintains chromosomal integrity by preventing end-to-end fusion of chromosomes.
- ✓ A key feature of that region is that it is not replicated by DNA polymerase only, it also needs an enzyme called **telomerase**. This region is associated with aging as the activity of telomerase decreases with age. Each time a cell divides, the telomeres get shorter. During life there is massive rounds of mitosis thus they get too short and the cell can no longer divide; it becomes inactive or "senescent" or it dies
- ✓ This can have advantages too, by protecting against cancer**.** Cells that ***are trying*** to transform into a cancerous cell undergoes massive rounds of replications and divisions. As it does so, telomeres are getting shorter and shorter until the cell dies. But if the cell ***was able to transform*** into a cancerous cell the activity of telomerase returns and the cell can now got for an unlimited amount of divisions. That's why cancer cells are immortal.

Sub-telomeric region :

The sequence of this region is not universal (as it is for telomeres). They can be common but **not identical among all chromosomes**. In other words there is some sequence homology between subtelomeres but they are not exactly the same.

- A karyotype is the number and appearance of chromosomes in the nucleus.
- The chromosomal complement for a normal female is indicated as : 46,XX
- The chromosomal complement for a normal male is indicated as : 46,XY
- To be examined by chromosome analysis for clinical purposes, cells must be capable of proliferation in culture. The most accessible cells that meet this requirement are white blood cells, specifically T lymphocytes.





This figure is skipped by the doctor and it's related to karyotyping (just read it)