University of Jordan
Faculty of Medicine

GENETICS & Molecular Biology

Number: 30 (Genetics2)

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Subject: **Detection of Mutations, Single Gene Disorders 1**

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Price:

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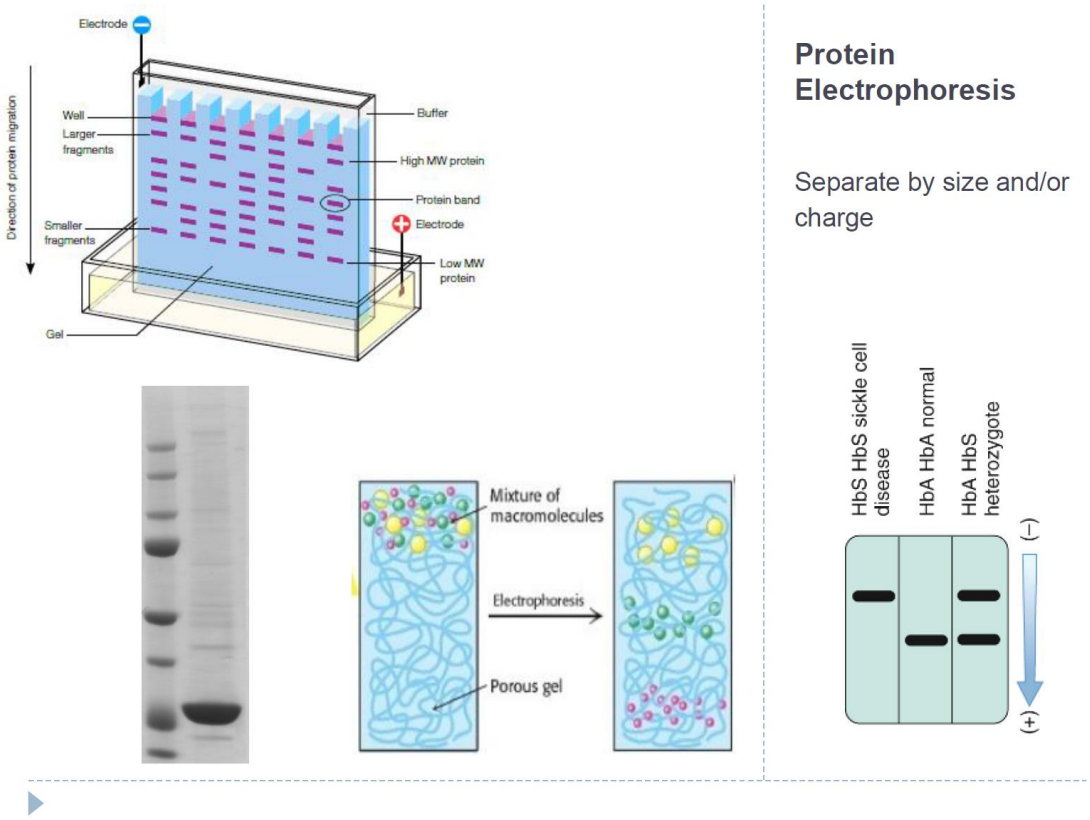
Medical Genetics Lecture 2 p.40-p.75

Topics of this lecture:
1- Detection and measurement of genetic variation.
2- Non-mutational genetic variation.
3- Single gene disorders (AD & AR).

Last time, we talked about genetic variation in the form of mutations. Now we will talk about different techniques by which these mutations are detected.

Techniques discussed in the next sections:
Protein electrophoresis (Western Blotting), southern Blotting and the use of restriction enzymes, PCR, DNA sequencing, and microarrays.



Western blotting

- Western blotting is used for proteins, whereas southern and northern blotting are used for DNA and RNA, respectively. We can still use western blot for detection of mutations because the protein product of a mutated gene will be different from the normal one.
  - For example, if there's a non-sense mutation that results a smaller protein and a mis-sense mutation that makes a larger protein, their rate of migration through the gel will be different from that of normal proteins. Separation of proteins is based on size and charge, but if we coat them with negative charges, the size would be the only determinant of protein migration. Hence, larger proteins will tend to be held on the top of the gel, while smaller ones will migrate down faster.

See the figure above

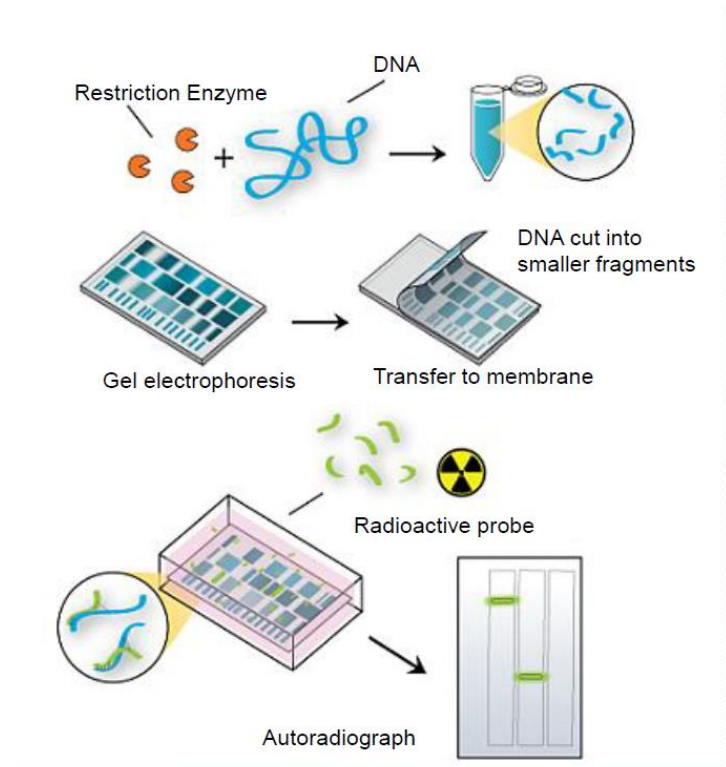
Ex: Detection of mutations in sickle cell disease using western blotting.

1- Normal Hemoglobin (in the middle).

2- Sickle cell trait (the individual is heterozygous): the individual has a normal protein and an abnormal one, so we will end up with two bands, one representing the normal protein and the other representing the abnormal one.

- We had two bands because normal and abnormal proteins are different, so they migrate differently on the gel.

3- Sickle cell anemia: the individual has the two alleles mutated, so we will end up with one band that's different from the normal band.



Southern Blotting & Restriction Fragment Analysis

Restriction enzymes cleave human DNA at specific sequences (restriction sites).

Restriction digest → Restriction fragments

You can detect:
- Insertions
- Deletions
- Mutations affecting restriction sites (e.g. sickle cell disease)

<u>Southern Blotting</u>

- The entire sequence of DNA is very long, so it's not logical to put it on the gel to start migrating. Therefore, we use restriction enzymes to cut it into small pieces.
- For most of the time, these enzymes cut at specific sites (restriction sites). So, if you know the sequence of your DNA, you can figure out the number and size of the fragments you will get based on the restriction enzyme you are going to use.
- Since we know the size and number of fragments, we can differentiate between normal DNA sequences, in which the expected number of fragments will show up on the gel and mutated sequences (i.e. If there's an insertion/ deletion/ mutation at the restriction site, the fragments will change).

→ → You can detect insertions, deletions and mutations at restriction sites.

Ex: Sickle cell anemia, the mutation is at one of the restriction sites and thereby you can detect it using restriction fragment length analysis.

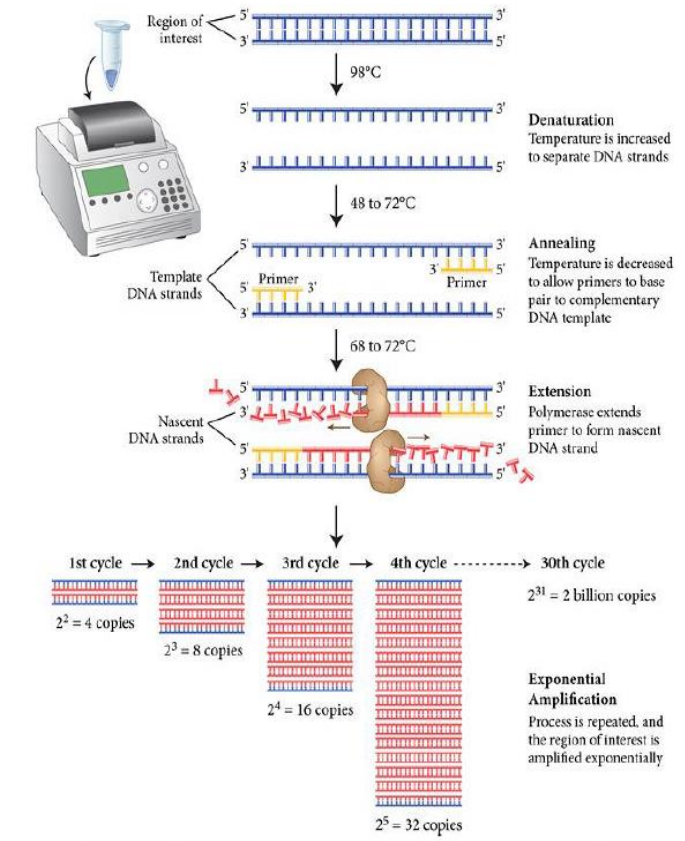
- How can we detect the fragments that correspond to a specific region of DNA?
  Use gel electrophoresis → cut up the DNA → put it into the well and let them run through the gel. Based on their size (there is no charge here) you would separate them, larger fragments on top, and smaller fragments downward.
- Since you might get lots and lots of fragments, you can specifically look for a unique fragment that is meant to be a fragment of disease. You get a complementary probe that has a fluorescent marker on it or a traditional radio-labeled probe and you put an x-ray film on that membrane. It will end up with that particular place showing up on the photo as a band, we call it autoradiogram.

Why do we transfer the gel into a membrane?

1- Fixation of the bands

Proteins and DNA move through the gel in straight directions because you apply a charge, otherwise, they will move and diffuse in all directions. To prevent this random diffusion of proteins and DNA in all directions, we fix the bands on the membrane.

2- These membranes can be used again and again, instead of running the gel over and over.



Polymerase Chain Reaction

Advantages:

Can be used with extremely small quantities of DNA

Faster than cloning

No radioactive probes required

Disadvantages:

Known sequence required

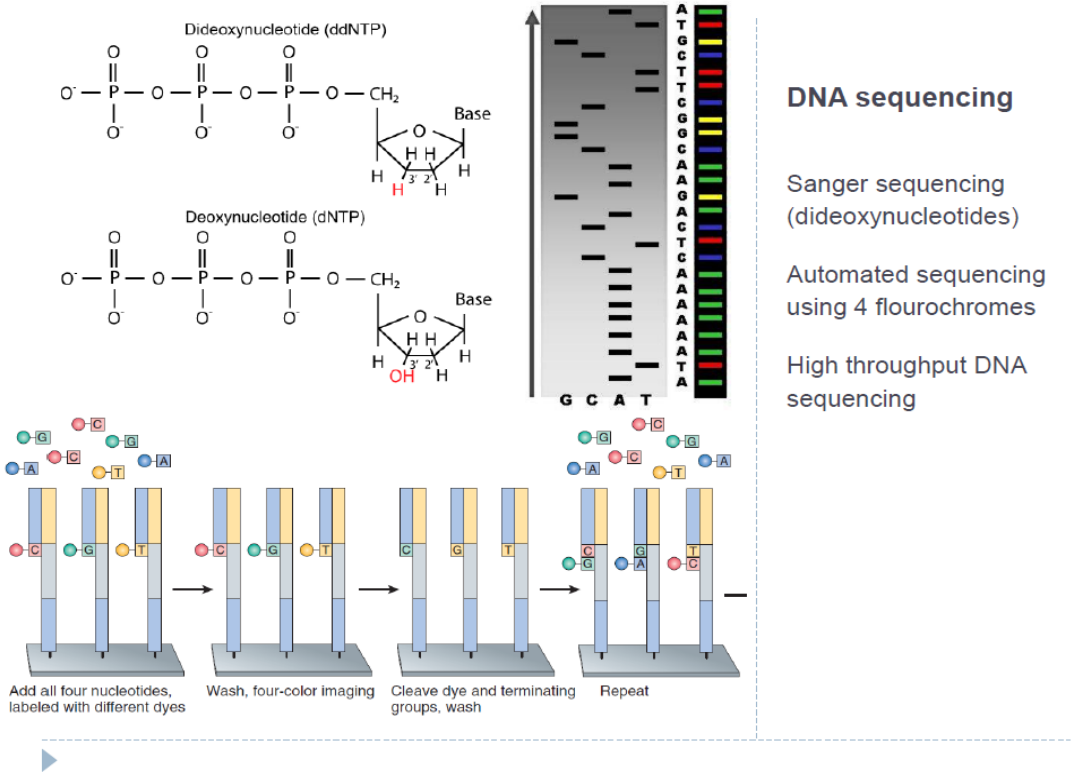
Contamination

Limited to a few Kb

It is used to amplify DNA. The problem of it is that

- You need to know the sequence.
- Mutations, specifically at the primer site, would affect the process.
- Non-specificity of the primers, and that's why primers should be 15 to 20 nucleotides long to decrease the chances of having the primer binding to a non-specific site. Also, when you design a primer, you can check using a software against other genes that contain that particular sequence, if there were any chances of non-specific binding, you pick another sequence.
- Limited by length, so you cannot amplify very long sequences.

- We use a stable form of DNA polymerase that is derived from the bacterium *Thermus aquaticus*. And the high temperature is what separates the two strands instead of the enzyme helicase that works in our bodies.



Sanger Sequencing (dideoxynucleotides method):

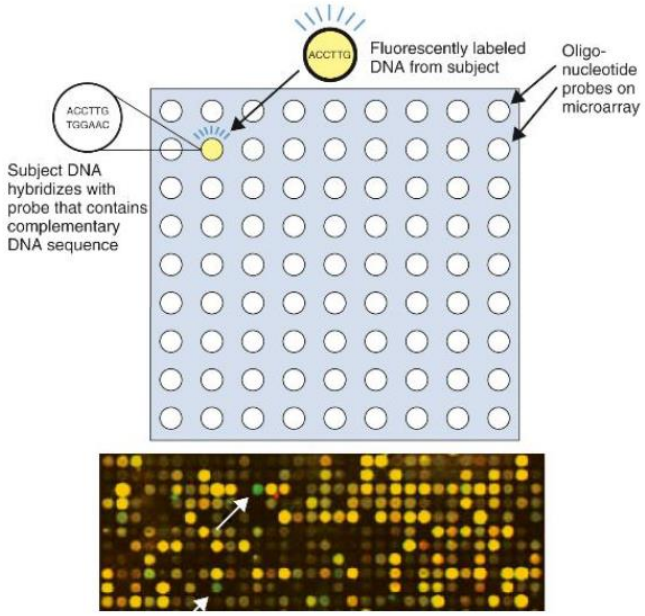
- Why are we using dideoxynucleotides? They terminate the growing chain.
- Four reactions, each reaction contains all normal nucleotides and one dideoxynucleotides. So, the sequence will be terminated many times randomly, depending on which dideoxynucleotide is incorporated.
  - Being a random process makes DNA sequencing a limited technique that cannot be used for very long sequences.
  Let's break it down to simple probabilities; let's assume that the amount of normal nucleotides to the amount of dideoxynucleotides is 50:50, what is the chance of stopping at the first dideoxynucleotide location? 50%.
  What is the chance of stopping at the second dideoxynucleotide location? 0.5*0.5= 0.25.
  Third: 0.5*0.5*0.5 and so on. Now flip it around, the same probability is the probability of continuing, so that means: the likelihood of getting longer sequences is less and less and less. And that's why, to partially solve the problem, we add excess nucleotides versus dideoxynucleotides. Nevertheless, the likelihood of getting very long fragments is less and less and less,

so you are limited to a few hundred base pairs in length.
- Limitations of DNA sequencing:
a- we have to run 4 reactions.
b- sequencing of long sequences is difficult, and time-consuming.

See this animation before you read:
http://www.sumanasinc.com/webcontent/animations/content/highthroughput2.html

However, what we use now is the “high throughput DNA sequencing or what is also called “next generation" sequencing. Here we use nucleotides that have a chlorofluorosence, each one of the nucleotides have a different color and we have machine that can read these chlorofluorescents. Now you can have all the nucleotides and the machine will read whichever one is incorporated. And rather than entering the whole long sequence of DNA, you chop the DNA into small segments and you run these fragments separately on the same machine and the machine will read each of the different fragments, that is why it is called “massively parallel” sequencing.

Now all the genome can be sequenced in less than a week compared to decades! You can get a patient and sequence his/her whole genome. It is cheaper than it used to be, but it is still not that cheap.



Microarrays

DNA
Known mutations only

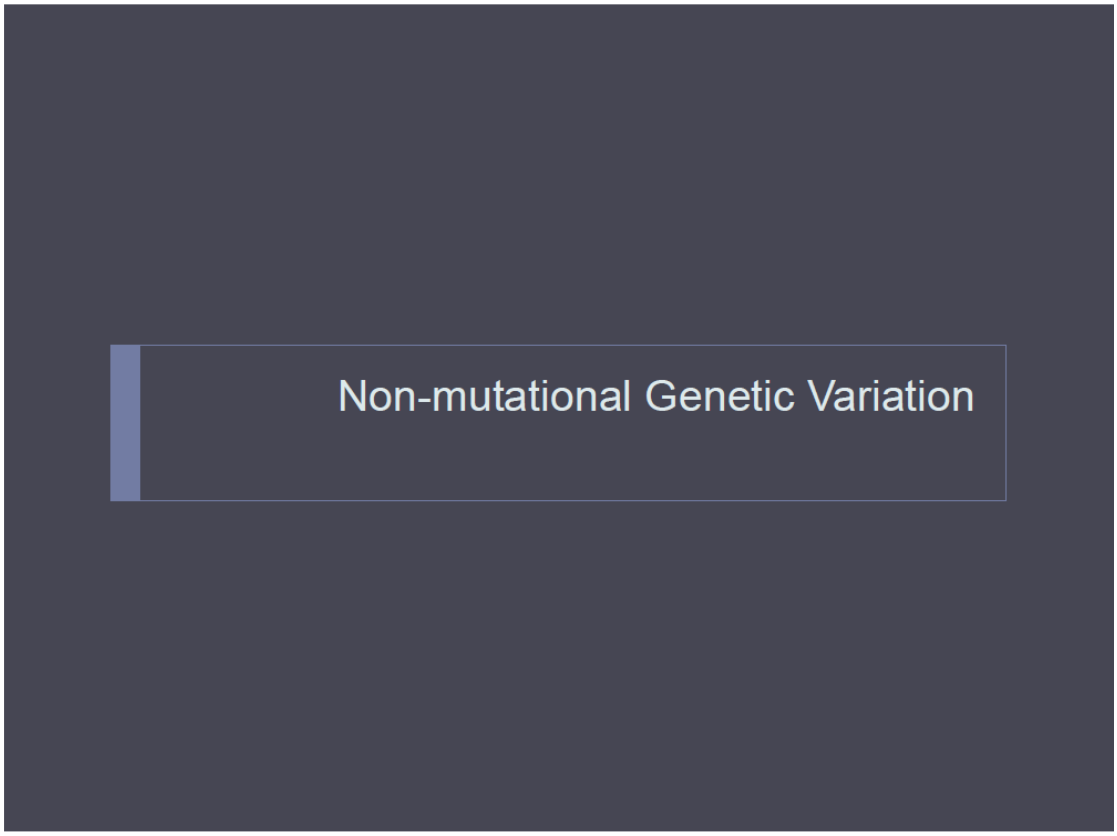
mRNA
Expression

What would you detect in microarrays?

- **Gene expression**, you are looking for mRNA. You have a complementary sequence to your mRNA on the microarray, and your mRNA sticks to it. If it sticks to it more than a normal sample then the gene is over-expressed, and if less, then it's under-expressed.

DNA microarray, you have multiple and multiple copies of the same gene with different mutations. If your DNA binds to that particular part of microarray you have that particular mutation.

- The problem of detecting mutations using microarrays is that you can't detect unknown mutations; you have to know the sequence.



Pages (56-58)

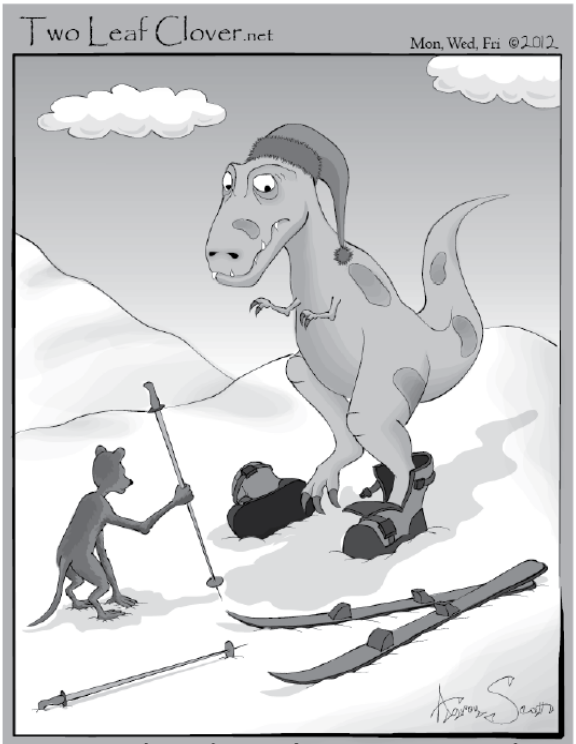
There are three main causes of non-mutational genetic variation that are going to be discussed in the following sections, which are:

1- Natural selection

2- Gene Flow

3- Genetic Drift

1- Natural Selection:



One contributing factor to the mass extinction was the dinosaur's inability to deal with a changing global climate.

Natural selection

See page 56 in the book.
Examples on natural selection and its role in genetic variation:

1- Sickle cell mutation:
Homozygous --> they die early
Heterozygous --> the malarial parasite doesn't survive well in the RBCs of these individuals.
So, there was selection for the mutation in heterozygous individuals and against it in homozygous ones.
In areas where there's no malaria, there's selection against the mutation.

2- Adaptation to live in oxygen-deficient areas (at very high altitudes).
- HIF pathway natural selection

3- Lactose intolerance

2- Gene Flow

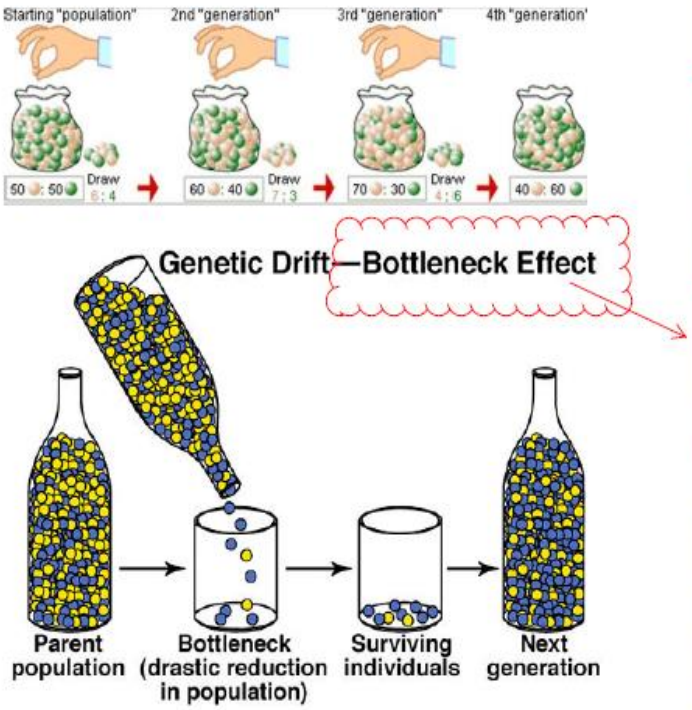
If you marry someone from an American descent, African descent or European descent, this adds more genetic variability to your progeny. This is called Gene Flow

Gene flow

See Page 58

- Gene Flow occurs when populations exchange migrants who mate with one another.

- Examples:
CF --> common in Europeans
Sickle cell disease --> common in Africans
So, sickle cell disease is less common in African Americans than Africans because of genetic flow between African Americans and European Americans. For the same reason, African Americans have CF more than Africans



Genetic Drift See page 57

Genetic drift occurs mainly in small, isolated populations. It may occur in large populations in these two cases:
1- Bottleneck effect
2- Founder effect

- Bottleneck effect:
If there was a catastrophic event that kills off the majority of the population, the remaining individuals will not have the same genetics as those who died and this ultimately contributes to genetic variation.

- Founder effect: Finland's population

2- Gene Drift

- Probability rules:
  If you have a population of ten, and half of them are autosomal recessive to a certain gene. After several generations, the expected percentage of autosomal recessive versus normal gene will remain the same across generations.

  - But actually this doesn't occur. When you flip a coin, you expect to get a percentage of 50% tails and 50% heads, but you might end up with 40:60 rather than 50:50. So, that means that the population after that are not starting from the same place (50:50 point) and that would affect their progeny. This is called genetic drift. This genetic drift is very common in small isolated populations, whereas in larger the populations, the percentage is more likely to the same.

-Genetic drifts tend to occur in small, isolated populations. In large populations, genetic drifts can be a result of either bottlenecks or founder effect.

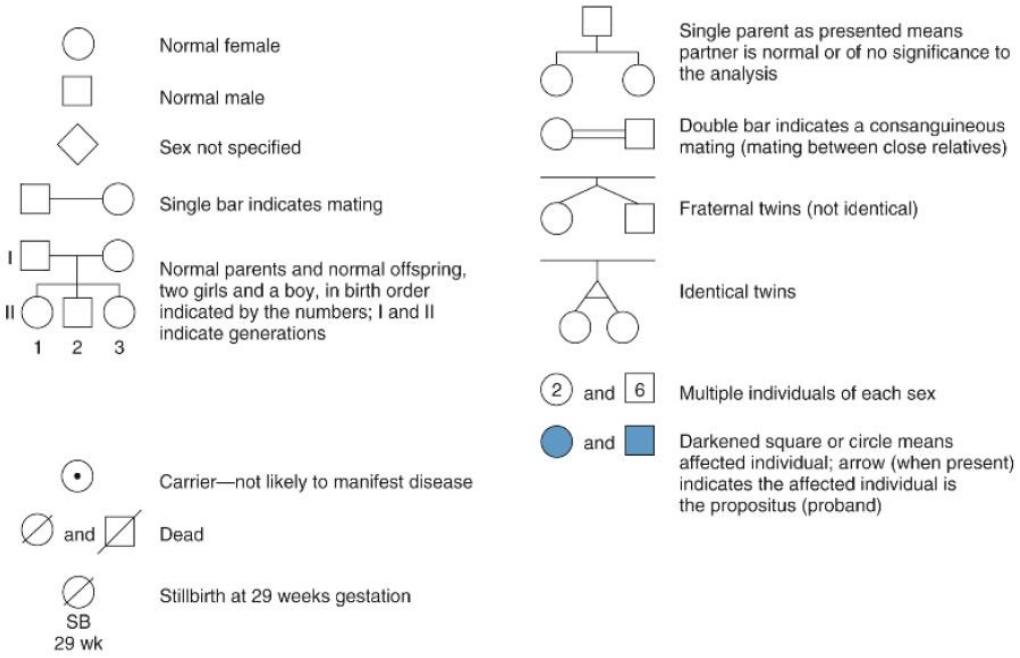
- In Jordan, there's genetic drifts that result from inter-marriage within the same family (i.e. we are dividing the whole population into small populations, in which genetic drifts are common).

  - Examples on Genetic Drift due to "founder effect":
  Finland's population was founded primarily by a small number of individuals (the founders) some 100 generations ago. That's why Finland's population has distinct characteristics that are different from other European populations. For example, they have increased frequency of genetic diseases that are rare elsewhere in Europe. On the other hand, CF and PKU which are common in other European countries are relatively rare in Finland.

  - Genetic drift in Finland proves that genetic drift may be good (CF and PKU) or bad (other genetic diseases).

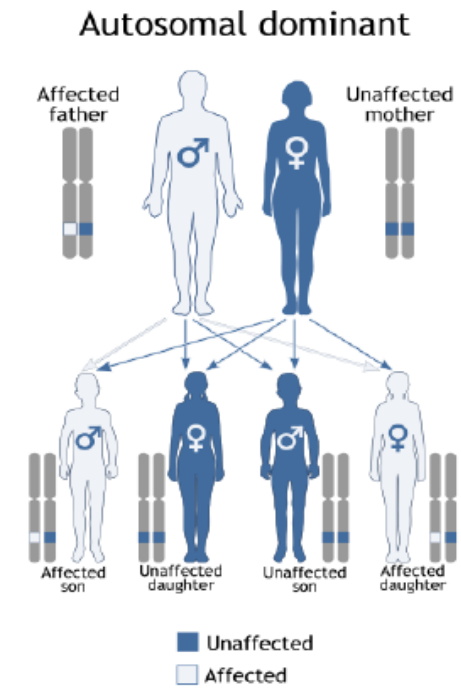
The name “Single Gene Disorders” is over-simplified; while one gene can produce a disease, there are multiple factors that affect it. Among these are environmental factors, genetic modifiers, polymorphisms, other mutations … etc.

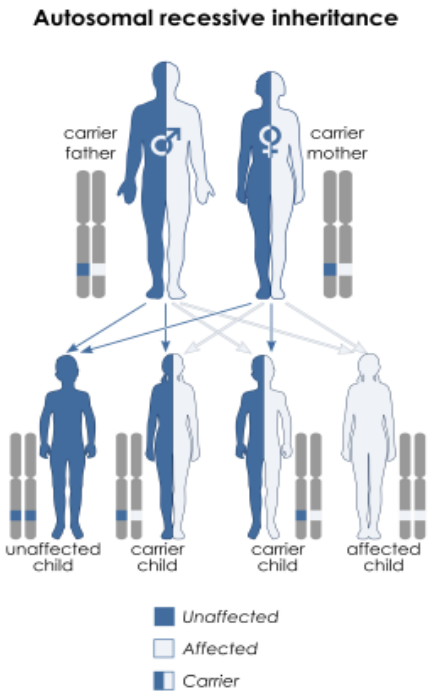
Basic Pedigree



- Proband: is the the first person diagnosed with the disease in the pedigree.
  Proband = Propositus (proposita for a female).

  - Pay attention to all symbols used in the pedigree to able to read it well.





<u>Autosomal Dominant</u>

-The disease is expressed by one gene homozygous and heterozygous

Penetrance: Not everybody with the disease-causing genotype would exhibit the disease phenotype.

-Reduced penetrance (or incomplete penetrance), and age-dependent penetrance. So you would look to the progeny and you would find that only 25% of them show the disease, you might think it is an autosomal recessive disease, be careful, it could be incomplete penetrance. An example is Huntington disease; during this time when the patient is not diseased he/she is by

<u>Autosomal Recessive</u>

-There are carriers

-There could be a disease with only one copy and that is called haploinsufficiency (having half of the protein is not enough for normal functioning). However, the symptoms are more severe when homozygous. That is where natural selection would increase the likelihood of being heterozygous for sickle cell anemia in Africa because sickle cell trait protects you from malaria.

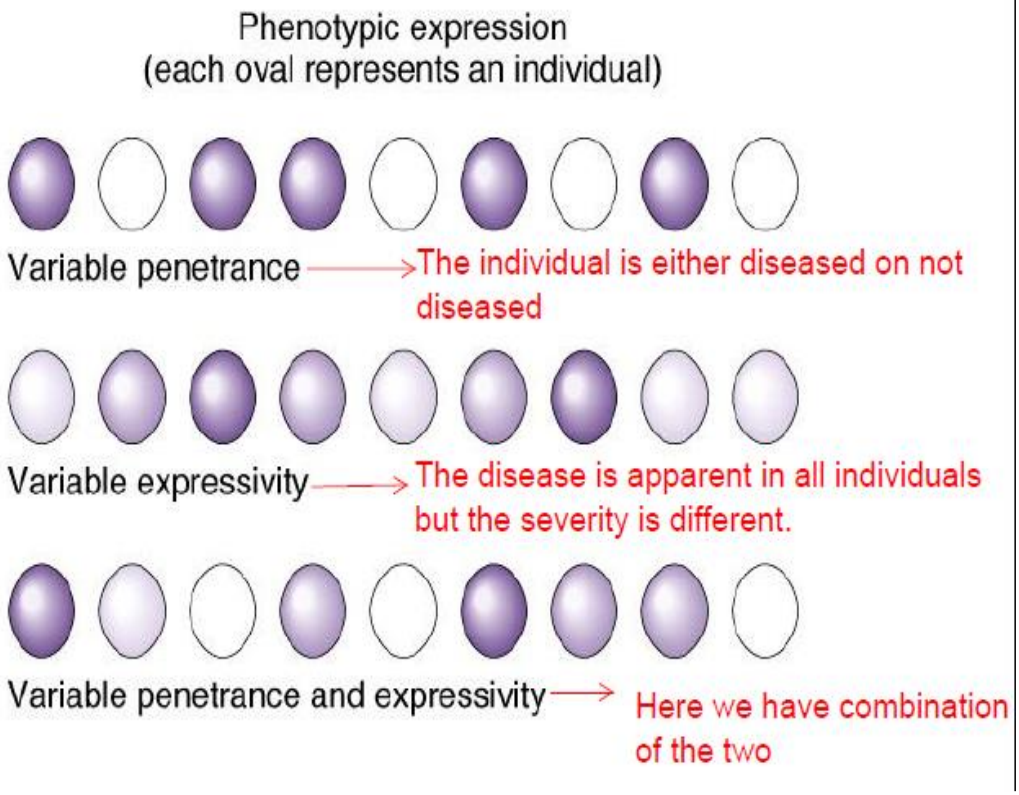
-Very frequently the presentation is at birth.

-There is no penetrance

definition a carrier. So there are actually carriers for autosomal dominant diseases, they are diseased but by now we don't know that they are.

-Let's say that a diseased person and a normal person are having an autosomal dominant disease, what are the percentages of having a diseased child? This depends on whether the diseased person is homozygous or heterozygous, if homozygous then 100% the offspring will be diseased, if heterozygous then 50%.

-Variable expression; remember those environmental modifiers, genetic modifiers, and polymorphisms, etc. so the severity of the disease might vary between patients depending on internal and external factors.



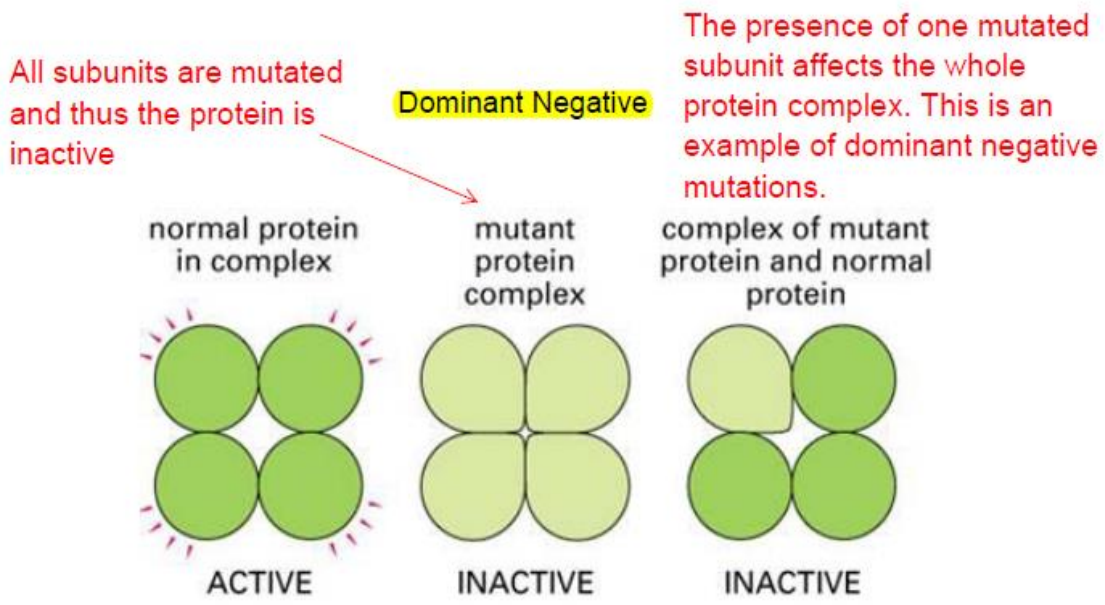
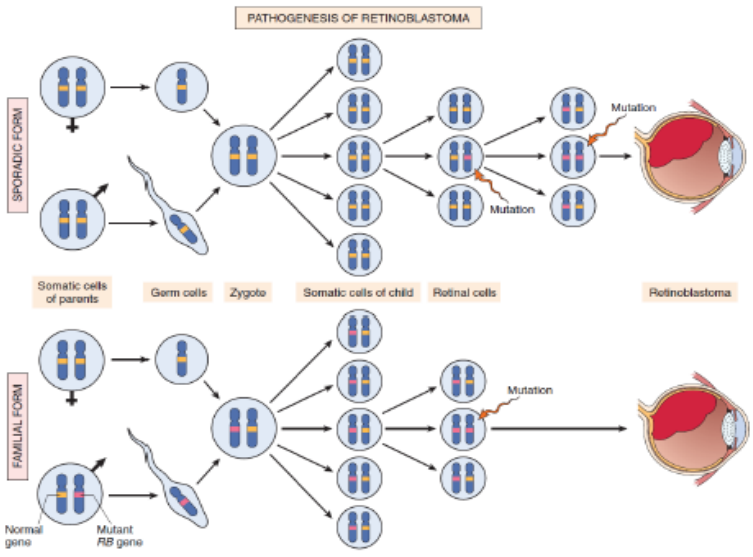


Figure 8–67. Molecular Biology of the Cell, 4th Edition.



***RB* (Reduced Penetrance)**

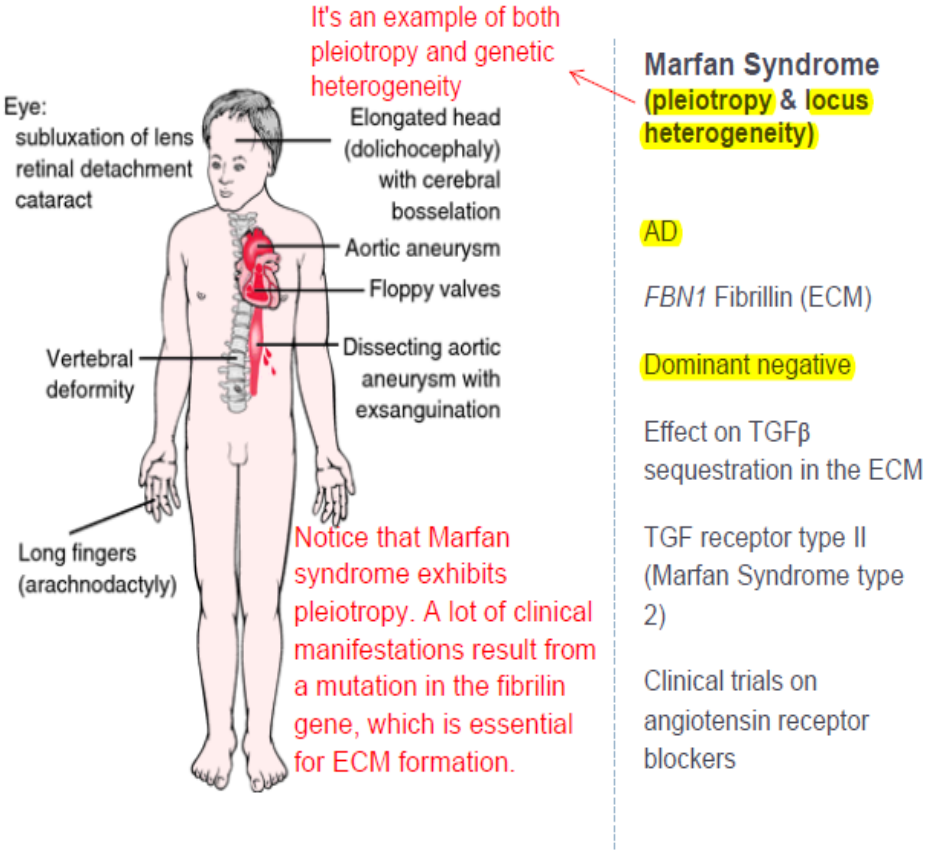
Knudson "two-hit" hypothesis

Two defective copies needed

Familial: -inherited
-somatic mutation

Sporadic: 2 somatic mutations

Retinoblastoma is an example of reduced penetrance, as the individual born with one defective allele doesn't have a tumor. Only when the other allele is lost by a somatic mutation, the individual develops the disease. Shortly, the individual is born normal with no tumor although it's AD mutation and this is by definition, reduced penetrance.



- Genetic Heterogeneity: different mutations in different genes may cause the same disease (i.e. one disease may develop from one of many different mutations).
- Pleiotropy: One mutation may result in multiple phenotypical pictures.
Note: All these clinical manifestations may be classified under the same disease. Ex: Marfan syndrome (multiple manifestations of one disease).

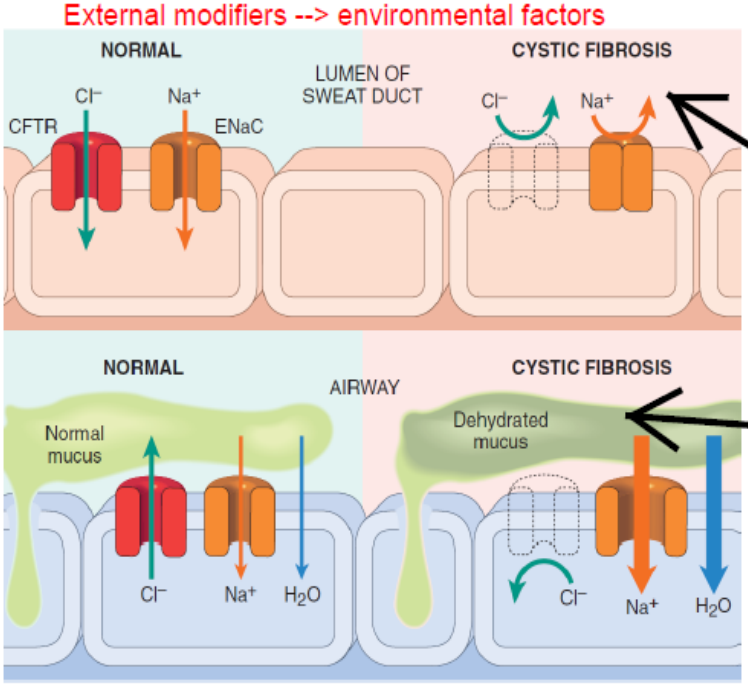
- Marfan syndrome is an example of pleiotropy because it results from a mutation if the fibrilin gene that's important in the ECM. And since the ECM is important in all tissues, Marfan syndrome has a lot of manifestations and thus exhibits pleiotropy.

- It's also an example of locus heterogeneity as it can be caused by mutations either in the fibrilin gene or TGFβ gene.

- It's dominant negative.

- TGF-beta sequestration is affected by the mutation of Marfan syndrome.

Internal modifiers --> polymorphisms may increase or decrease the severity of the disease
External modifiers --> environmental factors



Cystic Fibrosis
(modifiers & allelic heterogeneity)

AR

CFTR Chloride channel

>1300 mutations (mild-severe)

Tissue specific effects

Viscid mucous secretions
- Chronic pulmonary infections
- Pancreatic insufficiency

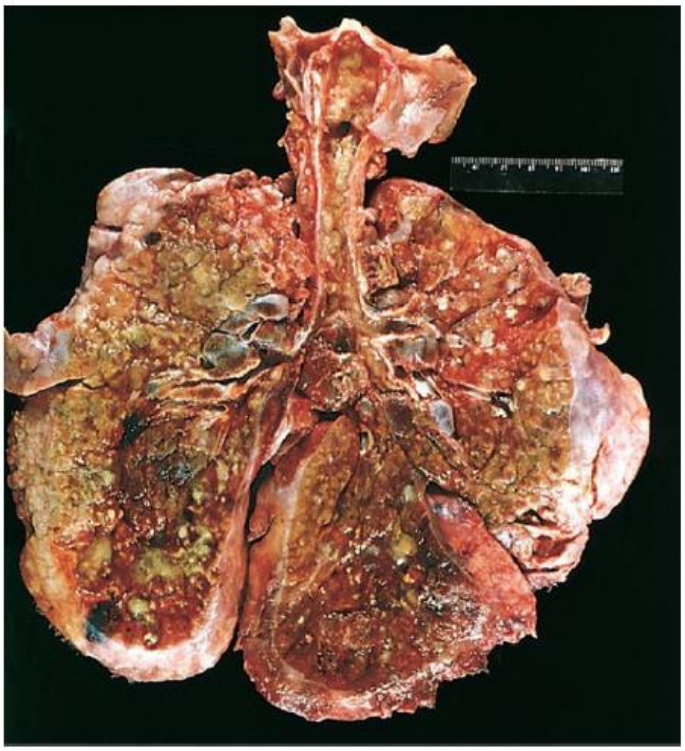
Innate immunity
polymorphism modifiers

Cystic Fibrosis:
- Autosomal recessive disease where you have an abnormality in the CFTR chloride channel.
- If you have this abnormality your sweat would have high levels of chloride, your mucous-producing cells would be affected (viscid mucus secretions that is very thick and could not be expelled). Also, you would have respiratory problems and pancreatic insufficiency.

Modifiers: there are polymorphisms that can increase or reduce the severity of the disease.

Environmental modifiers, for example if you are exposed to certain bacteria, the prognosis could be worse.

So these are examples of internal and external modifiers.



Cystic Fibrosis
(modifiers & allelic heterogeneity)

See page 61

AR

CFTR Chloride channel

>1300 mutations (mild-severe)

Tissue specific effects

Viscid mucous secretions
- Chronic pulmonary infections
- Pancreatic insufficiency

Innate immunity polymorphism modifiers

Homework
Page 74

Consanguinity

- This sheet was re-designed in this way by Hashim Ahmad. Please tell us your opinion about this way of presenting Dr.Mazin's sheets. Dr. Mazin summarizes everything in a brilliant way in his slides, that's why we've made this. Waiting for your feedback. Special thanks to Laila Al-Hafez.

  Good Luck

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