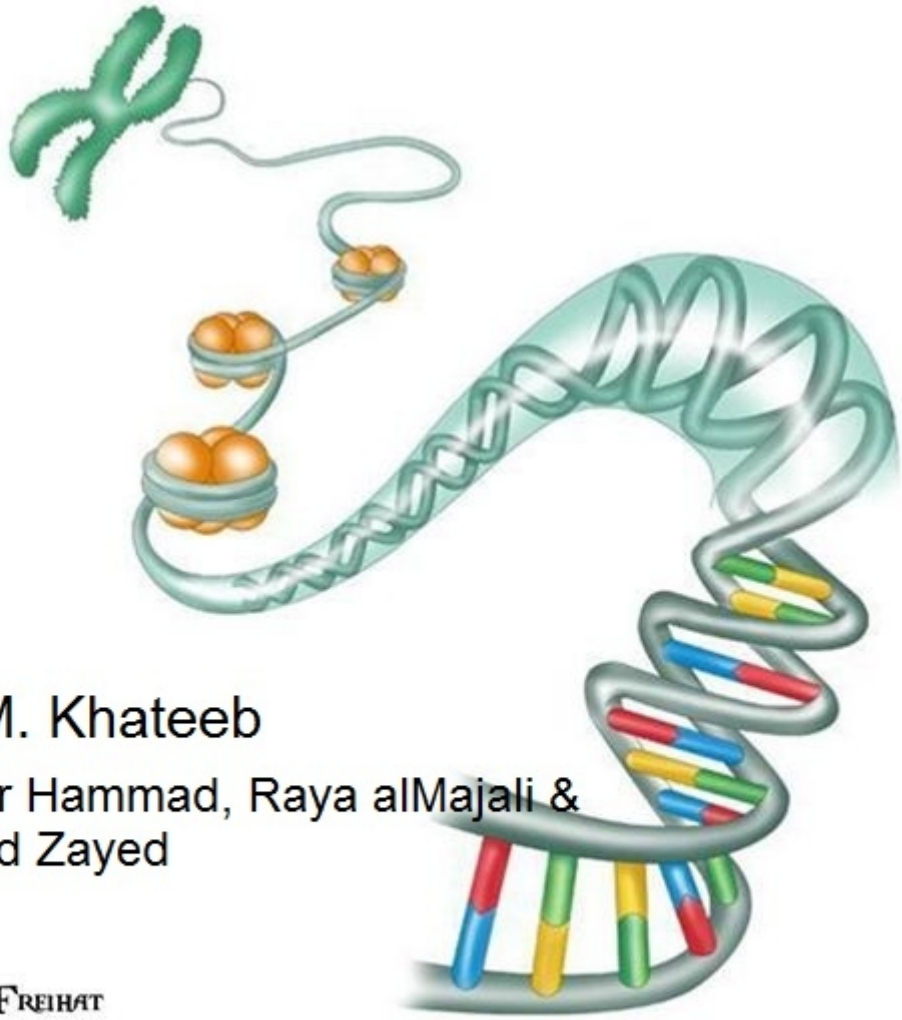
University of Jordan
Faculty of Medicine
Batch 2013-2019

Medical Committee
The University of Jordan

Genetics & Molecular Biology

○ Slides ● Sheet ○ Handout ○ other.....



Sheet#: 9

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Designed by Nadeen Al-Freihat

Tumor Genetics

احكوا بسم الله وابدؤا بهمة , اتذكروا انه بعد **3** أشهر بنصير سنة ثالثة

المادة بدها شدة حيل, حاولوا تركزوا على الاشياء الي عليها شرح وموفقين

The following sheet includes two lectures:D

- Today we will talk about ▪

- ☒ Types of Genetic Alterations in Cancer
- ☒ Evidence that Mutations Cause Cancer
- ☒ Multistage Model of Carcinogenesis
- ☒ Oncogenes, Tumor Suppressor Genes

Genetic Mechanisms of Tumors

There are many **abnormalities**at the cellular, genetic, and epigenetic level that ultimately reprogram a cell to undergouncontrolled cell division,.

Types of chromosomal abnormalities

- Gene deletions which will end up with amplifications
- Mutations
  - interstitial mutation(inside the gene)
  - Point Mutations
- Genetic Instability

All these**abnormalities** lead to DNA damage

Genomic instability is often associated with cancer and can be indicative of a poor prognosis for some types of cancer.

- Microsatellite Instability (MSI)
- Chromosomal Instability (CIN)

DNA can be damaged by physical;biological and chemical mutagens. The DNA in every cell in our body is constantly in danger of being damaged. But cells contain many different proteins whose job is to repair damaged DNA. Most DNA damage is repaired immediately, with no ill effects. But if the DNA damage is so severe**(irreparable**). The cell will activate the pathways of p53 to induce ***APOPTOSIS***.Sometimes mutations in important genes mean that a cell no longer understands its instructions, and starts to multiply out of

control. It doesn't repair itself properly, and it doesn't die when it should. This can lead to **cancer.**

- ✓ Cancers arise when critical genes are mutated, causing unregulated proliferation of cells.
- ✓ These rapidly dividing cells pile up on top of each other to form a tumor.
- ✓ When cells detach from the tumor and invade surrounding tissues, the tumor is **malignant** and may form secondary tumors at other locations in a process called **metastasis.**
- ✓ A tumor whose cells do **not i**nvade surrounding tissues is **benign**.

☒ Note: The most common types of cancer treatment, such as surgery, chemotherapy, radiation therapy, targeted therapy and many others

❖Etiologic agents:

- Environmental (chemical, physical, and biological) (85%)
- Hereditary (familial cancer syndromes) (15%)

✓ **Familia Clustering of Cancer**
Epidemiologicalstudies (:P) show anincreasedrelativerisk of cancer in individualswith a familyhistory of cancer.

✓ Normal response to the Environmental Signals is the alteration of Growth Factors,Steroid factors and cell-cell interactions, which ends up in:

- Differentiation
- Growth and Death
- Mitosis

The vast majority of cancers are related to ENVIRONMENTAL conditions

Signaling Molecules

- Growth Factors

- Receptors for Growth Factors and Hormones
- Intracellular Signal Transducers
- Nuclear Transcription Factors
- Cell-Cycle Control Proteins
- Receptors signaling outside the cell where the signal molecule is picked up by a receptor ,then there will be transient signal through cascade including molecules and proteins(transduction factors till eventually reaching the nucleus to turn on or off a certain genes)

Oncogenes and the cell cycle

- ☒ The cell cycle has multiple checkpoints(on each transition states)
- ☒ The cell cycle checkpoints play an important role in the control system by sensing defects that occur during essential processes such as DNA replication orchromosome segregation, and inducing apoptosisor cell cycle arrestin response until the defects are repaired.
- ☒ Specific cyclins made at specific times for each transitionG1/S, S/G2, G2

1-(G0-G1): where Quiescent cells which are cells that are not actively replicating (found in the G0 phase) can emerge to G1 phase by stimulation of certain growth factors.

2-(G1-S) transition point known as the *restriction point*, in which we need to be sure that our DNA is corrected and repaired during G1 .

3-(G2-M) checkpoint it is a transition state between G2 and M phase where we want to check and make sure that the replicated DNA is not mutated.

- In tumor cells, cell cycle checkpoints are often deregulated due to genetic defects in the machinery that alternately raises and lowers the abundance of the cyclin/CDK complexes

Oncogenes and Tumor-Suppressor Genes

- **Oncogene:**dominant-acting stimulatory genes that cause cancer
- **Proto-oncogenes:** responsible for basic cellular functions in normal cells; when mutated, they become oncogenes.
- **Tumor-Suppressor Genes:** Inhibit cancer and recessive acting; when mutated, normal cells become cancerous.
- Functions of oncogens are

1- Regulation function

- ✓ Tumor suppressor genesare needed in **Go-S** transition
- ✓ DNA repair genes responsible for **S-G2**transition
- ✓ Oncogenes are important for cell passage from **G2-M**

2-Chromosomal translocation

3-onco -virus insertion

4-Gene amplification

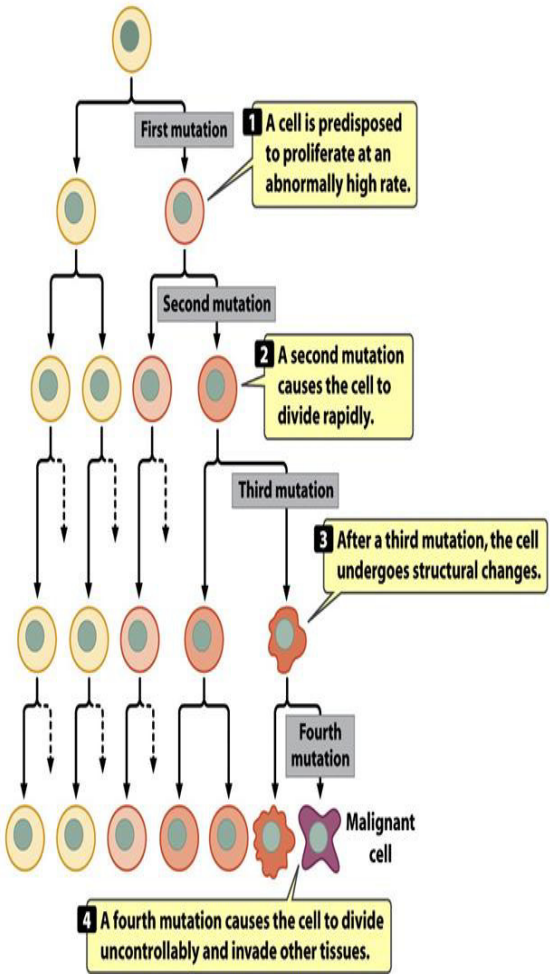
Cancers develop through an accumulation of **somatic mutations** (**not a single**) in **proto-oncogenes** and **tumor suppressor genes**(**4-7 mutations are** necessary for full Transformation)such as

- ✓ **Point mutation**
- ✓ **Duplication or deletion of chromosomes**
- ✓ **Loss of heterozygosity**

Tumor formation, growth, and metastasis depend on the accumulation of mutations in several**different genes.**

- **Single mutation is not enough to develop cancer**

Now look at the figure below which illustrates the <u>accumulation of mutations</u> and their effect



The morphology of the cell resembles the **<u>NORMAL</u>** cell at the first three hits, but at the fourth hit the morphology will appear abnormal.

<u>Common Inherited Cancer Genes</u>

- Familial Retinoblastoma(RB gene mutation)
- Li-Fraumenisyndrome(P53 mutation)
- familial adenomatous polyposis syndrome(APC gene mutation)

<u>NOTE</u>:RB, APC and p53 aretumor suppressor genes

- Breast cancer(BRCA 1and BRCA 2 mutation)
- P16 Familial Melanoma
- RET proto-oncogene and multiple endocrine neoplasia

We will explain some of these cancers:

1- <u>Retinoblastoma</u>

- ✓ Deletions 13q14 or mutations of the RB1 gene
- ✓ Cell cycle regulatory protein that inhibits G1 to S phase transition
- ✓ 80% de novo mutations (sporadic)
- ✓ High rate of loss of heterozygosity in tumorTissue(meaning one allele is mutated and the other is normal),in SPORADIC cases

Stop hereplz(ما خلصت الشيت لسا بنسمي);there are definitions ,we should explain them

Penetrance: The proportion of genotypes that actually show expected phenotypes(شو؟؟؟؟)

For some traits, the phenotype might not occur as often as the genotype. For example, say everyone in populationcarries the same allele combinations for a certain trait, yet only 85% of the population actually shows the phenotype expected from those allele combination.

What is the importance of this information?

Penetrance is the probability a person with the genotype will show the phenotype. If the penetrance of *retinoblastoma* of 90%, then it means 90% of individuals carrying the mutation will express the trait. Note that it says nothing about the severity

- ➢ Penetrance of *retinoblastoma* is 90%
- ➢ Obligate carriers(NON –penetrance carriers) are 10%
- ➢ to get the disease we need to lose both alleles

Sporadic (non hereditary) **vs**Familial(hereditary)

in a **familial** case all cells in the body have a one mutated allele in RB gene due to inheritance of it by families, so a one single somatic mutation where the normal RB gene is lost in retinoblast as a result of somatic mutation **(BILATERAL form)**

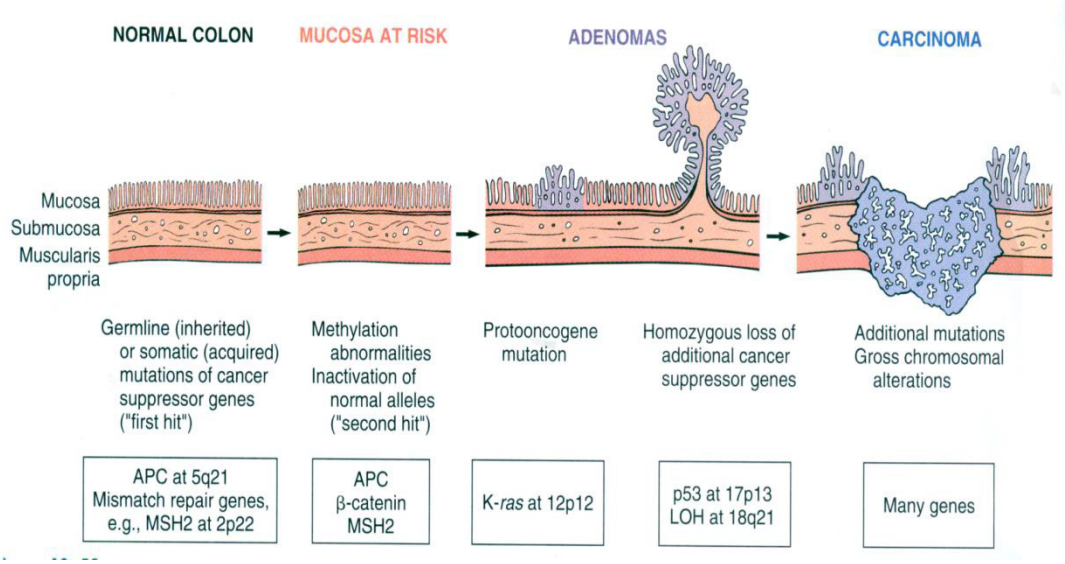
In sporadic case: both RB alleles are normal alleles not mutated ,so to get the disease we need to lose the normal alleles due to somatic mutation of both alleles, here we need two somatic mutations to get the cancer (UNILATERAL form)

2- The Familial Polyposis(Autosomal Dominant)

- ✓ The familial polyposis gene (APC), which strikingly predisposes to colon cancer.
- ✓ This tumor suppressor gene has been shown to function as a major regulator of the Wnt pathway,
- ✓ Cytogenetic analysis showed an interstitial **deletion** of chromosomal region **5q**(deletion of the APC gene) can be considered as causative for FAP.

The sequence of mutations in Familial Adenomatous Polyposis

1- Del 5q21→without pathological abnormalities
2- Another mutation which is Del 5q21→ without pathological abnormalities
   - ☒ (Most of these mutations cause the production of an APC protein that is abnormally short and presumably nonfunctional.)
3- Third mutation in 12p12→ leads to overgrowth of epithelial cells
4- P53 mutation → the ADENOMA is developed
5- The last mutation is del (17p)→carcinoma with manifestation
   - ☒ There are five different mutations(the figure below)
   - ☒ Genotype and phenotype varies among patients
   - ☒ There is a familial tendency

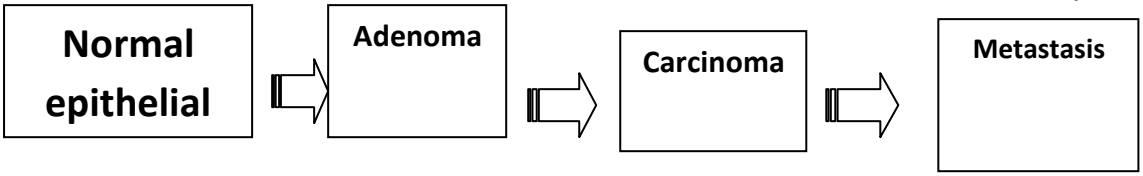


3- Hereditary non polyposis colorectal cancer(HNPCC)

- ✓ It is an autosomal dominant genetic condition
- ✓ Penetrance of HNPCC is 90%

☒ **Cancer and repair: HNPCC**

- Mismatch repair defects.



- **In each step there is a new Mismatch repair defect**

DNA mismatch repair genes have been identified that, when mutated, cause susceptibility to hereditary non polyposis colorectal cancer (**HNPCC**). Mutational inactivation of both copies of a DNA mismatch repair gene results in a profound repair defect and progressive accumulation of mutations throughout the genome.

- Examples of cancers that need a multiple mutations in different genes
  - Astrocytoma
  - Androgen-Independent Prostate Cancer

4-Breast Cancer (BRCA1 and BRCA2)

- High (60-80%) lifetime risk of breast cancer, both genes.
- Increased **ovarian cancer** risk (BRCA1>BRCA2)
- Surveillance for both indicated; mammography, MRI gene
- Consider prophylactic surgery to prevent this type of cancer
- It is an Autosomal dominant disease

❖ SOLID TUMORS SARCOMAS

One example is t(11;22) seen in **Ewing's sarcoma** in which the DNA binding domain of a transcription factor FLI1 is fused with the trans -activation domain of EWSR1 gene

* RECURRENT ABNORMALITIES EPITHELIAL TUMORS:

- ✓ Small cell carcinoma of the lung del(3)
- ✓ Wilm's tumor del(11)
- ✓ Breast Her-2/neu amplification

* Uterine leiomyomas and leiomyosarcomas

- ✓ Benign tumors such as leiomyomas also show recurrent chromosomal abnormalities such as t(12;14) and deletion of 7q
- ✓ Leiomyosarcomas show complex chromosomal rearrangements(loss and gain in the same tumor)

* Mechanisms Leading to Loss of Heterozygosity:

- **Heterozygosity** :Human somatic cells, contain two copies of the genome, one from each parent (chromosome pair);
- Loss of heterozygosity due to loss of one parental copy in a region.
- Loss of normal alleleresults in
  - ✓ Chromosome loss
  - ✓ Deletion
  - ✓ Unbalanced translocation
  - ✓ Loss and reduplication
  - ✓ Point mutation
  - ✓ Mitotic recombination

That was written by Noor Hammad

Chromosome abnormalities and cancer

Chromosomes during division of the cell may have certain abnormalities:

1) Non disjunction segregation of chromosomes:

When a normal diploid cell during division has an **error in the centromere** so the chromatids won't be separated from each other and we will end up with a **tetraploid**.

2) Problem in replication or repair error of that abnormality:

We will end up with **chromosome aberrations (translocation, deletion, amplification).**

3) Spindle error:

We will have **aneuploidy.**

Chromosomal translocations

1- The most famous translocation is the one between chromosomes **22 and 9** (Philadelphia chromosome translocation). It's a reciprocal translocation between these two chromosomes.

It'll end up in a **Chronic Myelogenous Leukemia.** The translocation in this case will end up with a new gene which becomes an oncogene and that oncogene will activate the production of certain proteins which will cause the tumor (the proteins).So there is **insertion** of one gene into another.

2- Translocation between chromosomes **15 and 17** (gain duplication) which causes **APL (Acute Promyelocytic Leukemia "from wiki" or Acute Plasma Lymphocyte Lymphoma "as the dr said").**

**Note that If we looked at the normal immune cell development, these abnormalities may start at the stem cell or they might be seen at the functional B-lymphocyte or T-lymphocyte.

For example , At the stem cell we can find the 9-22 translocation which will cause chronic myelogenous leukemia or we may have translocation from 9-22 which will cause Acute Lymphoblastic Leukemia (ALL) .or Acute Megakaryocytic Leukemia which results from a translocation from 1 to 22, or AML results from translocation (8,21). These mutations may happen also in mature lymphocytes when they respond to a stimulus.

**Note: In non-Hodgkin's lymphoma there are more than 20 chromosomal aberrations that may cause it.

**What will happen in these translocations ?

We will mention two examples of translocations that happen in BurkittLymphoma : translocation between chromosome 8 and 14 (so the gene responsible of the heavy chain of immunoglobulins and c- myc gene will be translocated resulting in activation of the c-myc gene thus cancer) and the translocation between chromosomes 8 and 21 (AML1-ETO translocations).

Chromosomal deletions

Examples on these are solid tumors like: breast carcinoma, small cell lung carcinoma (in chromosome 3) ,Familial polyposis and other tumors .

In the third column of this table you will see genes that are associated with these cancers.

Chormosome Region	Disorder(s)	Associated TSG
lq	Breast carcinoma	Unknown
3p	Small~celllung carcinoma	Unknown
5q	Familial polyposis coli; colorectal carcinoma	MCC
11 p	Wilms tumor; rhabdomyosarcoma	WTI
13q	Retinoblastoma; breast carcinoma; osteosarcomas	RB 1
17p	Colorectal carcinoma; breast cancer	TP53
18q	Colorectal carcinoma	DCC
22	Neurofibromatosis, type 2	Unknown

Control and the prevention of genetic diseases

Normally, there is no effective treatment for genetic diseases . For example if a person has thalassemia there is no effective treatment or drug for this disorder.

In human genetic diseases there are a lot of complex factors that affect the genotype and phenotype, the extremely important factors are the one that cause activation of those diseases.

In the last two decades there were a significant development in the knowledge of the causes of these diseases and other information related to these diseases .

The prevention of these diseases are like any other disease have a primary prevention, secondary prevention or tertiary prevention (vaccine, environmental changes or Antibiotic treatment) .

A) Primary prevention :

When we talk about early detection and interaction with the condition .to be able to do it there should be certain programs . These programs should be adapted by the government and there should be rules to do it.

-For example :for screening . what type of screening can we use ?

1- Carrier screening:

which means to test the person if there is a carrier in the family or a person who is affected .

2- Premarital screening: (before marriage)

to know if there is a carrier or not.

3- Neonatal screening: for the new born.

4- Prenatal diagnosis:during pregnancy

5- Preimplantation diagnosis and others.

-What makes us use screening tests for a certain disease and not for the other diseases? (principles and conditions of doing screening)

1) prevalence of the disease in the population: screening is done for diseases with high prevalence in the population.

2) cost of the test: we have to choose a cheap test.

3) the test should be highly sensitive and specific.

4) there should be a conformation test for it (a test that confirms that the previous test was true) .

5) there should be a diagnostic urgency : how much we have to wait in diagnosing the disease after birth, we screen for disease which can be diagnosed as soon as possible.

6) Government mandate: every child should be screened and each affected person should be treated, because it would be a

waste of time and effort to screen for a disease and the treatment of it isn't provided for patients.

B) Secondary prevention :

the prevention of clinical manifestation of the disease , for example : thalassemia patient if we treat him well if we give him a good supply of blood and if we give him vaccines …etc

C) Tertiary prevention :

For example, if a female patient's genes showed the tendency for having breast cancer, she would surgically remove her breast as a tertiary preventive method.

Premarital screening which is very important, the population screening should be decided by the government depending on whether this population is at high risk or not for certain diseases.

The main goal of this screening is to reduce the prevalence of a certain diseases in the community. So the beta thalassemia in Jordan has a prevalence around 1 in every 2500 births every year, so the expected number is around 80-90 new cases will be born in every year with thalassemia. Now, around 1400 cases are receiving proper treatment and follow-up.

To sum up; The goals of Premarital screening:

Conclusive counseling of identified carriers

1. Can influence marriage decision
2. Allows informed reproductive decisions
3. Marks up individuals for prenatal diagnosis

4. The ultimate goal is to reduce the birth incidence of beta-thalassaemia in Jordan

When the program started there was:

1- Education of the public about the meaning of screening.

2- Training of health professionals (very important).

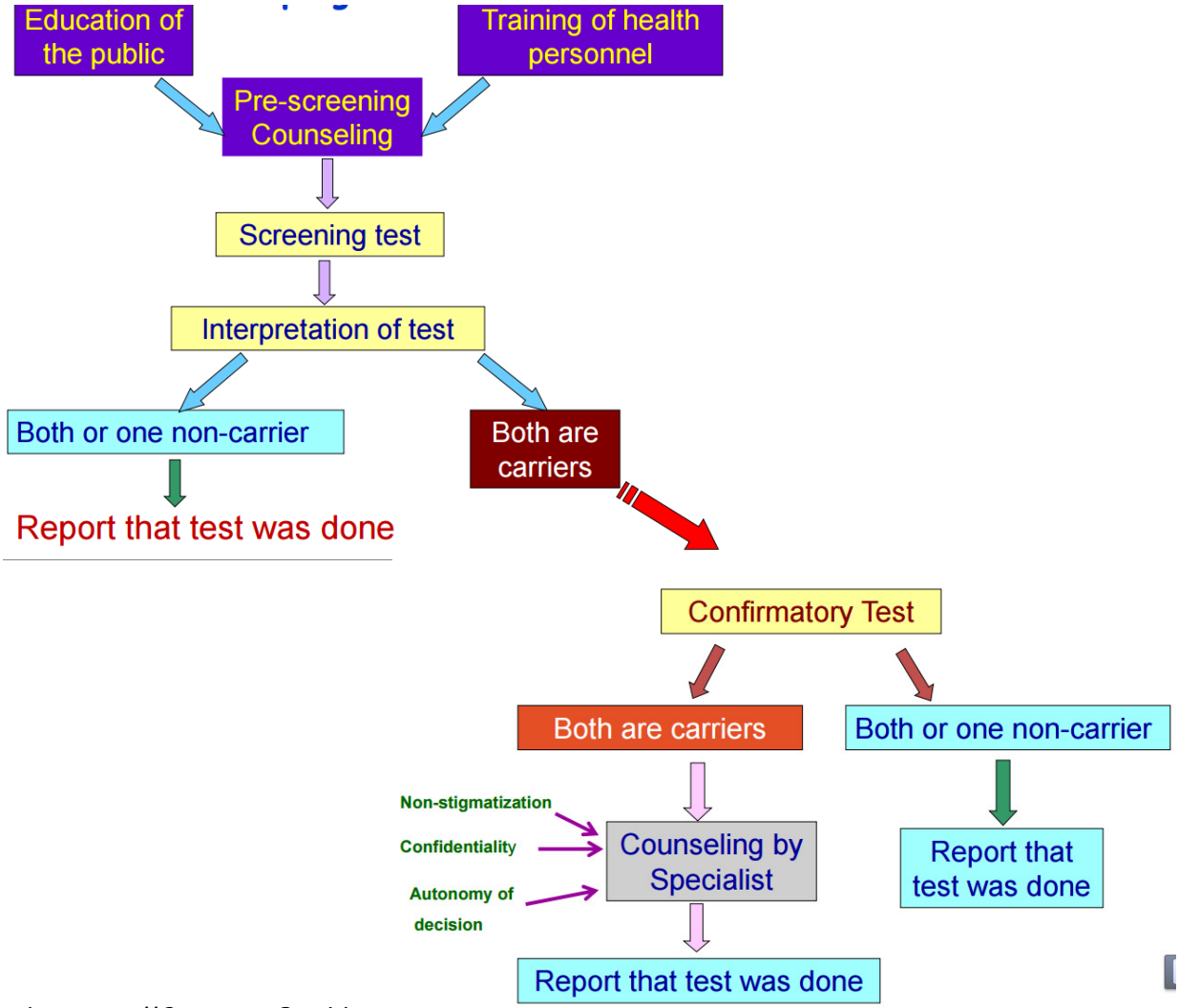
3- Pre-screening counseling; to tell people why and how we are screening.

4- Doing the screening test.

5- Interpretation to the test.

Then depending on the results the decision will be made.

This figure summarizes all what the doctor said: (read all the details)



There are many **successful programs** for premarital screening around the world, such as:

1- premarital screening for beta-thalassemia in **Cyprus**:

The success of a genetic screening program can be judged on the basic of a reduction in the births of affected babies .

- In 1974: Births incidence of Beta-thalassaemia was 1 in 250 (10 times of that of Jordan)

Introduction of a comprehensive screening program to determine carrier status of young adults and premarital couple.

- In 1984: incidence of affected babies declined by over 95%.
- In 1990: no new birth of beta-thalassemia, because it's allowed in their country to have abortion if the fetus had the disease.

2- Neonatal screening:

- Disorder produces irreversible damage before onset of symptoms
- Treatment is effective if you start early
- Natural history of disorder is known

Benefits of newborn screening:

1. Reduce mortality and morbidity of inherited disease
2. Identify congenital disorders
3. Improve patient outcomes through early detection and treatment
4. Minimizing the impact of disease
5. Offering essentially a "normal" life
6. Offer a cost benefit to society

That was written by Raya Al Majali

Genes or diseases

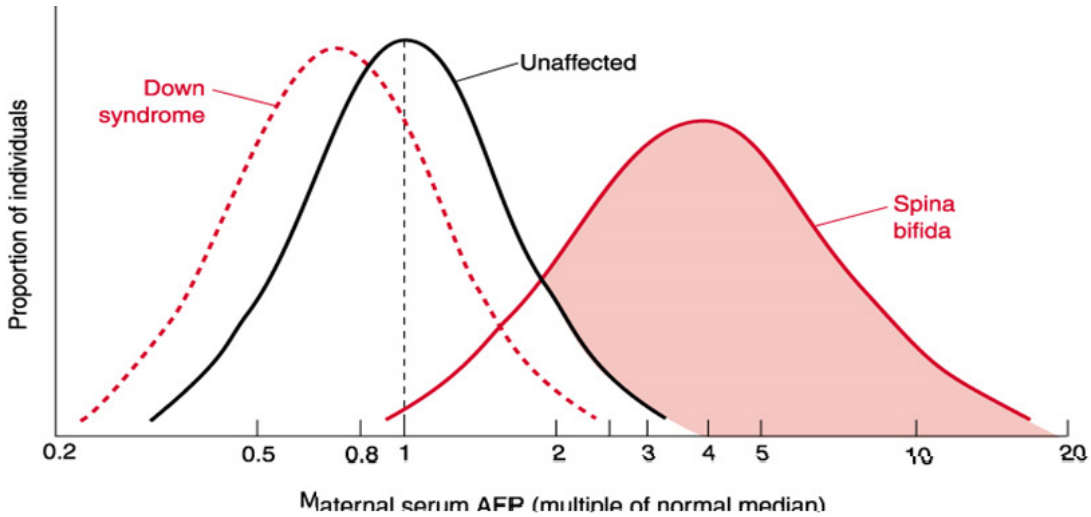
If you know the gene and if you know the mutations you can do prenatal diagnose for any disease you think, that is the rule of prenatal diagnose.

The **non-invasive** ones we can measure the level of alpha vetoprotein in the mothers serum that generally we can use it in the second trimester and it can give us a certain indication especially between 15 to 22 weeks of gestation.

The tests are variable it could be one test or two tests or three tests and it could be four tests, these tests which we use them are beta estrogen, unconjugated estriol, inhibin A tests and others.

The value of these tests it could be around 60% to 75% of Down syndrome in a mother her age is less than 35 years, if the mother is older than 35 the value of these tests will increase around 10%. And if she is older than 80

it's reduce significantly to 18% still you have 15 to 20 % they are undiagnosed.



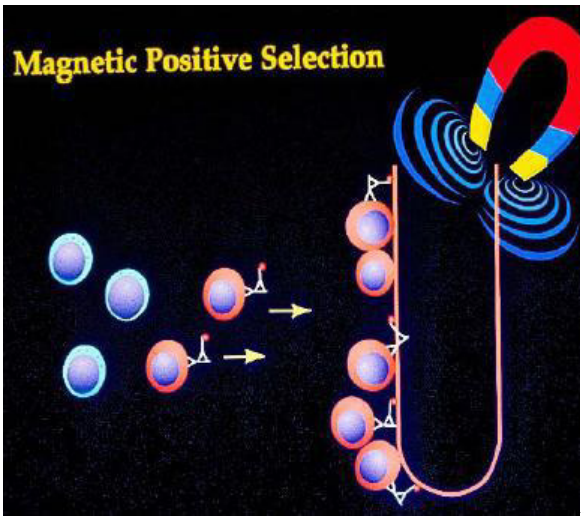
This is what we see when we screen for it when we look for AFP protein in the mothers circulation.

The black line (middle figure) represent the Normal state in any

pregnancy if there is Down syndrome the amount could be less than normal (shifting to the left).
If the mother or the baby has spina bifida the amount of AFP protein will be high (shifting to the right).
There are other possibilities for elevated AFP:-
-multiple gestation (pregnancies)
- fetal demise, premature delivery, growth retardation
-Abdominal wall defect.
-congenital nephrosis.
-maternal liver disease.
The other **Non-invasive** tests where we can look for the presence of white blood cells in the mother circulation and DNA in the mother circulation.
Generally for every 10 million cells in the circulation of the mother we can find one cell belonging to the Fetus, so isolation of this cell will not be an easy task.

In addition, cell-free fetal DNA or RNA is found in maternal circulation, this may prove easier way to isolate and to test than the fetal cells and we can use them for the diagnoses the fetal cells which have specific receptors (transferrin receptor) that are not found in the mother cells so if we produce antibody specific to the transferrin receptor and that antibody if we tag it with iron nano-particle we will end up with antibody and has iron on its surface.

So when we isolate WBCs from the mother circulation and added these antibodies to them, the antibodies will react with the fetal cells after that we will use a magnet on the tube and all the fetal cells will attach to the tube.
Or we can find DNA or RNA and



isolate and test them to look for the abnormalities.

The last N0n-ivasive procedure is ULTRA SOUND:-

- •Noninvasive, uses reflected sound waves converted to an image
- •Transducer placed on abdomen
- •See physical features of fetus, not chromosomes
- •May ID some chromosomal abnormalities by physical features

Experience doctor can diagnosis certain condition by using the ultra sound procedure, for example in Down syndrome if we see a gap between the neck and skin and this gap was 6mm so we know that we have down syndrome (normally this gap measure 3mm).

Invasive procedures:-

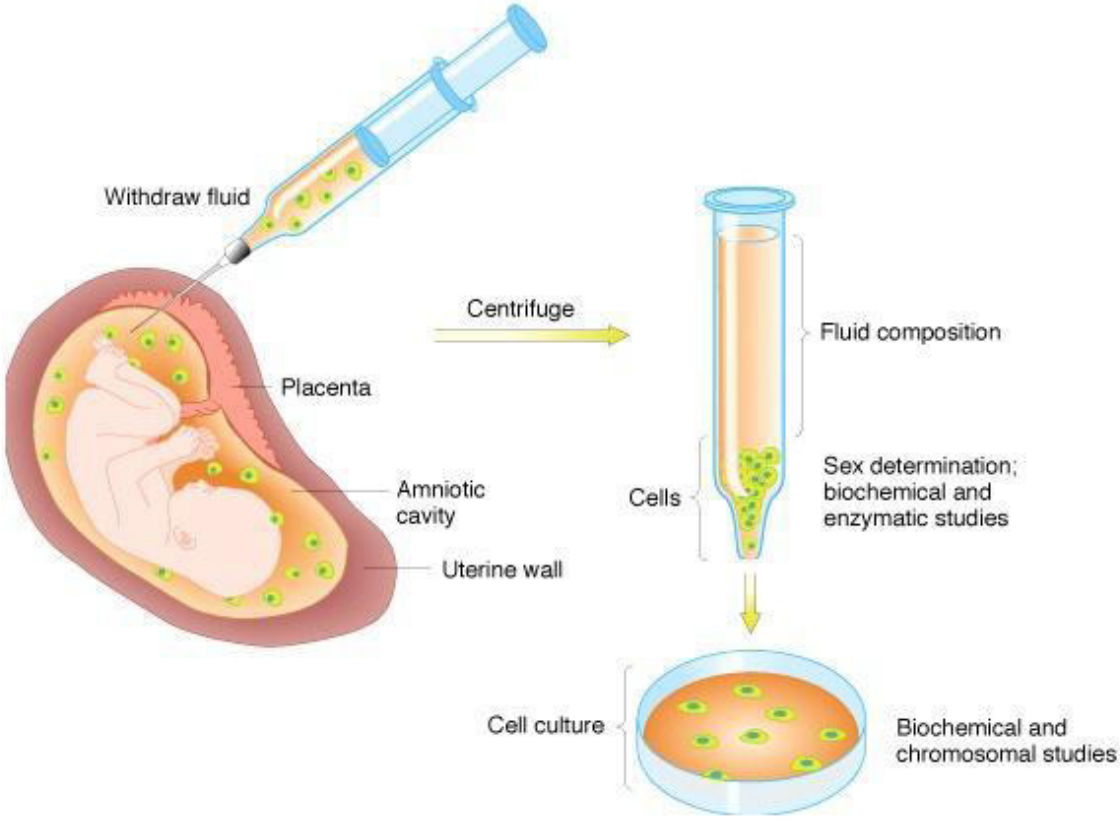
Amniocentesis: is to extract fluid –by a needle- from around the fetus and generally we take around 20-30 ml from around the fetus, and in that fluid there are supernatant & fetal cells. Then we culture these cells and look for any problems in the Chromosomes and we can analyze the amniotic fluid for AFP levels.
The time frame of this procedure is week 15-17 of gestation, we can do it earlier but there is a risk that we will induce abortion.

Advantages:

- Can examine AFP levels for spinal defects
- Can be performed by an Ob/Gyn vs. perinatologist
- Fetal loss rate very low (0.5%) - for late Amniocenteses

Disadvantages

- Early amniocentesis has a higher risk of miscarriage (5%)
- Longer wait time for patients than CVS – 1-2 weeks
- also have some risk of mosaicism



-The second Invasive procedure is **Chorionic Villus Sampling (CVS)**:-

- Essentially a placental biopsy
- Tissue biopsy from the villous area of the chorion is aspirated transvaginal or transabdominally depending on the experience of the doctor.

* We must clean the specimen from the mother blood so we don't contaminate it.

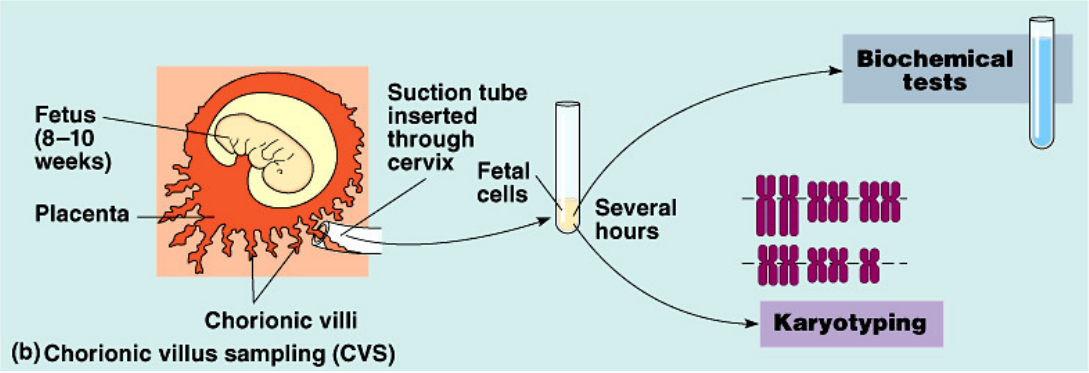
-The time frame for CVS is 8-10 weeks of gestation.

Advantages:

- first trimester diagnosis
- diagnostic results provided 99% of the time.
- post-CVS fetal loss rate low (1%)
- results usually obtained in 5-7 days

Disadvantages

- looks only at extra embryonic material - will not detect a defect arising after embryonic material partitioned off
- confined placental mosaicism may be a problem (2%)
- only gathers cells, not fluid - can't measure AFP
- **Can't identify NTDs.**



The third invasive procedure is **Cardocentesis** :-
obtaining blood from the fetus.
The timeframe of it is 19-21 weeks of gestation.

Advantages:

- Rapid diagnosis time, fetal blood cells only need to be cultured for a few days to provide good chromosomes

Disadvantages:

- Must be performed by a perineonatologist because of difficulty in accessing the umbilical vein
- Higher fetal loss than with CVS or Amnio (2-3%).

The forth invasive procedure is **Fetoscopy**:
skin biopsy from the fetus
it helps in the diagnosis of structural abnormalities and specefic genetic dermatoses (epidermolysisbullosa)
the Timeframe is 15-18 weeks of gestation.

What technique do you use?

Depends upon what you are looking for

Chromosomal abnormalities - need to look at chromosomes - need live fetal cells obtained from amniocentesis or chorionic villus sampling

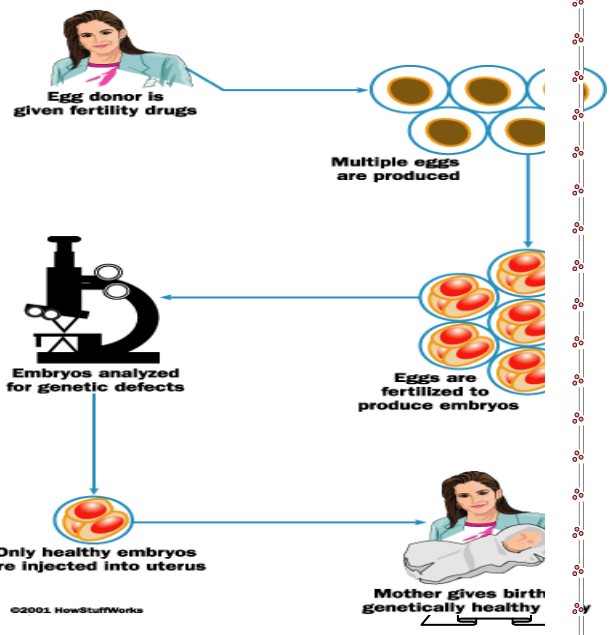
Hormone or enzyme levels - need cells or fluid

Direct mutation analysis - need DNA (fetal cells)

Tests: Karyotyping, FISH, CGH, Molecular, Biochemical.

The fifth invasive procedure is

Pre-implantation Genetic Diagnosis (PGD):-
we fertilize the egg in Vitro and when the Zygote reach eight or sixteen cells, we extract a cell or two and examine these cells for the deficit which we are looking for, then we



inject it in the uterus if it's healthy
By this technique we can select the Sex of embryo as we want then implant it (XX OR XY) (Sex selection)
But ethically we use it only for Sex-Related diseases or expecting a Disease.

PGD Applications:-

- All known single-gene disorders
- Chromosomal rearrangement
- HLA-matched siblings
- Cancer predisposition genes
- Late-onset disorders
- Monogenic disorders
- Translocations together with aneuploidy
- Couple who carry a genetic disorder

*What is HLA-Typing??
A child is Born with a Disease (cystic fibrosis, Anemia…) and the only effective treatment is bone marrow transplantation so the parents using PGD fertilizing numerous Zygotes in the Vitro and select an Embryo very close or 100% match to the ill Child and Free of that disease then implant it then she will get pregnant.
The new baby if free from (cystic fibrosis, anemia...) they take bone marrow from the baby to his effected ill brother in order to treat him.

Advantages:

- Very early diagnosis
- Only transfer unaffected (or carrier) embryos

Disadvantages

- Cost is extremely high
- "Success"/implantation rate low
- Discard affected or unused embryos
- Which has raised ethical concerns .

Examples for people who undergo successful HLA-Typing:

Molly and Adam Nash
FanconiAnaemia

ZainHashmi
Beta thalassaemia from Kuwait.

Therapy of Genetic disorders:-
There is NO effective therapy the best way to do prevention.

Current Therapy of Genetic Disorders:-

•Preventive

•Metabolic Manipulation

•Gene Product Replacement

•Cell or Organ Transplantation

•Gene Therapy

1) Preventive:-

- Preventive screening
  - -Neonatal screening
  - -Population screening
- Prenatal diagnosis
- Preimplantationdiagnosis.

Genetic counseling is also very important way for prevention.

2) Metabolic Manipulation

–Dietary Restriction

• (Lactose restriction for Lactase deficiency; phenylalanine restriction for phenylketonuria) we don't give anything that harms him.

–Dietary Supplementation:-
if he doesn't have a certain enzyme we supply it for him

• (Biotin for Biotinidase deficiency, Starch for G-6-P deficiency)

–Chelation and enhanced excretion:-
when he's having thalassaemia or sickle cell anemia and he's getting a blood transfusion that results in Iron overload or copper as in Wilson Disease
we extract the excess so it doesn't harm the patient

–Metabolic inhibitors:-

•(allopurinol for gout, Statins for hypercholesterolemia,).

3) Gene Product Therapy:-

- **Hormone, protein or enzyme replacement**
- Hormone supplementation:
- Hypothyroidism: Thyroid hormones
- Congenital adrenal hyperplasia: Cortisol
- Growth hormone
- Hemophilia: Clotting factors
- Diabetes: Insulin
- Enzyme replacement
- Gauchers: Beta glucosidase
- Pompe: Alpha glucosidase
- SCID: Adenosine deaminase

4) Cell or Organ Transplantation:-

Cells:
- Bone marrow,

Stem cells: Embryonic, adult SC Mesenchymaland Peripheral

Organs

Kidney: Fabry Disease

Liver: Tyrosinemia

STEM CELL THERRAPY:-

We can use now completely closed systems to transplant stem cells from the donor to the acceptor.

Embryonic vs Adult Stem Cells

- **Totipotent**
  Differentiation into ANY cell type
- **Known Source**
- **Large numbers can be harvested from embryos**
- **May cause immune rejection**

- **Multi or pluripotent**
  Differentiation into some cell types, limited outcomes
- **Unknown source**
- **Limited numbers, more difficult to isolate**
- **Less likely to cause immune rejection, since the patient's own cells can be used**

5) Gene therapy :-

We use replacement therapy to replace the defective gene also we can transfer the gene or you can manipulate the gene as well as we can use stem cells for the treatment.

There is no successful case that has been reported till now