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Basics of Genetics

- ✓ To understand the following concepts:
  - Genetics
  - Cells
  - Chromosomes
  - Genes
  - DNA
- ✓ To understand the basic concepts in molecular biology
- ✓ To understand the processes of meiosis and mitosis
- ✓ Types of mutations

What is Genetics ? :Genetics is the science of **heredity** and **variation** in living organisms.

Chromosome

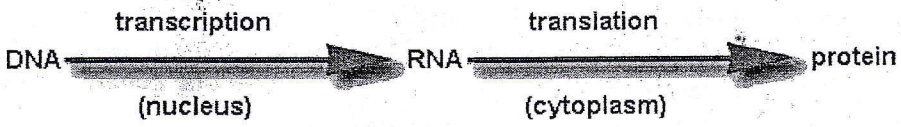
(1) Chromatid - one of the two identical parts of the chromosome
(2) Centromere - the point where the two chromatids touch, and where the microtubules attach.
(3) Short (p) arm.
(4) Long (q) arm

- DNA, or deoxyribonucleic acid, is the hereditary material in humans and almost all other organisms.
- The information in DNA is stored as a code made up of four chemical bases: adenine (A), guanine (G), cytosine (C), thymine (T).
- DNA bases pair up with each other to form units called base pairs. A pairs with T – 2 hydrogen bonds, C pairs with G – 3 hydrogen bonds
- Each base is also attached to a sugar molecule and a phosphate molecule. Together, a base, sugar, and phosphate are called a nucleotide. Nucleotides are arranged in two long strands that form a spiral called a double helix.

DNA Replication

- An important property of DNA is that it can replicate, or make copies of itself.

How are DNA sequences used to make proteins ???



The Central Dogma:
DNA encodes the information to make RNA.....and RNA molecules function together to make protein.

Cell Division Processes: Prokaryotic cell division, Eukaryotic cell division - Mitosis - Meiosis
Eukaryotic cells divide by the process of Meiosis and Mitosis to form Somatic cells or Germ line (Sex) cells.

Mitosis:

- Mitosis is the process by which the diploid nucleus (having two sets of homologous chromosomes) of a somatic cell divides to produce two daughter nuclei, both of which are still diploid.

Meiosis:

- Gametes — eggs and sperm — are created through a process called meiosis.
- Meiosis serves to reduce the chromosome number for that particular organism by half.

What is a Mutation ??? A mutation is a permanent change in the DNA sequence of a gene.

How does this happen?

- The DNA sequence is interpreted in groups of three nucleotide bases, called codons.
- Each codon specifies a single amino acid in a protein.

Types of Mutations

Point Mutation Original: The fat cat ate the rat. Point Mutation: The fat hat ate the rat	**Frame-shift mutation** Original: The fat cat ate the rat. Frame Shift: The fat caa tet her at
Deletion Original: The fat cat ate the rat. Deletion: The fat ate the rat	**Insertion** Original : The fat cat ate the rat. Insertion: The fat cat xlw ate the rat
Inversion Original : The fat cat ate the rat Insertion : The fat **tar eht eta tac**	**DNA expression mutation**: Mutations that do not change the protein itself but change where and how much of a protein is made. These types of changes in DNA can result in proteins being made at the wrong time or in the wrong cell type. Changes can also occur that result in too much or too little of the protein being made.

http://learn.genetics.utah.edu/units/basics/tour/

Mendelian theory of inheritance

Brief Outline……

- ✓ Mendelian theory of inheritance
- ✓ Different mechanisms of inheritance in human beings based on the Mendelian theory
- ✓ Drawing and analyzing data available from family history in the form of pedigrees
- ✓ Multiple alleles with special reference to blood groups in humans

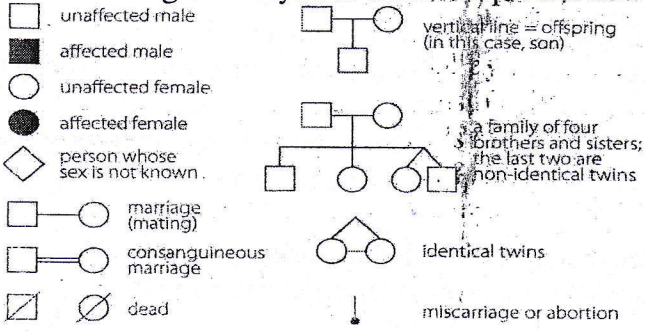
Mendel's Laws

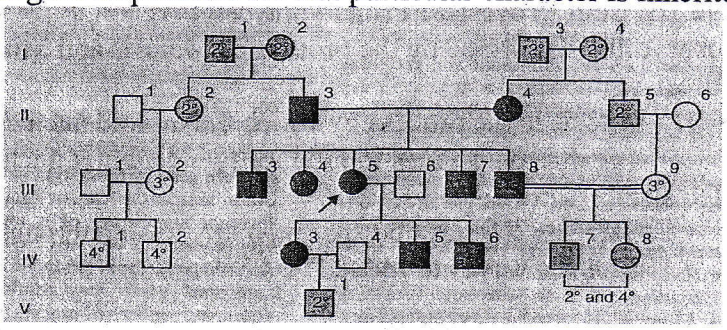
1. Genetic characters are controlled by unit factors that exist in pairs in individual organisms.
2. When two unlike factors responsible for a single character are present in a single individual, one unit factor is dominant to the other, which is said to be recessive.
3. During the formation of gametes, the paired unit factors separate or segregate randomly so that each gamete received one or the other unit factor with equal likelihood.
4. Two independent characters segregate independently of each other

Punett Squares: Devised by Reginald Punett

What is the relevance of Mendel's laws to humans??

- To determine how different genetic diseases are passed on from one generation to the next in familie
  - crosses cannot be designed
  - large number of offsprings cannot be produced
- Traditional way - construct family tree – "pedigree" – indicates expression of a character in each member.
- Pedigree analysis is done – to predict how the gene responsible for that particular character is inherited





- Proband / propositus / index case – Member through whom a family with a genetic disorder is first brought to attention
- Consultand – Person who brings the family to attention by consulting a geneticist – may be proband or unaffected relative of proband
- First-degree relatives – parents, sibs, offsprings of proband
- Second-degree relatives – grandparents, grandchildren, uncles, aunts, nephews, nieces
- Third-degree relatives – first cousins etc

Locus (loci): Location on a given chromosome of any particular gene.
Alleles: Different forms taken by a given gene – contains different genetic information (eg: brown eyes / black eyes) but determines same character (eye colour)
Mutant allele – Other versions of the gene that differ from wild-type allele
Mutation – A permanent change in the nucleotide sequence or arrangement of DNA.
Genotype – Set of alleles that make up a persons genetic constitution
Phenotype – The observable expression of a genotype as a morphological, clinical, biochemical or molecular trait
Single-gene disorder – Determined by alleles at a single locus.
Homozygous / homozygote - Having identical alleles for a single character. Homozygous dominant (RR) / recessive (rr)
Heterozygous / heterozygote – Having different alleles for a single character (Rr)

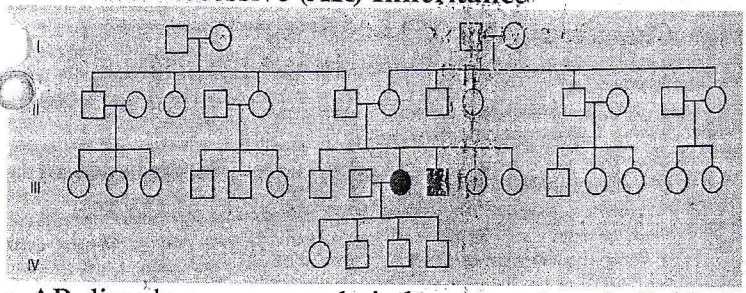
- Dominant inheritance – Any phenotype expressed in heterozygotes
- Recessive inheritance – Any phenotype expressed only in homozygotes
- Incompletely dominant – When phenotype of heterozygous genotype has intermediate severity between both homozygotes (Eg: Pink flowers)
- Co-dominant – When expression of each allele can be detected even in the presence of the other (Eg: ABO blood group system)

Genetic disorders with classical Mendelian Inheritance
Patterns of single-gene disorders depend on 2 factors:

- Chromosomal location of the gene locus – autosomal or X-linked
- Phenotype (Expression)

– Dominant – Disease condition is expressed when only one chromosome of a pair carries the mutant allele – normal allele on other chromosome of the pair.
– Recessive – Disease condition is expressed only when both chromosomes of a pair carry a mutant allele.

Four basic patterns of single-gene inheritance
Autosomal Recessive (AR) Inheritance

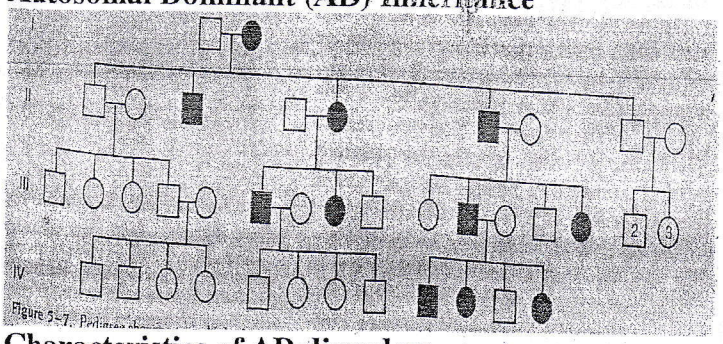


- AR disorders – occur only in homozygotes – individuals with two mutant alleles – no normal allele
- One normal gene copy in a heterozygote – compensates for the mutant allele – prevent occurrence of disease
- Homozygous recessive individual – inherits the mutant alleles one from each parent – parents heterozygous carriers

- Carriers of AR disorders: - clinically unrecognizable - far more common than homozygous affected individuals
- Mutant alleles exist in families – for several generations – without appearing in homozygous form
- When carrier mates with carrier – child may reveal presence of mutant gene
- Consanguinity

Exercise 1: 3 types of matings: Mutant allele *r* and normal dominant allele *R*. Carrier X Carrier, Carrier X Affected, Affected X Affected

Autosomal Dominant (AD) Inheritance



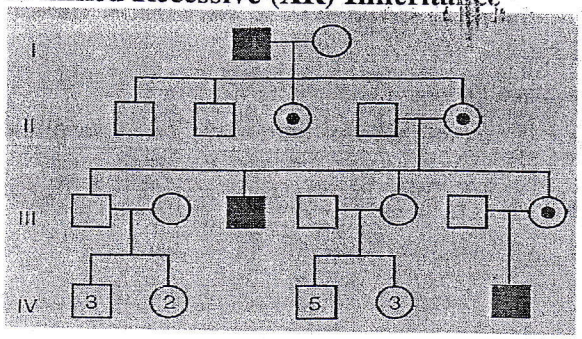
- Parents of affected individual hav to be affected - whose parents are also affected – and so on as far bac as the disorder can be traced – or until occurrence of original mutation
- No carriers
- Males and females – equally likely to transmit the disorder – to children of either sex
- Male-to-male transmission – males can have unaffected daughters

Characteristics of AD disorders

- Incidence of some AD disorders – quite high\
- Occurs in homozygous dominant and heterozygous individuals
- Much more severe in homozygotes than in heterozygotes

Exercise 2: Matings: Mutant allele *A* and normal allele *a*: Affected X Normal, Affected X Affected

X-linked Recessive (XR) Inheritance



- Expressed phenotypically in males
- Predominantly affect males – affect homozygous females (very rare) - rarely manifest in heterozygote females

Eg: Duchenne Muscular Dystrophy, Haemophilia

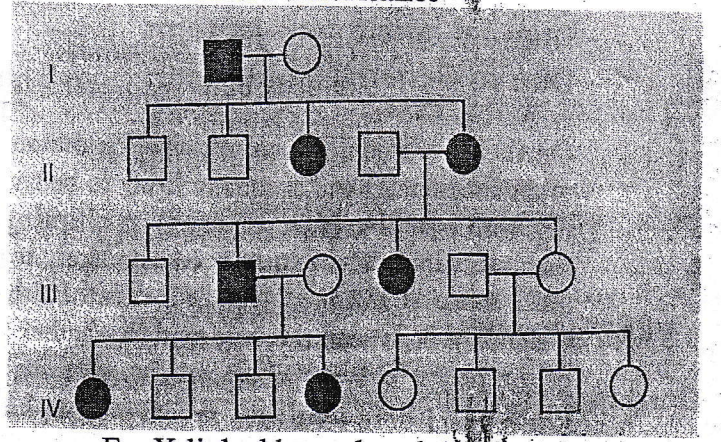
- Sex chromosomes distributed unequally to males and females
- Phenotypes on X chromosome – show characteristic sex distribution and pattern of inheritance
- X chromosome – more than 1400 genes – 40% associated with disease phenotypes

- Males are hemizygous with respect to X-linked genes

X Inactivation – almost entirely silenced – genes appear not to transcribe

Exercise 3: Affected male X Normal female, Normal male X Carrier female, Affected male X Carrier female

X-linked Dominant Inheritance



- Eg: X-linked hypophosphatemic rickets, Incontinentia pigmenti – skin rash

Characteristics of XD inheritance

- Always expressed in heterozygotes
- Affected male – has all affected daughters no affected sons
- Each affected female – has 50% chance of passing the trait to each child of any sex – (same as autosomal dominant pattern – because females have 2 autosomes and 2 X chromosomes)
- Expression usually milder in females as compared to males – since they are almost always heterozygotes

Exercise 4: Affected male X Normal female, Normal Male X Affected female, Affected male X Affected fem

Maternal Inheritance of mitochondrial disorders

- Eg: Mitochondrial myopathies

Polygenic inheritance

- Occurs when a trait is controlled by several gene pairs
- Eg: height, shape, weight, eye colour, skin colour, metabolic rate, Intelligence and many forms of behaviour.
- The inheritance of EACH gene follows Mendelian rules.

Multiple alleles : Blood Groups in Humans

- Multiple alleles are different forms of the same gene
- The sequence of the bases is slightly different in the gene located on the same place of the chromosome.

Gene of the ABO blood group system

- ABO gene – chromosome 9
- There are 3 different alleles for blood type: A B O
- A is dominant to O, B is also dominant to O, O is recessive, A and B are both codominant.

What are the PHENOTYPES and POSSIBLE GENOTYPES of those phenotypes ?????

Exercise : HOME WORK

AB x AA, OO x BB, AB x OO, BO x BO, OO x AB, AB x AO, OO x AA, AB x AA

Adult Onset Genetic Disorders

The objectives of this lecture are --

- ✓ To know the various types of adult onset disorders
- ✓ To understand the molecular and genetic mechanisms behind these disorders
- These disorders manifest themselves after childhood and adolescence.
- For example, heart disease, stroke, cancer, and diabetes - all cause death and disability during middle-age and the senior years.

Diabetes (Type 2): Genetic aspects

- More than 250 candidate genes have been tested, with little success.
- Standard oral glucose tolerance test will diagnose asymptomatic cases of type 2 diabetes and detect impaired glucose tolerance that is associated with a 40% risk of developing type 2 diabetes in 3-5 years.

Coronary artery disease (CAD) / Coronary Heart Disease (CHD)

Risk factors of CAD: In addition to other FAMILY HISTORY

Hypertension: Genetics : Genetic factors may contribute to an estimated thirty percent of cases of hypertension

- Adult African American men are most at risk for developing hypertension and cardiovascular diseases.
- A large percentage of people with hypertension have genetic abnormalities of their arterioles, This genetic abnormality makes the walls of the arteries stiff so there is greater resistance to the blood flowing through them.
- More than 50 genes have been examined in association studies with hypertension, and the number is constantly growing.
- Across populations, small effects were noted in portions of a variety of chromosomes including 1, 2, 5, 9, 10 and 14.
- Medication could be tailored to the individual based on her genetic makeup.

Adult Onset Neurodegenerative Disorders: <u>CAG trinucleotide repeat mutation</u>

- The stable and nonpathological alleles have 10-30 repeats,
- Unstable pathological alleles have expansions ranging from 40-100 repeats.

Several adult onset neurodegenerative disorders are associated with expansion of CAG repeats.

	Inheritance	Location	Normal range	Affected range
Huntington disease (HD)	AD	4p	6-28	40-121
spinocerebellar ataxia 1 (SCA 1)	AD	16p	6-39	41-81

Machado-Joseph disease (MJD/SCA 3)	AD	14q	13-36	68-79
Kennedy disease or spinal and bulbar muscular atrophy (SBMA)	XR	Xq	12-34	40-62
Dentatorubraopallidoluysian atrophy (DRPLA)	AD	12p	13-36	68-79

What is Huntington's Disease?

- It is a brain disorder that affects a person's ability to think, talk, and move
- In the United States, about 1 in every 30,000 people has Huntington's disease

Clinical manifestations

- Occurs in middle adulthood, Uncontrolled movements, dementia and emotional and psychiatric disturbance Death usually occurs within 15 years of diagnosis following a downhill course.
- The pathology involves generalized atrophy of the brain, especially in the corpus striatum and cerebral cortex.
- The disease destroys cells in the basal ganglia, the part of the brain that controls movement, emotion, an cognitive ability.

The brain cells of HD patients accumulate clumps of protein that become toxic, resulting in cell death.
Mode of Inheritance: Autosomal dominant

Gene for HD

- The gene for HD is located on chromosome 4 at band p16.3, Protein product is called huntingtin.
- Individuals with 29-39 CAG repeats fall into a gray zone in which individuals may or may not manifest HD

Genetic testing

- Predictive testing is now available for Huntington disease to identify individuals with the mutation be onset of symptoms.
- This testing presents ethical and logistical challenges due to the late onset and severity of the disorder an limited treatment options.
- Many individuals have requested predictive testing to help make decisions concerning family planning health and life insurance, and choice of employment.

Haemoglobinopathies and bleeding disorders

- To know about the various types of haemoglobinopathies and bleeding disorders
- To understand the molecular and genetic mechanisms and diagnostics for these disorders

Thalassaemias

- Inherited disease of faulty synthesis of hemoglobin – reduced rate of synthesis of globin chains – causes anaemia – characteristic presenting symptom

Diagnosis

- Early diagnosis difficult – due to presence of sufficient amount of HbF – ensures balance in number of globin chains (α and γ - that make of HbF) – protects infant from ineffective process of RBC production
- Complete blood count – Hb levels, Mean Corpuscular Value (MCV - no. and vol. of RBCs), M n Corpuscular Haemoglobin (MCH – Hb concentration),
- Microscopic examination of RBCs – carriers – pale red colour vs dark red, various shapes (poikiocytosis) vs round and concave
- Total Iron Binding Capacity (TIBC) and ferritin levels (to exclude iron deficiency)
- Thalassaemia trait and type – Hb electrophoresis – Quantitative measurement of HbA (main componenet), HbA2 (minor component of adult Hb) and HbF (foetal Hb) – for carrier detection
- DNA analysis – to determine - α or β-thalassemia trait

Treatment

- Severe thalassemia - treated by safe blood transfusions – alongwith iron chelation therapy – improves survival and quality of life
- Mouse models studies - assessing potential of gene therapy
- Few patients - cured by bone marrow transplantation.

Mode of inheritance: Autosomal recessive pattern

α-thalassaemia: Adult hemoglobin - composed of two alpha (α) and two beta (β) polypeptide chains.

wo closely linked genes of the haemoglobin alpha gene (*HBA1* and *HBA2*) – each encodes an α-chain – both genes located on chromosome 16 – thus, 4 loci encoding α-chain.

α-thalassemia – deficient synthesis of α-chains - hence excess of β-chains are formed in adults - bind oxygen poorly - form unstable tetramers – "HbH" - leading to low concentration of oxygen in tissues (hypoxemia)

- 4 genetic loci for α-globin – 2 maternal and 2 paternal origin – severity of α-thalassaemias – related to number of affected α-globin loci
- 1 locus affected – minimal effect – 3 loci enough to permit normal Hb production – no anaemia – "silent carriers"
- 2 loci affected – "α-thalassaemia trait" – nearly normal erythropoiesis – mild anaemia – incorrectly diagnosed as iron deficiency anaemia and treated with iron
- 3 loci affected – "HbH disease" – 2 unstable Hb present in blood–
  4 loci affected – "Hydrops foetalis"

Gene structure

- HBA1 gene – encodes α1-globin - on centromeric side
- HBA2 gene – encodes α2-globin – located at 5' edge of α-globin cluster – 20 kb away from ζ gene

Genetic etiology and analysis

- Deletions – PCR-based methods/ Southern Blot Analysis

β-thalassaemia

- Single Haemoglobin beta gene (*HBB*) – encodes β-chain – located on chromosome 11 – 2 loci encoding β-chain
- β-thalassemia - lack of β-chains - excess α-chains form insoluble aggregates inside red blood cells - aggregates cause death of red blood cells and their precursors – at high concentrations – toxic - cause very severe anemia - spleen becomes enlarged as it removes damaged red blood cells from the circulation.
- β-globin loci – severity of disease varies

- 1 locus affected - β-thalassaemia minor/trait – mild anaemia – sometimes asymtomatic
- 2 loci affected - β-thalassaemia major / Cooley's anaemia
- Thalassaemia intermedia – intermediate condition – between major and minor forms

Other "abnormal" types of adult Hb – HbS (sickle cell anaemia), HbE, C, D, Lepore – combine with β-thalassemia

Genetic etiology and analysis:

- More than 200 gene mutations
- Common mutations detected by PCR-based methods – reverse dot blot analysis, primer-specific amplification, mutation scanning methods (DHPLC), sequence analysis methods used

Sickle Cell Disease (SCD)

- RBCs change shape – deoxygenation – polymerization of abnormal sickle Hb – damage to RBC membrane – cells get stuck in blood vessels – deprives further tissues of O2 – causes ischaemia and infarction – organ damage and stroke

Clinical manifestations

- typical Hb levels – 6-9 g/dl – pallor, tachycardia, fatigue
- spleen affected before end of childhood – increased risk of encapsulated organisms – preventive antibiotics and vaccination recommended

Diagnosis

- diagnosis clinically
- microscopic examination of peripheral blood smear – sickle RBCs, nucleated red blood cells seen
- Haemoglobin electrophoresis – abnormal Hb forms detected
- HPLC analysis – detect presence of significant quantity of HbS
- Genetic analysis – sequence analysis – to determine mutations associated with specific Hb variants

Treatment

- Intravenous or oral antibiotics, Painful episodes – analgesics, O2 supplementation – for hypoxia, Blood transfusions, acute chest syndrome – treated with O2, analgesics, antibiotics and blood transfusion
- Gene therapy – in mouse SCA model – promising results

Mode of inheritance: Autosomal recessive
Gene location and structure: HBB Gene location – 11p15.5
Genetic Etiology: Point mutation – in β-globin chain of Hb – at position 6 – A to T – GAG to GUG - Glutamic acid replaced with Valine – association of 2 normal α-globin subunits with 2 mutant β-globin subunits – forms HbS

Haemophilia

- X-linked recessive disorder – single gene disorder
- Incidence is 1/10,000 male population
- Deficiency in coagulation factors - FVIII – Haemophilia A. FIX – Haemophilia B

Classification of haemophilia

Clotting factor activity	Degree of haemophilia	Characteristics
< 1%	Severe	Frequent spontaneous bleeds
2-5 %	Moderate	Few spontaneous bleeds
6-30%	Mild	Bleed only after trauma or surgery

- About one third of carriers have levels between 15 and 50%.
- Replacement therapy with virus-inactivated plasma concentrates.

Clinical manifestations

- Haematomas (bleeding under the skin)
- Haemarthroses (bleeding in the joints)
- Haematuria (bleeding in the kidneys)
- Intracranial haemorrhages – life threatening

Clinical Diagnosis

- History of joint and soft tissue bleeding
- Presence of arthropathy on physical examination
- Family history indicating X-linked inheritance
- Biochemical laboratory tests – Coagulation factor assays

FVIII Gene : Location

FVIII gene – Xq28 – from telomeric end towards centromere.

Salient features of the disorder (Genetic Aspects)

- Haemophilia predominantly affects males.
- All daughters of haemophilia patients are carriers, all sons are normal
- These carrier daughters will have either normal or carrier daughter or normal or affected son with each pregnancy
- There are two types of carriers. Classified according to family history. Obligate carriers are --- daughters of haemophilia patients, or who have one affected son and an affected male relative on the mother's side, or who have two affected sons

Possible carriers are women -- who have one or more relatives with haemophilia on their mother's side, or who have only one son with haemophilia and no other relatives with the disorder.

Methods of carrier detection

Risk assessment by pedigree analysis – Obligate and Possible carrier

- Test is predictive

Biochemical analysis – Assays of FVIII activity and vWF:Ag

- Levels and ratios of FVIII and vWF:Ag cannot be distinguished from those of normal women in 20% women.
- Test dependent of physiological condition of women (menstruation, pregnancy).
- Cannot be used for prenatal diagnosis.

Genetic analysis

- Direct method – Conclusive – Very expensive due to heterogeneous etiology of FVIII gene
- Indirect method (Polymorphisms) – Less expensive, Predictive in 99% cases, Non-informative if key females are homozygous (pedigree), DNA of many family members required

ebsites

- Haemophilia A Mutation Structure Test and Resource Site, HAMsTERS database; (http://europium.csc.mrc.ac.uk)
- Online Mendelian Inheritance in Man (http://www.nslij-genetics.org/search_omim.html)
- GeneReviews (www.geneclinics.org)

Cancer Genetics

- ✓ To know the classification related to cancer
- ✓ To become familiar with the various terminologies related to cancer
- ✓ To understand the process of carcinogenesis
- ✓ To understand the processes involved behind the causation of cancer at the genetic and molecular levels.

- Cancer is a group of diseases in which cells (malignant cells) are –
  - *aggressive* (grow and divide without respect to normal limits)
  - *invasive* (invade and destroy adjacent tissues)
  - and sometimes *metastatic* (spread to other locations in the body).
- As against these benign tumors are –
  - self-limited in their growth
  - not invasive
  - do not metastasize (although some benign tumor types are capable of becoming malignant)
- **Screening:** a test done on healthy people to detect tumors before they become apparent. Eg: A mammogram is a screening test.
- **Diagnosis:** the confirmation of the cancerous nature of a lump. This usually requires a biopsy or removal of the tumor by surgery, followed by examination by a pathologist.

Surgical margins: the evaluation by a pathologist of the edges of the tissue removed by the surgeon to determine if the tumor was removed completely ("negative margins") or if tumor was left behind ("positive margins").

Stage: a number (usually on a scale of 4) established by the oncologist to describe the degree of invasion of the body by the tumor.

Recurrence: New tumors that appear a the site of the original tumor after surgery.

Chemotherapy: Treatment with drugs.

Radiation therapy: Treatment with radiations.

Adjuvant therapy: Treatment, either chemotherapy or radiation therapy, given after surgery to kill the remaining cancer cells.

Prognosis: the probability of cure after the therapy. It is usually expressed as a probability of survival five years after diagnosis. Alternatively, it can be expressed as the number of years when 50% of the patients are still alive.

Cancers are classified by the type of cell that resembles the tumor and, therefore, the tissue presumed to be the origin of the tumor.

Carcinoma: Malignant tumors derived from epithelial cells. Eg: breast, prostate, lung and colon cancer.

Sarcoma: Malignant tumors derived from connective tissue, or mesenchymal cells.

Lymphoma and **leukemia:** Malignancies derived from hematopoietic (blood-forming) cells

Germ cell tumor: Tumors derived from totipotent cells (cells which can differentiate into any cell type). In adults most often found in the testicle and ovary; in fetuses, babies, and young children most often found on the body midline, particularly at the tip of the tailbone

Blastic tumor: A tumor (usually malignant) which resembles an immature or embryonic tissue. Many of these tumors are most common in children.

Malignant tumors (cancers) are usually named using **-carcinoma**, **-sarcoma** or **-blastoma** as a suffix, with the Latin or Greek word for the organ of origin as the root. Eg: a cancer of the liver is called *hepatocarcinoma,* a cancer of the fat cells is called *liposarcoma.*

For common cancers, the English organ name is used. the most common type of breast cancer is called *ductal carcinoma of the breast* or *mammary ductal carcinoma.*

Cancer Epidemiology

Cancer affects people at all ages, even fetuses, but risk for the more common varieties increases with age.T projected numbers for 2030 are 20 to 26 million new diagnoses and 13 to 17 million deaths.

Childhood cancers (from most common decreasing order)

Leukemia (usually Acute Lymphoblastic Leukaemia)- (30%), Relative survival for infants is good f neuroblastoma, Wilms' tumor and retinoblastoma, and fairly good (80%) for leukemia, but not for most oth types of cancer.

Factors causing cancers: Nearly all cancers are caused by abnormalities in the genetic material of th transformed cells (cells which acquire properties of cancer). These abnormalities may be due to the effects o carcinogens (cancer-causing substances), such as tobacco smoke, radiation, chemicals, infectious agents.

Other ways in which cancer-promoting genetic abnormalities are acquired are – randomly acquired throug errors in DNA replication, inherited from parents and thus present in all cells from birth. There are comple interactions between carcinogens and the host genome. That is why some people develop cancer after exposur to a known carcinogen.

There are newer aspects of the genetics of cancer causation that are now being recognized as important

- DNA methylation (addition of a methyl group to DNA)
- microRNAs (single-stranded RNA molecules of about 21-23 nucleotides in length, which regulate gen expression)

Signs and symptoms

Cancer symptoms can be divided into three groups:

- *Local symptoms*:
- *Symptoms of metastasis (spreading)*
- *Systemic symptoms*:

Diagnosis

- Most cancers are initially recognized either because signs or symptoms appear or through screening.

Investigation

- People with suspected cancer are investigated with blood tests, X-rays, CT scans and endoscopy.

Biopsy

- Definitive diagnosis of most malignancies must be confirmed by histological examination of the cancerous cells by a pathologist.
- Tissue can be obtained from a biopsy or surgery.
- Cytogenetics and immunohistochemistry may provide information about future behavior of the cancer (prognosis) and best treatment.

Genetics behind cancers!!

Genetic abnormalities found in cancer affect two general classes of genes.

1. Oncogenes
2. Tumour suppressor genes

1. ***Oncogenes*** (Cancer-promoting genes) are often activated in cancer cells, giving these cells new properties, such as – hyperactive growth and division, protection against programmed cell death (apoptosis), loss of respect for normal tissue boundaries, the ability to become established in diverse tissue environments.
   - ras oncogenes are the most commonly observed oncogenes in human tumours.

2. Tumor suppressor genes are often inactivated in cancer cells, resulting in the loss of normal functions in those cells, such as - accurate DNA replication, control over the cell cycle, orientation and adhesion within tissues, interaction with protective cells of the immune system.

What is "Carcinogenesis" ????: Process by which normal cells are transformed into cancer cells. Normally, there is balance between proliferation and programmed cell death (apoptosis) , Tightly maintained process, Mutations in DNA disrupt these orderly processes by disrupting the programming regulating the processes. In cancer, cells start dividing uncontrollably. Genes which regulate cell growth are damaged.

2 sets of genes are involved –

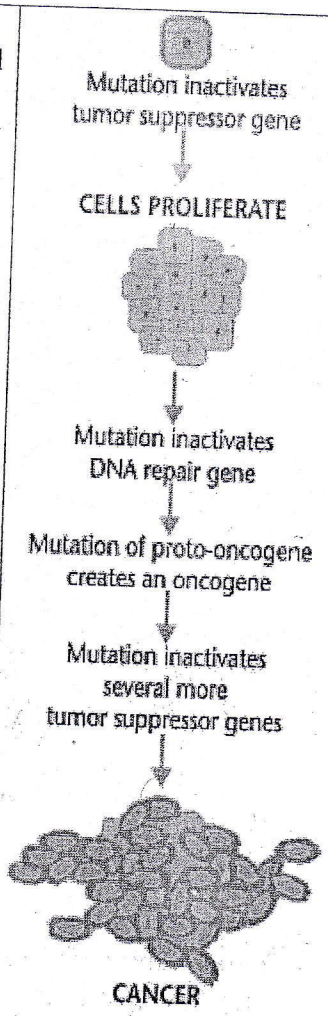
- Proto-oncogenes are genes which promote cell growth and mitosis, a process of cell division
- Tumor suppressor genes discourage cell growth, or temporarily halts cell division from occurring in order to carry out DNA repair.

Proto-oncogenes

- Mutations in proto-oncogenes can modify their expression and function, increasing the amount or activity of the product protein.
- When this happens, they become oncogenes (cancer-promoting genes)
- Thus cells have a higher chance to divide excessively and uncontrollably.
- The chance of cancer cannot be reduced by removing proto-oncogenes from our genome as they are critical for growth, repair and homeostasis of the body.
- It is only when proto-oncogenes become mutated, the signals for growth become excessive.

Tumour suppressor genes

- p53 gene, which is a transcription factor activated by many cellular stress including hypoxia and ultraviolet radiation damage.
- However, a mutation can damage the tumor suppressor gene itself, or the signal pathway which activates it, "switching it off".
- As a result, DNA repair cannot take place
- DNA damage accumulates without repair, inevitably leading to cancer.



Multiple mutations

- Several mutations in both proto-oncogenes and tumour suppressor genes are required for cancer to occur.

Non-mutagenic carcinogens: Substances which do not cause any mutations but cause cancer, Examples: alcohol and estrogen. These promote cancers by stimulating the rate of cell mitosis. Faster rates of mitosis - less opportunity for repair enzymes to repair damaged DNA during DNA replication – increased risk of a genetic mistake. A mistake made during mitosis can lead to the daughter cells receiving the wrong number of chromosomes - which may lead to cancer.

Role of viral infections: Viruses are responsible for 15% of human cancers. Viruses that are known to cause cancer are – Human papillomavirus (HPV) and cervical cancer, Hepatitis B and liver cancer

Etiology of cancer : It is impossible to tell the initial cause for any specific cancer. Up to half of all tumors have a defective p53 gene. This mutation is associated with poor prognosis, since those tumor cells are less likely to go into apoptosis (programmed cell death) when damaged.

Cytogenetics - Karyotyping

del(5)(q22q35) - Acute myeloid leukemia (AML), Myelodysplastic syndromes (MDS, bone marrow disorders)

del(6)(q15q21) - Chronic lymphocytic leukemia (CLL)

del(9)(p13) - Acute lymphoid leukemia (ALL, malignancy of Lymphoblasts)

i(6)(p10) - Retinoblastoma (most common (inversion) intraocular neoplasm of children)

(2;8)(p12;q24.1) - Burkitt's lymphoma (high grade B-(translocation) cell lymphoma, more frequent in young.)

A metaphase cell positive for the bcr/abl rearrangement using FISH (Fluorescent in situ hybridizat Philadelphia chromosome or Philadelphia translocation is a specific chromosomal abnormality associated w chronic myelogenous leukemia (CML).

- Website – Nature Journal– Cancer Molecular Diagnostics
- http://www.nature.com/nature/supplements/insights/cancerdiagnostics/

Collection of Human Samples

- ✓ To understand the various types of human samples collected for the purpose of diagnosis
- ✓ To know about the timing, collection, preservation and transport of human samples

What is the significance of collecting human samples?

- There is a need to understand the challenges and potential pitfalls in sample collection, processing and banking
- Several factors affect the quality and potential future use of biological samples
- Also, some points should be remembered during collection, processing and storage of human samples.
- Conducting of pilot study before actual sample collection

Informed consent: Participants are asked to give consent to use their sample for a particular study. Witl availability of newer technologies more experiments can be conducted on the same samples and consent shoul be taken for such unpredictable uses of their samples

Sample collection: For reliable and consistent sample collection – there should be clear communication bet the nurses and technical staff who collect the specimens, the patients and the researchers. Clear instruction should be given regarding timing of collection, fasting instructions, volumes required, specific containers to b used and size of needle for venipuncture. Written protocols should be prepared in this regard

Stability of samples depends on: anti-coagulants, Stabilizing agents, Temperature, Timing before initia processing, Sterile conditions, Enzymatic degradation. Containers or equipments

Safety issues in handling human samples: Precautions must be taken at all stages of work. Eg: HIV, Hepatitis or other transmissible or parasitic diseases. Personnel must be trained to handle human samples with necessary safety precautions for their own safety and for the safety of others involved in the process. Sharp items such as needles are especially risky and must be contained at all times. Strict adherance to laid down procedures and protocols is a must.

Sample banking: Adequate physical storage. Effective labelling system, Inventory management system, Bar-coding of samples can be done

To summarize ----------

(a) identify the appropriate tissue with preference to non-invasive approaches
(b) determine the timing of collection and examine the biomarker stability requirements
(c) obtain the necessary equipment for the processing facilities, develop the detailed protocols and flow charts, train the employees, carry out pilot studies on the efficiency of cryopreservation or DNA/RNA purification.
(d) organize the physical storage facilities and equipment and set up the barcoding and the electronic database management systems
(e) review all the legal requirements, including compliance with safety in handling human tissues, shipping of potentially infectious materials, human subject research approval and informed consent from the study subjects, laboratory and field QA/QC procedures

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Services related to genetics

- ✓ To understand the various services related to genetics

- Molecular Biology techniques
- Carrier and prenatal diagnosis

✓ To know about the applications of these techniques in the field of medicine with special reference t gene therapy and human genome project

Cytogenetics

Cytogenetics is a branch of genetics that's concerned with the study of chromosomes and cell division.
Cytogenetics is the study of normal and abnormal chromosomes.
This includes examination of chromosome structure, learning and describing the relationships betwee chromosome structure and phenotype, and seeking out the causes of chromosomal abnormalities.
Chromosomes are examined and characterized by obtaining an individual's karyotype
A karyotype is a description of the number and structure of the chromosomes.

Karyotyping

Chromosome banding is a standard and indispensible tool for cytogenetic analysis. Several banding techniques have been developed

Q banding: chromosomes are stained with a fluorescent dye such as quinacrine
G banding: produced by staining with Giemsa after digesting the chromosomes with trypsin
C banding: chromosomes are treated with acid and base, then stained with Giesma stain
Reverse banding (R-banding) requires heat treatment and reverses the usual white and black pattern that is seen in G-bands and Q-bands.
Each of these techniques produces a pattern of dark and light (or fluorescent versus non-fluorescent) bands along the length of the chromosomes.
Importantly, each chromosome displays a unique banding pattern, analagous to a "bar code", which allows it to be reliably differentiated from other chromosomes of the same size and centromeric position.

Preparing a Karyotype

Metaphase cells are required to prepare a standard karyotype, Any population of dividing cells can be used. Blood is easily the most frequently sampled tissue, but at times, karyotypes are prepared from cultured skin fibroblasts or bone marrow cells. None of the leukocytes in blood normally divide, but lymphocytes can readily be induced to proliferate. There are many protocols for preparing a karyotype from peripheral blood lymphocytes. A standard series of steps is as follows:A sample of blood is drawn and coagulation prevented by addition of heparin. Mononuclear cells are purified from the blood by centrifugation through a dense medium that allows red cells and granulocytes to pellet, but retards the mononuclear cells (lymphocytes and monocytes). The mononuclear cells are cultured for 3-4 days in the presence of a mitogen like phytohemagglutinin, which stimulates the lymphocytes to proliferate madly. At the end of the culture period, when there is a large population of dividing cells, the culture is treated with a drug (mitotic inhibitor) such as colchicin or colcemid, which disrupts mitotic spindles and prevents completion of mitosis. This greatly enriches the population of metaphase cells. The lymphocytes are harvested and treated briefly with a hypotonic solution (Carnoy's fixative). This makes the nuclei swell osmotically. This helps in getting preparations in which the chromosomes don't lie on top of one another. The swollen cells are fixed, dropped onto a microscope slide and dried. Slides are stained after treatment to induce a banding pattern. Once stained slides are prepared, they are scanned to identify "good" chromosome spreads (i.e. the chromosomes are not too long or too compact and are not overlapping). which are photographed. The images of each chromosome are then cut out and pasted in an orderly manner. Alternatively, a digital image of the chromosomes can be cut and pasted using a computer programme.

Karyotypes are presented in a standard form. First, the total number of chromsomes is given - followed by a comma and the sex chromosome constitution. This shorthand description is followed by coding of any autosomal abnormalities.

Cytogenetic maps: Diagrams identifying the chromosomes based on the banding patterns are known **cytogenetic maps**. These maps have helped people from both prenatal and oncological fields to quickly mo cytogenetics into the clinical lab where karyotyping allowed scientists to look for chromosomal alterations
Medical uses: *Cytogenetics* can determine which chromosomal translocations are present in the malignant cel to help in diagnosis and deciding the treatment course. In congenital disorders, such as Down's syndrom *cytogenetics* can determine the nature of the chromosomal defect - a trisomy, a mosaic, "balanced" translocatio a deletion, or an insertion in one - or both - of the parents, or in the fetus.
FISH (fluorescent in situ hybridization): **FISH** (Fluorescent *in situ* hybridization) is a cytogenetic technique th can be used to detect and localize the presence or absence of specific DNA sequences on chromosomes.
It uses fluorescent probes that bind to only those parts of the chromosome with which they show a high degree c sequence similarity. Fluorescence microscopy can be used to find out where the fluorescent probe bound to th chromosome. FISH is used for finding specific features in DNA. These features can be used in geneti counseling, medicine, and species identification. FISH for diagnosis of Philadelphia Chromosome
Translocation: A case of Down 46,XY,t(14;21)

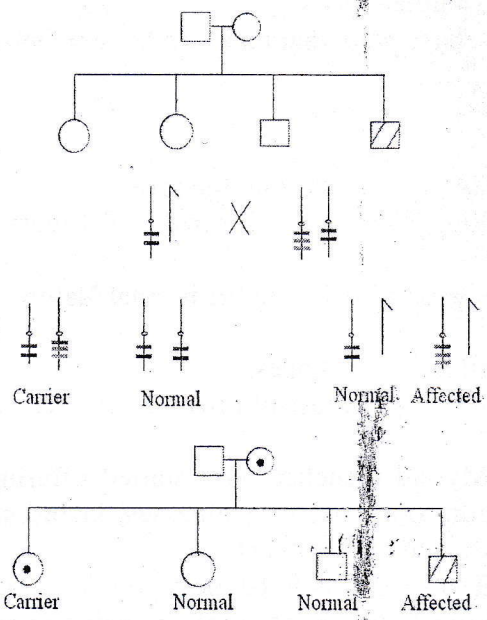
What is molecular biology???**Molecular biology** is the study of biology at a molecular level.
This field overlaps with other areas of biology and chemistry, particularly genetics and biochemistry.

Polymerase chain reaction (PCR) : The polymerase chain reaction is an extremely versatile technique for copyin DNA. PCR allows a single DNA sequence to be copied (millions of times), PCR can be used to cut DNA change particular bases of DNA. PCR can also be used to determine whether a particular DNA fragment is in a genomic library. http://www.maxanim.com/genetics/PCR/pcr.swf
Gel electrophoresis: Gel electrophoresis is one of the principal tools of molecular biology.
The basic principle is that DNA, RNA and proteins can all be separated by means of an electric field.
In agarose gel electrophoresis, DNA and RNA can be separated on the basis of size by running the DNA throug an agarose gel. Proteins can be separated on the basis of size by using an SDS-Polyacrylamide Gel, or on th basis of size and their electric charge by using a 2 dimensional gel.
Southern blotting: method for finding out the presence of a specific DNA sequence within a DNA sample.
Northern blotting: Northern blot is used to study the expression patterns of a specific type of RNA molecule relative comparison among of a set of different samples of RNA.
Western blotting: It is a technique used to analyze different proteins
High-performance liquid chromatography (HPLC) : This technique is used frequently in biochemistry.
It is used to separate components of a mixture by using a variety of chemical interactions between the substanc being analyzed and the chromatography column.
DNA Microarrays: Arrays make it possible to put down a large number of very small spots on a single slide
If each spot has a DNA molecule that is complementary to a single gene, one can analyze the expression of ever gene in an organism in a single expression profiling experiment
Carrier Detection and Prenatal Diagnosis: Various molecular techniques used in diagnostic studies
They have applications in carrier detection and prenatal diagnosis, Uptil now, no cure for genetic disorders
Prevention of transmission of genetic disorders to next generation is essential. This requires genetic counseling for which carrier detection and prenatal diagnosis services are a must.
Prenatal diagnosis includes – amniocentesis, chorionic villus sampling (CVS), percutaneous umbilical bloo sampling (PUBS), preimplantation diagnosis, Prenatal diagnosis
Recording family history, Confirming the diagnosis in the affected individual
Non-invasive techniques : Fetal ultrasound can identify anatomic features of a disorder. Eg: Prenatal diagnosis o alpha-thalassemia in at-risk fetuses. The diagnosis has been suspected as early as 12-13 weeks.
Maternal Serum Marker Screening for Down Syndrome
Ultrasound assessment of the fetus, including nuchal skin fold thickness – to estimate Down syndrome risk.
Amniocentesis : Routinely performed at 15 to 16 weeks gestation. First, an ultrasound examination is performe to assess fetal viability and number, gestational age, fetal anatomy, and placental location. Next, an optima pocket of amniotic fluid is identified, ideally avoiding the fetus, placenta and umbilical cord. Approximately 2(

milliliters of fluid are collected with second trimester amniocentesis. Complications and risks include spontaneous abortion due to : large number of needle insertions, using a needle greater than 18 gauge, discolored amniotic fluid. leakage of amniotic fluid, vaginal bleeding, needle puncture of the fetus.

Chorionic villus sampling (CVS) : Fetal tissue is obtained from the developing trophoblast for diagnostic studies in the first trimester. Performed at 10-13 weeks gestation – 10 weeks is the best time, Prior to the procedure, an ultrasound is performed to assess fetal viability, gestational age and placental position. The fetal nuchal translucency may be measured as well. Slightly higher risk of pregnancy loss associated with CVS compared to second trimester amniocentesis. Procedure is done using either a transcervical or transabdominal approach.

Genetic linkage analysis – Indirect method



Gene therapy

Gene therapy is a technique for correcting defective genes responsible for disease development.

Researchers may use several approaches for correcting faulty genes .....

- A normal gene may be **inserted** into a nonspecific location within the genome to **replace a nonfunctional** gene. This approach is most common.
- An abnormal gene could be **swapped for** a normal gene through homologous recombination.
- The abnormal gene could be **repaired** through selective reverse mutation, which returns the gene to its normal function.

How does gene therapy work?

- In most gene therapy studies, a "normal" gene is inserted into the genome to replace an "abnormal," disease-causing gene.
- The most common vector (vehical) to carry the gene is a virus that has been genetically altered to carry normal human DNA.
- Direct introduction of therapeutic DNA into target cells has some limitations
  - it can be used only with certain tissues and requires large amounts of DNA

What is the current status of gene therapy research?

- The Food and Drug Administration (FDA) has not yet approved any human gene therapy product for sale.
- Current gene therapy is experimental and has not proven very successful in clinical trials.

What factors have kept gene therapy from becoming an effective treatment for genetic disease ?

- Short-lived nature of gene therapy
- Immune response
- Problems with viral vectors
- Multigene disorders

- Preliminary attempts at gene therapy are exorbitantly expensive.

Some ethical and legal questions arise for gene therapy----

- Who will have access to these therapies?
- Who will pay for their use?

Human Genome Project (HGP)

- Completed in 2003,
- 13-year project
- Coordinated by the U.S. Department of Energy and the National Institutes of Health, the Wellcome Trust (U.K.) which funded the Sanger Institute in UK became a major partner; additional contributions :Japan, France, Germany, China.

Project goals were to ----

- identify all the approximately 20,000-25,000 genes in human DNA
- determine the sequences of the 3 billion chemical base pairs that make up human DNA
- store this information in databases
- improve tools for data analysis
- transfer related technologies to the private sector
- address the ethical, legal, and social issues that may arise from the project

- Sequence and analysis of the human genome working draft was published in February 2001 and April 2003 issues of *Nature* and *Science*.
- In May 2006, the sequence of the last chromosome was published in the journal Nature.

Benefits of HGP

- The work on interpretation of genome data is still in its initial stages.
- It is anticipated that detailed knowledge of the human genome will provide new avenues for advances in medicine and biotechnology.
- For example, a number of companies, such as Myriad Genetics has started offering easy ways to administer genetic tests that can show predisposition to a variety of illnesses, including breast cancer, disorders of hemostasis, cystic fibrosis, liver diseases and many others.
- Also, the etiologies for cancers, Alzheimer's disease etc will be better understood from genome information and possibly may lead in the long term to significant advances in their management.
- A researcher investigating a certain form of cancer may have narrowed down his/her search to a particular gene.
- Deeper understanding of the disease processes at the level of molecular biology may determine new therapeutic procedures.
