MEDICAL GENETICS- INTRODUCTION

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- Importance of Genetics to Medicine
- **WORLD HEALTH ORGANIZATION EXECUTIVE BOARD (EB116/3) 116th Session 21 April 2005 describes:**
- Globally, at least 7.6 million children are born annually with severe genetic or congenital malformations
- 90% of these infants are born in mid- and low-income countries.
- In the developed world, genetic and congenital disorders are the second most common cause of infant and childhood death
- occurring with a birth prevalence of 25-60 per 1000
- Infants and Infant Deaths
- 3-5% of all births result in congenital malformations (Robinson, Linden, 1993)
- 0.5% of all newborns have a chromosomal abnormality (Robinson, Linden, 1993)
- 7% of all stillborns have a chromosomal abnormality (Robinson, Linden, 1993)
- 20-30% of all infant deaths are due to genetic disorders (Berry, et al, 1987)
- 30-50% of post-neonatal deaths are due to congenital malformations (Koekelman, Pless, 1998)
- Children and Adults (age 1 and above)
- **11.1% of pediatric hospital admissions are for children with genetic disorders (Scriver, et al, 1973)**
- **18.5% of pediatric hospitalizations are for children with congenital malformations(Scriver, et al, 1973)**
- **50% of individuals found to have mental retardation have a genetic basis for their disability (Emory, Rimoin, 1990)**
- **12% of adult hospital admissions are for genetic causes (Emory, Rimoin, 1990)**

- **15% of all cancers have an inherited susceptibility (Schneider, 1994)**
- **10% of the chronic diseases (heart, diabetes, arthritis) which occur in the adult populations have a significant genetic component (Weatherall, 1985)**
- Prevalence of more common conditions for referral
- **Down syndrome (1/600 live births and increases with advanced maternal age)**
- **Pregnant and age 35 or above (risk of chromosome aneuploidy)**
- **Cystic Fibrosis (1/2500 Caucasian Americans)**
- **Fragile X syndrome (1/1,000 males and 1/800 female carriers of which 30% will be mentally retarded)**
- **Sickle cell disease (1/500 of African American births)**
- **Hemophilia - Factor VIII Deficiency (48/100,000 male births)**
- **Duchenne muscular dystrophy (200/million male births)**
- **Hemochromatosis (1/450 individuals)**
- **Breast cancer (1/8 women of which 5-10% of will have a genetic predisposition)**
- Medical Genetics
- Why We Need Knowledge in Genetics?
  - **realize when genetic factors play a role**
  - **effectively use family hx & genetic tests**
  - **be able to explain genetics concepts**
  - **deal with "risk" & genetic predisposition**
  - **realize personal and societal impact of genetic information**
  - **protect genetic privacy**
  - **use genetics to individualize patient care**
  - **use genetics to preserve health**
- History of Genetics
- People have known about inheritance for a long time.

- Horses appeared in Paleolithic cave art as early as 30,000 BC
- Mid 1800's Discoveries
- Three major events in the mid-1800's led directly to the evelopment of modern genetics.
- 1859: **Charles Darwin** publishes *The Origin of Species*, which describes the theory of evolution by natural selection.
- 1866: **Gregor Mendel** publishes Experiments in Plant Hybridization, which lays out the basic theory of genetics.
- 1871: **Friedrich Miescher** isolates "nucleic acid" from pus cells.
- Gregor Johann Mendel (1822 - 1884)
- Friedrich Miescher (1844-1895)
- Major Events in the 20th Century
- **1900: rediscovery of Mendel's work by Robert Correns, Hugo de Vries, and Erich von Tschermak .**
- **1902: Archibald Garrod discovers that alkaptonuria has a genetic basis.**
- **1904: Gregory Bateson discovers linkage between genes. Also coins the word "genetics".**
- **1910: Thomas Hunt Morgan proves that genes are located on the chromosomes (using Drosophila).**
- More 20th Century Events
- **1926: Hermann J. Muller shows that X-rays induce mutations.**
- **1944: Oswald Avery, Colin MacLeod and Maclyn McCarty show that DNA can transform bacteria, demonstrating that DNA is the hereditary material.**
- **1953: James Watson and Francis Crick determine the structure of the DNA molecule, which leads directly to knowledge of how it replicates**
- **1966: Marshall Nirenberg solves the genetic code, showing that 3 DNA bases code for one amino acid.**
- **1972: Stanley Cohen and Herbert Boyer combine DNA from two different species *in vitro*, then transform it into bacterial cells: first DNA cloning.**
- **2001: Sequence of the entire human genome is announced.**
- Type of genetic disease

- **Chromosomal**
- Mostly rare
- No clear pattern of inheritance
- Usually low risk to relatives
- **Single gene (mendelian)**
- Numerous though individually rare
- Clear pattern of inheritance
- High risk to relatives
- Type of genetic disease
- **Multifactorial**
- Common disorders
- No clear pattern of inheritance
- Low or moderate risk to relatives
- **Somatic mutation**
- Accounts for mosaicism
- Cause of neoplasia
- Estimated prevalence of genetic disease per 1000 population
- **Single gene**
  - Autosomal dominant 2–10
  - Autosomal recessive 2
  - X linked recessive 1–2
- **Chromosomal abnormalities 6–7**
- **Common disorders with appreciable genetic component 6-10**
- Congenital malformations 20
- Total 38–51

- **CHROMOSOME FEATURES**
- Chromosomes are long coiled pieces of DNA, with supporting proteins.
- Genes are short regions of this DNA that hold the information needed to build proteins
- Interphase chromosome
- **Structure of chromosome**
- **CHROMATIDS: two duplicated mitotic prophase chromosomes, each called a chromatid as long as it remains connected to "sister" chromatid.**
- **Chromosome classification**
- Chromosomes are placed into broad categories depending on the position of the centromere.
- **metacentric:** centromere in the middle, with arms of equal length. **Ch. 1**
- **acrocentric:** centromere near one end, with arms of very different lengths **Ch. 6**
- **sub-metacentric:** centromere near the middle, with arms of slightly different lengths. **Ch. 6**
- telocentric: centromere at one end, with only 1 arm (Such telocentric chromosomes are not seen in human cells.
- **EUCHROMATIN**
- When DNA is in it's least condensed form
- Transcribed
- Replicated early
- **HETERO CHROMATIN**
- When DNA is in it's most condensed form
- **Devoid of genes or has inactive genes**
- Not transcribed
- Replicated late
- **Constitutive heterochromatin**
- **-Centromere**
- **-q of Y chromosome**

- **-satellite of acrocentric Ch.**
- **Facultative heterochromatin -Transcriptionally inactive stage e.g. Barr body**
- Chromosome number in different mammals
- **Chromosome classification**
- **Chromosome classification is based on International System for Human Cytogenetic Nomenclature (ISCN) from 1985.**
- **Karyotype**
- **Autosomes are divided into groups A (1 to 3), B (4 & 5), C (6 to 12), D (13 to 15), E (16 to 18), F (19 & 20) and G (21 & 22).**
- **Sex chromosome XX or XY**
- Human Chromosomes
- 46 chromosomes, or 23 pairs.
- 44 of them are called autosomes and are numbered 1 through 22. Chromosome 1 is the longest, 22 is the shortest.
- The other 2 chromosomes are the sex chromosomes: the X chromosome and the Y chromosome.
- Males have and X and a Y; females have 2 X's: XY vs. XX.
- **Normal human karyotype: Male 46, XY**
- **Normal human karyotype: Female 46, XX**
- **Abnormal Karyotypes**
- **ABNORMALITY IN NUMBER**
- **45, X 48, XXXY**
- **47, XY,+21**
- **46, XY+18, -21**
- **70, XXY,+22**
- **45,X/46,XX/47,XXX**
- **STRUCTURALLY ALTERED CHROMOSOMES**

- **46, X,i(Xq)**
- **46, XY,t(2;12)(p24;q15)**
- **46,XY,r(4)(p16q34**
- **Visualizing chromosomes**

Obtain tissue from person

- Fetal tissue: amniocentesis
- chorionic villi sampling
- fetal cell sorting
- Adult tissue: blood (white blood cells)
- cheek swab (buccal cells)
- skin cells
- tissue biopsy

- **CHROMOSOME ANALYSIS OR CYTOGENETIC STUDIES**
- **TECHNIQUE: Collect venous blood → Isolate lymphocytes → Culture → Add PHA → Add Colchicine → Add hypotonic saline → Fix cells → Spread on slides → Stain → Photograph → *Karyotype***
- **STAINS:**
  - **G banding by Giemsa stain**
  - **Q banding**
  - **R banding**
  - **High resolution banding**
  - ***In situ hybridization***
- Chromosome banding
- **CHROMOSOME BANDS: Alternate light & dark stained areas, constant morphology between chromosomes and individuals. Each is numbered & starts with 11 e.g. 11.1**
- **G banding: produced by staining with Giemsa after digesting the chromosomes with trypsin**

• CYTOGENETIC DISORDERS

- **Types of chromosomal disorders**
- Abnormalities in number
- Abnormalities in structure
- **NUMERICAL**
  CHROMOSOME
  MUTATIONS
- **EUPLOID CHANGES**
- Any abnormal euploid condition above triploid can be called “polyploid”.

  The number of sets of chromosomes in a polyploid is designated by the prefix.

  Thus, 3N = triploid
  4N = tetraploid
  5N = pentaploid
  6N = hexaploid
  etc.
- Two types of polyploidy
- **Autopolyploidy:** all of the chromosome sets come from the same species.
  - **Failure of cell division (2N --> 4N)**
  - **produce diploid (not haploid) gametes**
  - **Allopolyploidy**: the chromosome sets come from two or more different species.
  - **2 different species hybridize**
- **Allopolyploidy**
- Allopolyploid- usually a plant contains multiple sets of chromosomes derived from different species.
- Hybrids are usually sterile, because they do not have sets of homologous chromosomes and therefore pairing cannot take place.
- Diploid and Polyploid Varieties of Apples

- **TRIPLOIDY 69+XXX or XXY or XYY**
  - **20% of chromosomally abnormal abortions**
  - **1st trimester-focal trophoblastic hyperplasia, partial mole**
  - **2nd trimester- growth retardation, foetal defects**
  - **Live births are rare, survive for only brief period.**
- **Pathogenesis**
  - **Fertilization error e.g. disprmy**
  - **Failure of meiosis in germ cells i.e. fertilization of a deploid ovum by a haploid sperm & *vice versa*.**
- **Triploidy**
- **TRIPLOIDY**
- **69+XXX or XXY or XYY**

NO RECURRENCE RISK

- extra set of chromosomes is of paternal origin, resulting from dispermy
- extra set of chromosomes is of maternal origin, resulting from failure of extrusion of the polar body,
- Triploidy
- A - Placental tissue
- B - Severe assymitrical growth restriction in 13 week foetus (USG)
- TRIPLOIDY
- **A** - 3-4 syndactyly in triploidy
- B - Partial hydatidiform mole
- **TETRAPLOIDY 92+ XXXX or XXYY**
- Karyotype 92+XXXX or XXYY
- Chromosomally abnormal abortions
- Most are lost during 1st trimester

- Ongoing pregnancy rare
- Growth retardation, multiple malformation
- Pathogenesis: Failure of the 1st cleavage division resulting in doubling in number immediately after fertilization.
- ***NO RECURRENCE RISK***
- ***Aneuploidy:*** **If the chromosome number is <u>not</u> an exact multiple of the haploid number**
- **Aneuploidy is almost always harmful.**

  Imbalanced gene dosage causes the negative effects of aneupoidy.
  Aneuploidy for all chromosomes occurs frequently.

  The reason only trisomy 13, 18 or 21 is seen in live births is because other autosomal aneuploides are embryonic lethal conditions.
- **Aneuploidy is usually due to <u>nondisjunction</u>**
  Non-disjunction during meiosis
- **Trisomy 21**
  Down Syndrome
- **The best known human aneuploidy is Trisomy 21 Down Syndrome (47, +21)**

  This was the first chromosomal mutation to be associated with a particular genetic disease in humans

  Occurs worldwide

  Most common of chromosomal disorders (1 in 800 live birth in the US)
- **Clinical Features**
- **Prenatal →Cystic hygroma, Low maternα feto protein level**
- **Infancy → Characteristic physical featur**
- **Childhood & Adult → 40% have congenital heart disease**
- **Mental retardation (IQ 25-50)**
- **10 to 20 fold increased risk of Acute lukaemias.**

- **Presenile dementia (changes like Alzheimer's)**
- **Abnormal immune response (serious infections, thyroid autoimmunity)**
- Frequency of Dysmorphic Signs in Neonates with Trisomy 21

Dysmorphic sign	Frequency (%)
Flat facial profile	90
Poor Moro reflex	85
Hypotonia	80
Hyperflexibility of large joints	80
Loose skin on back of neck	80
Slanted palpebral fissures	80
Dysmorphic pelvis on radiographs	70
Small round ears	60
Hypoplasia of small finger, middle phalanx	60
Single palmar crease	45

- **Incidence of Some Associated Medical Complications in Persons with Down Syndrome**

Disorder	**Incidence (%)**
Mental retardation	>95
Growth retardation	>95
Early Alzheimer's disease	Affects 75% by age 60
Congenital heart defects	40
Hearing loss	40 to 75
Ophthalmic disorders	60
Epilepsy	5 to 10
Gastrointestinal malformations	5
Hypothyroidism	5

- Leukemia 1
- Atlantoaxial subluxation with
- spinal cord compression <1
- Increased susceptibility to infection Unknown
- Infertility >99% in men; anovulation in 30% of women
- **The incidence, or risk, of Down syndrome is related to <u>maternal</u> age as the following chart shows:**

<u>Mother's age</u>	**<u>Incidence</u>**
20	**1/1550**
25	**1/1050**
30	**1/1200**
35	**1/350**
40	**1/70**
45	**1/25**
48	**1/9**

- **Most often occurs by nondisjunction of chr. 21 during meiosis; in theory could occur in either father or mother, but 95% of these trisomies have defective ovum as source**
- **The reasons for this <u>maternal age effect</u> are not known but it is seen in all aneuploidies, for all chromosomes**
- **All ova are formed by birth and arrested in meiosis;**
- **increased age and the syndrome due to more nondisjunction in older ovum**
- **<u>Chromosome In Dawn's Synd:</u>**
- **95% are TRISOMY 21**
- **4% Robertsonian translocation of 21q to ch. 14 or 22 (t (14q 21a)).**
- **1% are Mosaics (usually 46/47 mosaics)**
- **Obligate Dawn's Synd. region is 21q 22.2 & 21q 23.3**
- **Recurrence risk – 1/200 – 1/100,**
- **Translocation type 1-3% male carriers 10-15% Female carriers**
- **Translocation-type (Robertsonian translocation) Down syndrome has familial pattern of inheritance.**

The entire long arm of 21 is translocated to another chromosome, giving the individual in effect an extra 21

The two types of Down syndrome are clinically indistinguishable.

- Prenatal Screening for Down Syndrome

Screening tests for "high risk" pregnancies

If +ve then further diagnostic testing.

- quick and easy
- more chances of "false-positives" or "false-negatives"
- Diagnostic tests: +ve result very likely the patient has Down baby.
- More expensive and require an elaborate procedure
- Maternal Serum Screening

Combination of different markers on mother's blood

- **Triple test**: alpha-fetoprotein (AFP), unconjugated estriol (uE3), and human chorionic gonadotropin (hCG)
- Quadruple screen: inhibin A is added
- These tests are independent measurements, and when taken along with the maternal age, can calculate the risk of having a baby with Down syndrome.
- These are done in the 15th to 18th week of pregnancy.
- **Alpha-fetoprotein is made in the yolk sac and in the fetal liver. In Down syndrome, the AFP is decreased in the mother's blood**
- **Estriol is a hormone produced by the placenta, using ingredients made by the fetal liver and adrenal gland. Estriol is decreased in the Down syndrome pregnancy.**
- **Human chorionic gonadotropin produced by placenta. The beta subunit, is increased in Down syndrome pregnancies.**
- **Inhibin A is a protein secreted by the ovary, and is designed to inhibit the production of the hormone FSH by the pituitary gland. The level of inhibin A is increased in the blood of mothers of fetuses with Down syndrome.**
- Ultrasound Screening

- The main usefulness of ultrasound is to confirm the gestational age of the fetus
- a strong association between the size of a collection of fluid at the nack of the fetal neck, called nuchal transluceny, and the risk of Down syndrome
- Several other items that can be found during an ultrasound exam {echogenic bowel, echogenic intracardiac focus, and dilitation of the kidneys (pyelctasis)}
- However, these markers as a sign of Down syndrome are still controversial
- Even the best combination of ultrasound findings and other variables is only predictive and not diagnostic.
- For confirmatory diagnosis, the chromosomes of the fetus must be examined (Amniocentesis, Chorionic Villus Sampling)
- **Chromosome analysis in Foetal Down Syndrome**

Diagnostic procedure	Gestational age when test is done (weeks)	Risk of fetal loss (%)
_____		
Chorionic villus sampling	10 to 12	0.5 to 1.5
Early amniocentesis	12 to 15	1.0 to 2.0
Second-trimester	15 to 20	0.5 to 1.0 amniocentesis

- **Trisomy-13**
  Produces Patau syndrome
  Frequency: 2 in 10,000 live births

  Features:
  Cleft lip and palate
  Small eyes
  Polydactyly
  Developmental retardation
  Most die before 3 months

- **Trisomy-18**
  Produces Edwards syndrome

Incidence: 2.5 in 10,000 live births
About 80% are female

Features:
Elongated skull
Low-set malformed ears
Mental and developmental retardation
90% of infants with Edwards syndrome die within 6 months

- **Edward's syndrome – Trisomy-18**
- **Sex Chromosome Aneuploidy**
- MONOSOMY
- Autosomal monosomies are lethal
- **X chromosome monosomy are seen**
- **TURNER SYNDROME**
- **Complete or partial monosomy of X chromosome**
- **Characterized by hypogonadism in phenotypic females**
- **Karyotype**
- **57% are 45 X0**
- **Deletion of small arm 46Xi (Xq)**
- **Deletion of portions of small or long arm**
- **Mosaics 45X/46XY, 45X/47XXX**
- **Only 1% fetuses with 45 X0 survive, 99% aborted**
- *Karyotypic heterogeneity is responsible for significant variation in phenotype*
-
- 1st indication is delayed sexual development
- Sexual infantilism, short stature, webbing of neck
- Primary and secondary amenorrhoea.
- Rarely fertile, offsprings increased chance of Ch. abnormality

- Phenotypes include short stature, webbing at back of neck, incomplete sexual development, hearing impairment

• SEX CHROMATIN BODY

- **Many of the genes on X escape inactivation eg. MIC-2**
- **Genes inactivated are DMD, G 6PD, HPRT etc.**
-
- **Molecular basis of X- inactivation: X- inactive specific transcript gene XIST X 13.**
- **Barr Body**

Inactivated X chromosome can be seen in females cells as the Barr body - Murray Barr (1949)

- **Manifesting heterozygote**
- **A carrier of an X-linked trait who expresses the phenotype of the trait.**
- A higher proportion of normal X chromosomes if inactivated in a given individual, may result in appearance of symptoms of disease in various degrees.
- Ornithine transcarbamylase deficiency (an enzyme deficiency resulting in high blood levels of ammonia and impaired urea formation),...
- Severe disease in males who inherit the mutant X chromosome).
- However, can also affect females who are “manifesting heterozygotes” presenting with severe disease during infancy or later in life during times of metabolic stress—for instance, during viral...
- **HERMAPHRODITISM**
- **Genes on the Y chromosome**

There are three classes of genes on the Y.

Genes shared with X chromosome define the pseudoautosomal regions (PAR)

Genes similar to X chromosome genes are X-Y homologs

Genes unique to the Y including SRY gene

- **Abnormal sexual phenotypes result from mutations in genes involved in sexual development.**

SRY gene Normal female development

Anti-Mullerian hormone **Mullerian ducts persist in male**

- **gene**

Testosterone gene **Early development as female**

Masculinization at puberty

DHT converting enzyme **External structures lack signal**

and develop as female,

internal structures are male.

- Abnormal Development **Hermaphroditism**
- **True hermaphrodism:**
  - **possessing both male and female sexual anatomy**
  - **example: one ovary, one testis, vaginal opening and penis**
- **Pseudohermaphrodism:**
  - **ovaries or testes, but not both**
  - **if ovaries, then male external sexual anatomy**
  - **if testes, then female external sexual anatomy**
- **TRUE HERMAFHRODITISM**
    - Very rare
- Have both TESTICULAR and OVARIAN tissue.
- Internal & External sex organs variable
- Sex hormones also variable
- Majority XX, some XY some XX/ XY
- **PSEUDO HERMAPHRODITISM**
- Have gonad of one sex i.e. testis OR ovary
- Ambiguous genetalia
- Various cause (cytogenetic, mendelian, Teratoganic)

- MALE PSEUDO HERMAPHRODITISM
- Hetergenous group. genetically as well as clinically
-
- **TESTICULAR FEMINZATION**
- X Linked disorder
- genetic males (XY) with a female phenotype
- - gonadal sex correct - gonads differentiate to testis
- - produce MIH – females duct system has degenerated
- produce testosterone and DHT
- **FEMALE PSEDOHERMAPHRODITISM**
- **CONGENITAL ADRENAL NYPERPLASIA (Adrenogenital synd.)**
- **Several genetic & clinical forms, all are AR**
- **Block in a specific step in cortisol biosynthesis**
- **Increased ACTH secretion**
- **Hyperplasia of adrenal gland**
- **Masculinization of female fetus**
- **CONGENITAL ADRENAL NYPERPLASIA**
- **Most common form is 21 – hydroxylase deficiency**
- **Results in 3 different clinical presentations:**
    - **Salt losing**
    - **Simple virilizing**
    - **Late onset virilization**
- **Diagnostic dues – Absence of testis in scrotum**
    - **Presence of a uterus**
    - **Elevated 17- ketosteroid.**

• DNA, GENE AND PROTEIN

- Gregor Mendel (1822-1844) father of modern genetics
- **MENDELISM**
- **1866 Gregor Mendel ► inheritance of "factors" in pea plants.**
- **Mendel's laws ► based on mathematical probabilities ► Predictions of resulting phenotypes when certain crosses were made in the garden pea**
- **Mendel postulated dominant and recessive traits in heredity.**
- Mendel's workplace
- Mendel's monohybrid traits
- **Gregor Mendel experiment**
- Mendel's laws-
- Unit inheritance (Uniformity): Blending of characters do not occur (character of parents may not be expressed in F1, could reappear in later generations)
- Law of segregation: Members of a single pair of characteristics (genes) always segregate and pass to different gametes.
- Independent assortment: Members of different gene pairs assort to the gametes independently i.e. there is random recombination of the paternal and maternal chromosomes in the gametes.
- **Alkaptonuria and Inborn Errors of Metabolism**
- **1908 Archibald Garrod Proposed that some human diseases are due to "inborn errors of metabolism" that result from the lack of a specific enzyme.**
- **Garrod studied the recurrence patterns in several families, realized it followed an autosomal recessive pattern of inheritance, and**
- **postulated that it was caused by a mutation in a gene encoding an enzyme involved in the metabolism of a class of compounds called alkaptans.**
- **He published *The Incidence of Alkaptonuria: a Study in Chemical Individuality* in 1902.**
- AMINO ACIDS

- **DNA (Deoxyribonucleic Acid)**
- DNA is a polymer.
- The monomer units of DNA are **nucleotides**, and
- the polymer is known as a "**polynucleotide**."
- Each nucleotide consists of
  - a 5-carbon sugar (deoxyribose),
  - a nitrogen containing base attached to the sugar, and
  - a phosphate group.
- **DNA (Deoxyribonucleic Acid)**

Four different types of nucleotides found in DNA, differing only in the nitrogenous base.

They are given one letter abbreviations as shorthand for the four bases.

- A is for adenine
- G is for guanine
- C is for cytosine
- T is for thymine
- **DNA**
  Deoxyribonucleic Acid

Adenine and guanine are purines.

Cytosine and thymine are pyrimidines.

bases that bond to form specific pairs:

adenine can only pair with **thymine**

cytosine can only pair with **guanine**

Within the DNA double helix, A forms 2 hydrogen bonds with T on the opposite strand, and G forms 3 hyrdorgen bonds with C on the opposite strand.

The combination of **base pairs** *cannot vary*

- **Deoxyribose Sugar**

- The deoxyribose sugar of the DNA backbone has 5 carbons and 3 oxygens.
- The carbon atoms are numbered 1', 2', 3', 4', and 5' to distinguish from the numbering of the atoms of the purine and pyrmidine rings.
- The hydroxyl groups on the 5'- and 3'- carbons link to the phosphate groups to form the DNA backbone.
- Deoxyribose lacks an hydroxyl group at the 2'-position when compared to ribose, the sugar component of RNA.
- Nucleoside
- A nucleoside is one of the four DNA bases covalently attached to the C1' position of a sugar.
- The sugar in deoxynucleosides is 2'-deoxyribose.
- The sugar in ribonucleosides is ribose.
- Nucleosides differ from nucleotides in that they lack phosphate groups.
- The four different nucleosides of DNA are deoxyadenosine (dA), deoxyguanosine (dG), deoxycytosine (dC), and (deoxy)thymidine (dT, or T).
- Nucleoside
- **Nucleosides can be produced by de novo synthesis in the liver,**
- **But they are more abundantly supplied via nucleic acids in the diet, whereby nucleotidases break down *nucleotides* (such as the thymine nucleotide) into *nucleosides* (such as thymidine) and phosphate.**
- **Nucleosides can be phosphorylated by specific kinases in the cell on the sugar's primary alcohol group (-CH2-OH), producing nucleotides, which are the molecular building-blocks of DNA and RNA.**
- DNA backbone
- The DNA backbone is a polymer with an alternating sugar-phosphate sequence.
- The deoxyribose sugars are joined at both the 3'-hydroxyl and 5'-hydroxyl groups to phosphate groups in ester links, also known as "phosphodiester" bonds.
- **DNA Double Helix**
- DNA is a normally double stranded macromolecule.
- Two polynucleotide chains, held together by weak thermodynamic forces, form a DNA molecule.

- **Features of the DNA Double Helix**
- Two DNA strands form a helical spiral, winding around a helix axis in a right-handed spiral
- The two polynucleotide chains run in opposite directions
- The sugar-phosphate backbones of the two DNA strands wind around the helix axis like the railing of a sprial staircase
- The bases of the individual nucleotides are on the inside of the helix, stacked on top of each other like the steps of a spiral staircase.
- DNA REPLICATION
- DNA replication is a semi-conservative process. One strand serves as the template for the second strand.
- DNA replication is initiated at a region on a chromosome called an origin of replication.
- Enzyme called **DNA Helicase** binds to the origin and unwinds the DNA in both directions from the origin.
- DNA REPLI-CATION
- HOW DNA CODES PROTEINS ?
- Geneticists have long said that humans and chimpanzees, the closest living relative of the humans, share 98.5 % of their DNA
- There are obvious similarities between chimpanzees and humans, but also high differences in body structure, brain, intellect, and behavior etc.
- The specific DNA sequences encode protein or protein variants that other species lack and vice versa.
- A sequence of three bases coding for an amino acid is codon
- **Genes**
- **GENE: Unique sequence DNA that codes for a protein which give rise to a phenotype**
- The basic unit of genetic information
- They determine the nature and the function of the cell.
- **A genome** is the full set of genes in each cell of an organism.
- Gene

- **There are two general types of gene in the human genome:**
- **non-coding RNA genes**
  - **represent 2-5 per cent**
  - **encode functional RNA molecules**
- **protein-coding genes.**
  - **represent the majority**
  - **expressed in two stages: transcription and translation.**
  - **show incredible diversity in size and organisation**
  - **have no typical structure.**
  - **However, several conserved features.**
- The boundaries of a protein-encoding gene are defined as the points at which transcription begins and ends.
- The core of the gene is the coding region
- The coding region begins with the initiation codon, which is normally ATG.
- It ends with one of three termination codons: TAA, TAG or TGA.
- On either side of the coding region are DNA sequences that are transcribed but are not translated.
- **The main characteristics are:**
    - **Coding and non coding exons**
    - **Introns**
    - **Translation start site (ATG)**
    - **Splice sites (GT, donor and AG, acceptor)**
    - **Translation termination site (STOPs: TAG, TGA and TAA)**
- Both the coding region and the untranslated regions may be interrupted by introns.
- Most human genes are divided into exons and introns.
- The exons are the sections that are found in the mature transcript (messenger RNA)

- the introns are removed from the primary transcript by a process of splicing
- The smallest protein-coding gene in the human genome is only 500 nucleotides long and has no introns. It encodes a histone protein
- The largest human gene encodes the protein dystrophin. This gene is 2.5 million nucleotides in length and it takes over 16 hours to produce a single transcript.
- **Definitions:**
- **ALLELE: Alternative forms of both normal and abnormal genes**
- may be variations of normal

e.g., blood group alleles

may result in a medical disorder

e.g., cystic fibrosis, hemophilia, Marfan disease

- **LOCUS**

Genetic locus: specific position of each gene on the chromosomes

- **LOCUS: The physical location of gene on a chromosome is fixed**
  - ***Since human chromosomes are paired, individuals have two alleles at two loci, one on each chromosome.***
- Genotype & phenotype
- **GENOTYPE: Genetic constitution of an individual, which is the specific allelic makeup of an individual.**
- **PHENOTYPE: Observed expression of a gene, is the end result of the genetic and environmental factors.**
- **HOMOZYGUS: Conditions having identical allele at one locus, which can be either normal or abnormal.**

  both alleles the same [dd, DD]
- **HETEROZYGUS: Two different alleles at one locus. Usually one normal and one abnormal or mutant allele. alleles are different [Dd]**

Hemizygous: only one copy (genes on the X chromosome in males)

Trait: observed expression of the gene

- **DOMINANT CONDITION: This implies that a single copy of the allele is enough for the condition to be expressed. It is seen both in heterozygote and the homozygote.**
- **RECESSIVE CONDITION: Seen in homozygote. The allele must be present in both chromosomes.**
- ***Compound heterozygote: Two different mutant allele at one locus.***
- ***Double heterozygote: Two mutant alleles that are each at a different locus.***
- **HETEROGENEITY**

Genetic heterogeneity (locus heterogeneity)

- mutations of different genes causing the same disease

Clinical heterogeneity (allelic heterogeneity)

- mutations of the same gene causing different diseases

- **PEDIGREE CHART**
- Drawing a family history (pedigree) chart is a helpful shorthand method of in documenting
  - affected relatives,
  - identifying patterns of inheritance in families, and
  - identifying those at risk for genetic conditions.

  powerful tools in human genetic studies is pedigree analysis.
- **Standard symbols for the construction of pedigrees.**
- **Pedigree symbols**
- **Pedigree chart construction**
- **POSSIBLE COMBINATION OF GAMETS**
- **Pedigree chart AND Punnet square**

ARE NOT SAME

MENDELIAN DISORDERS

- **Genes and human disease**
- We all carry genes that are potentially hazardous.
- Some are hidden in recessive form and we may never know that we carry them.
- Some will only exert their influence through interactions with environmental triggers.
- Others are manifest from or even before our birth.
- Monogenic diseases result from modifications in a single gene occurring in all cells of the body.
- Though relatively rare, they affect millions of people worldwide.
- Over 10,000 of human diseases are known to be monogenic.
- Pure genetic diseases are caused by a single error in a single gene in the human DNA.
- The nature of disease depends on the functions performed by the modified gene.
- **AUTOSOMAL DOMINANT INHERITANCE**
- The trait appears in **every generation** without skipping
- Every affected child **has an affected parent**
- *Most common scenario in clinical practice:* Heterozygote affected mate with normal homozygote person. In this situation 50% of the child will inherit the trait.
- **Unaffected do not transmit** the trait
- **Both sexes are affected** equally.
- The defective product of the gene is usually a structural protein, not an enzyme
- AD pedigree
- **Factors Which May Alter Presentation of AD Pedigree**
  Exceptions to clear cut Mendelian inheritance
- **New mutations** e.g. Achondroplasia
- **Reduced penetrance** e.g. Polydaetyly
- **Variable expressivity** e.g. Neurofibromatosis
- **Genetic heterogeniety** e.g Sensinnuronal deafness

- **Phenocopy** e.g. Conradi syndrome Vs. Warfarin embryopathy
- **Variation due to sex** e.g. Huntington's disease
- **The Smallest Girl In The World**
- **Lethal alleles**
- ***Some allele combinations are lethal.***
- **Mexican hairless dogs result from a mutation in a**
- **gene that shows lethality**
- **hh hairy the wildtype trait**
- **Hh hairless one mutation present**
- **creates a visible phenotype**
- **HH dies two mutation are lethal**
- Reduced penetrance
- **Expressivity**
- **<u>Co-dominance: ABO blood gr., HLA genes</u>**
- ***Intermediate inheritance :*** *Sickle cell trait*
- **Multiple alleles :**An individual has two alleles, but a population can have many alleles within the individual members.
- <u>Gene</u> <u>Genotype</u> <u>Phenotype (B1.group.)</u>
- OAB OO O AO / AA A
- BB / BO B AB AB
- **Epistasis: Bombay blood group**
- hh genotype = no H protein.

All ABO genotypes appear as type O.

- **Phenocopy**

A trait caused by the environment that appears inherited.

- **environmental influence cause an effect similar to a phenotype under genetic control.**

Exposure to teratogens

- **Thalidomide causes limb defects akin to rare inherited phocomelia.**

Infection

- **Rubella in pregnant mothers causes deafness mimicking inherited forms of deafness.**
- **Genetic heterogeneity**

Individuals with identical phenotypes may reflect different genetic causes.

- Deafness
- Albinism
- Cleft palate
- Poor blood clotting
- **AUTOSOMAL DOMINANT DISORDERS**
- **Examples**
- Familial polyposis coli
- Dentinogenesis imperfecta, Achondroplasia, Marfan's syndrome, Familial hypercholesterolemia, Hungtington's disease.
- **AUTOSOMAL RECESSIVE INHERITANCE**
- Rare traits appear characteristically **in siblings**. Parents and relatives are normal.
- Commonest clinical scenario: Mating of 2 heterozygotes where segregation frequency is **25-50-25**
- **Both sexes are affected** in equal number
- For rare traits, chance of finding **parental consanguinity** is increased
- All children of two affected parents are affected
- AR

Glycogen Storage Diseases are genetic enzyme deficiencies associated with **excessive glycogen accumulation** within cells.

Some enzymes whose deficiency leads to glycogen accumulation are part of the inter-connected pathways shown here.

- Consanguinity
- **X LINKED RECESSIVE INHERITANCE**
- Incidence is **much higher in males** than females
- The trait is **passed from an affected man through all his daughter to average half of their sons**.
- Trait **never transmitted directly from father to son**
- Trait may be transmitted through a series of **carrier females**
- **Carries show variable expression** of the trait.
- X-linked recessive
- **<u>Special featurs:</u> Sporadic case may be due to new mutation Heterozygous females- subtle clinical features, int. enzyme levels**
- **Heterogeneity: Albinism as AR, Ocular albinism as X linked.**
- **<u>Example:</u> Duchanne muscular dystrophy, Haemophilia, Becker muscular dystrophy, Lesch-Nyhan syndrome**
- **X Linked recessive pedigree**
- **X Linked recessive pedigree**
- Duchenne Muscular Dystrophy
- XLR
- Affects one in 3500 to 5000 newborn males
- 1/3 of these with previous family history
- 2/3 sporadic
- Progressive muscle weakness
- Defects in muscle proteins
- Death of muscle tissue
- Mother carries the recessive gene and passes it to her child

- Trait is usually expressed in

  males only

- **X LINKED DOMINANT INHERITANCE**
- **Affected male have no normal daughter & no affected son.**
- **Affected heterozygous female transmit the condition to ½ their children of either sex.**
- **Affected homozygous female transmit to all their children.**
- **Affected females are more common than affected males.**
- Examples: Xg blood group systems, Vit. D resistant rickets, Browning of the enamel of the teeth, Albright's hereditary osteodystrophy , Taybi Syndrome
- XD Pedigree
- XLR vs XLD
- **SEX LIMITED INHERITANCE**
- **In some X-linked recessive diseases (Duchenne muscular dystrophy) expression of the disease phenotype is limited exclusively to males.**
- **In some X-linked dominant traits, such as incontinentia pigmenti expression is limited to females, males do not survive to term.**
- **There are autosomal diseases that are limited to expression in only one sex e.g. Precocious puberty and beard growth (expressed only in males), hereditary form of prolapsed uterus in females**
- Sex-Influenced Traits
- The trait is dominant in one sex but recessive in the other sex
- The best human example is male pattern baldness.
- Baldness is dominant in males: heterozygotes and homozygotes both become bald.
- In females, baldness is recessive: only homozygotes (which are relatively rare) become bald. Also, females tend to lose hair more evenly than men, giving a sparse hair pattern rather than completely baldness.

SINGLE GENE DISEASES THAT DO NOT FOLLOW MENDEL'S LAW

Disorders due to triplet repeat mutation

MITOCHONDRIAL INHERITANCE

Uniparental Disomy and Genomic Imprinting

Gonadal mosaicism

Disorders due to
triplet repeat mutation

Long repeating sequences of three nucleotides, in most cases C and G

Examples: Fragile X syndrome (CGG), Myotonic dystrophy (CTG), Huntington's disease (CAG)

Disorders due to triplet repeat mutation

In the 1940's, geneticists noticed that more males than females were mentally retarded.

Led to the hypothesis that there were 1 or more genes on the X that had a major effect on mental development

Among mentally retarded males, there is a subpopulation which shows a peculiar karyotype:

Their X chromosomes are often broken at a particular site when their cells are cultured in media lacking folic acid.

Fragile X syndrome

Familial mental retardation gene-1 (FMR-1) at Xq27.3 contains tandem repeats of CGG

CGG repeats in normal persons 6 to 46

In transmitting male & carrier female 50 to 230 (premutation)

In affected persons 230 to 4000 (full mutation)

MITOCHONDRIAL INHERITANCE Maternal inheritance

Unlike nuclear DNA, mtDNA is inherited strictly from mother to child.

Any sperm mitochondria that might enter the fertilised egg are destroyed by embryo's cellular machinery

MITOCHONDRIAL INHERITANCE Maternal inheritance

Almost all mitochondrial DNA is maternally inherited

All children of an affected mother an affected & all children of affected father are normal

mtDNA encodes enzymes involved in oxydative phosphorylation. Rich tissue are skeletal & cardiac muscle, kidney, CNS.

Example:_ Kearns- Sayre synd., Laber's optic neuropathy, mitochondrial myopathy

Mitochondrial inheritance prdigree

Mitochondrial inheritance

Expression of disorders is quite variable because of uneven distribution of normal & mutant mtDNA in daughter cells after cell division.

Uniparental Disomy
and
Genomic Imprinting

Uniparental Disomy

Uniparental disomy: Presence of two copies of a chromosome (or part of a chromosome) from one parent and none from the other.

Discovered in 1988 in a child with cystic fibrosis and short stature who received two copies of the same chromosome 7 with a mutant CF gene from her carrier mother, and none from her noncarrier father.

GENOMIC IMPRINTING

Differential expression of genetic traits depending on whether it has been inherited from mother or father.

What is imprinted gene?

For many years a gene was assumed to have the same function, whether it was inherited from the mother or the father.

We now know this is not the case, as the DNA of some genes is modified during gametogenesis and as a result may have altered expression, becoming either inactivated or activated.

Genes that are susceptible to parent specific modification in this way (termed epigenetic, because the modification does not entail mutation of the DNA code) are referred to as imprinted genes.

GENOMIC IMPRINTING

Therefeore,

genetic information in a portion of certain chromosomes is inactivated when inherited from one sex parent but not when inherited from the other.

only one copy of the genes is transcribed in imprinted regions, the other remain genetically silent .

Prader-Willi syndrome (PWS)

The first recognized example of uniparental disomy of an imprinted part of the genome

a multiple congenital anomaly/mental retardation syndrome characterized by infantile hypotonia, feeding problems and failure to thrive, dysmorphia and hypogonadism followed by obesity, mental insufficiency and short stature.

Prader-Willi syndrome results from the absence of the paternal contribution to long arm of chromosome 15 (either by deletion or maternal disomy) which is genetically active and necessary for normal development.

Prader-Willi syndrome (PWS)

Approximately 70% of affected individuals have a small deletion of the long arm of chromosome 15, always occurring in the paternally-derived chromosome 15.

The remaining 30% of patients have *maternal uniparental disomy* for chromosome 15. That is, they have two otherwise normal copies of maternal chromosome 15 and no paternal 15.

The paternal contribution is necessary because the homologous maternally derived genes are inactivated or imprinted (perhaps by methylation).

Angelman syndrome

Angelman syndrome also involves imprinting of the same chromosome region - here the maternal contribution of the critical region is missing.

The critical genetic region which determines Prader-Willi synd. is *maternally* imprinted (i.e. inactivated when inherited from the mother), whereas the critical region which determines Angelman synd. is *paternally* imprinted (i.e. inactivated when inherited from the father).

Both disorders result when the expected active genetic contribution from one parent is missing, either by deletion or uniparental disomy.

Gonadal mosaicism

Mutation in early post-zygotic cells can affect only cells destined to become gonads.

A phenotypically normal parent who has germline or gonadal mosaicsm can transmit the disease to the offspring through mutant gametes.

Gonadal mosaicism

Mosaicism: If a person has two populations of cells -- one normal and one with genetic abnormality.

The symptoms are usually milder to none at all.

Gonadal mosaicism is a different situation.

In this case, the mosaicism is in the parent's ovaries or testes.

Any individual ova or sperm either has the mutation or not.

Therefore, if conception involves one of these mutant sex cells, the resultant child will not be mosaic, but will simply have the genetic disease caused by that particular mutation.

First in the Family: VHL Mosaicism

VHL is generally inherited as an autosomal dominant trait.

There are families in which a child with VHL has parents who do not have VHL. Some people with VHL do not have a VHL genetic mutation. And some unaffected parents are known to have more than one affected child.

Mosaicism may explain why a DNA mutation can not be detected in a person who has VHL tumors and cysts, or why unaffected parents may have one or more affected children.

Somatic Mosaicism. A portion of developing tissue will have the mutated VHL gene. Thus VHL may develop in some, but not all tissue sites.

Germline or Gonadal Mosaicism. Some of the egg or sperm cells have a VHL gene mutation.

● MUTATION

- ***Alteration or change in genetic material*** that is heritable

- ***Genome mutation***
- **Gene mutation**
- Large-scale mutations in chromosomal structure
- Small-scale mutations, such as those affecting a small gene in one or a few nucleotides
- SMALL SCALE MUTATION
- **Mutations** are changes in the nucleotide sequence of DNA
- Spontaneous / induced by mutagenic agents

- Mutations can be caused by copying errors in the genetic material during cell division,
- by exposure to ultraviolet or ionizing radiation,
- chemical mutagens,
- viruses,
- can occur deliberately under cellular control during processes such as hypermutation.
- MUTATION
- Mutations can be subdivided into
- **Germ line mutations** is any detectable, heritable variation in the lineage of germ cells. Mutations in these cells are transmitted to offspring
- **Somatic mutations** which are not transmitted to descendants in animals.
- A new mutation that was not inherited from either parent is called a **de novo mutation**.
- Affect coding / non. coding regions.
- Point mutation
- Often caused by chemicals or malfunction of DNA replication,
- Exchange a single nucleotide for another.
- Classified as transitions or transversions.
- Most common is the transition that exchanges a purine for a purine (A ↔ G) or a pyrimidine for a pyrimidine, (C ↔ T).
- Less common is a transversion
- exchanges a purine for a pyrimidine or a pyrimidine for a purine (C/T ↔ A/G).
- An example of a transversion is adenine (A) being converted into a cytosine (C).
- A point mutation can be reversed by another point mutation, in which the nucleotide is changed back to its original state (true reversion) or
- by second-site reversion (a complementary mutation elsewhere that results in regained gene functionality)
- DNA repair systems

- **Damage reversal**-
- **Damage removal**-
- **Damage tolerance**-
- DNA repair systems
- **damage reversal**--simplest; enzymatic action restores normal structure without breaking backbone
- **1. Photoreactivation**
- **2. Ligation of single strand breaks**
- DNA repair systems
- **damage removal**--involves cutting out and replacing a damaged or inappropriate base or section of nucleotides
- **1. Base excision repair**
- **2. Mismatch repair**
- **3. Nucleotide excision repair**
- DNA repair systems
- **damage tolerance**--not truly repair but a way of coping with damage so that life can go on
- **1. Recombinational (daughter-strand gap) repair**
- **2. Mutagenic repair (trans-lesion synthesis)**
- Types of mutation
- **Stable / Fixed**
- **Dynamic / instable**
- Point mutations that occur within the protein coding region of a gene may be classified into three kinds, depending upon what the erroneous codon codes for:
- **Synonymous / Silent mutations**: which code for the same amino acid (– No alteration of Pr. Product)
- **Non-Synonymous** – Alteration of Pr. Product
- Missense mutations: which code for a different amino acid (**Non-conservative / Conservative)**

- Nonsense mutations: which code for a stop codon and can truncate the protein.
- Types of mutations
- There are many different ways that DNA can be changed, resulting in different types of mutation.
- Synonymous mutation
- Substitution
- Exchanges one base for another eg. A to a G). Such a substitution could:
- change a codon to one that encodes a different amino acid and cause a small change in the protein produced. For example, sickle cell anemia is caused by a substitution in the beta-hemoglobin gene, which alters a single amino acid in the protein produced.
- change an amino-acid-coding codon to a single "stop" codon and cause an incomplete protein. This can have serious effects since the incomplete protein probably won't function.
- Missense mutation
- Insertion
- Insertions are mutations in which extra base pairs are inserted into a new place in the DNA.
- Deletion
- Deletions are mutations in which a section of DNA is lost, or deleted.
- Point mutation (Nonsense)
- Frameshift
- Since protein-coding DNA is divided into codons three bases long, insertions and deletions can alter a gene so that its message is no longer correctly parsed. These changes are called frameshifts.
- Frame shift mutation
- **RNA Mutation – RNA cleavage & Stability mutation / RNA splicing.**
- **Mutagens- Chemical. viral, radiation etc.**
- **Mutagenesis**
- ***– Mutagens always affect a single cell***
    - ***May act at any time of life***

- ***most are repaired***
- ***Most are recursive***
- **Mutation rate-**

- Point Mutations

Normal

Missense

Nonsense

Frameshift (deletion)

Frameshift (insertion)

THE BIG RED DOG RAN OUT.

THE BIG RAD DOG RAN OUT.

THE BIG RED**.**

THE BRE DDO GRA.

THE BIG RED ZDO GRA.

- Functional effects of murations
- **Loss of functions**
- **Dominant negative mutation**
- **Haploinsufficiency**
- **Gain of function.**
- Loss-of-function mutations
- are the result of gene product having less or no function. When the allele has a complete loss of function (null allele) it is often called an **amorphic mutation**.
- Phenotypes associated with such mutations are most often recessive.
- Exceptions are when the organism is haploid, or when the reduced dosage of a normal gene product is not enough for a normal phenotype (this is called haploinsufficiency).
- Gain-of-function mutations

- change the gene product such that it gains a new and abnormal function.
- These mutations usually have dominant phenotypes.
- Often called a neomorphic mutation.
- Dominant negative mutations
- altered gene product that acts antagonistically to the wild-type allele (also called **antimorphic mutations**)
- These mutations usually result in an altered molecular function (often inactive) and are characterised by a dominant or semi-dominant phenotype.
- In humans, Marfan syndrome is an example of a dominant negative mutation occurring in an autosomal dominant disease. In this condition, the defective glycoprotein product of the fibrillin gene (FBN1) antagonizes the product of the normal allele.
- Lethal mutations
- are mutations that lead to the death of the organisms which carry the mutations.
- Back mutation or reversion
- A is a point mutation that restores the original sequence and hence the original phenotype
- BIOCHEMICAL BASIS OF MENDELIAN DISEASE
- ENZYME DEFECTS
- Defective enzyme / Reduced amount of a normal enzyme
- Consequences – Accumulation of the substrate - Decreased end product
- Failure to inactivate a damaging sub.
- DEFECTS IN RECEPTOR & TRANSPORT SYSTEM.
- Familial hypercholesterolaemia, Hartnup's disease
- ALTERATION OF NON-ENZYME PROTEINS
    - Structure / Function / Quantity
- Sickle cell anemia – HbS
- Thalassemia - or β globin chains

- Marfan's synd. – Collagen cross linkage defect.
- ADVERSE REACTION TO DRUGS
- G6PD deficiency → Antimalerial → Severe hemolysis
-
-

MULTIFACTORIAL INHERITANCE

- **Multifactorial inheritance affects greatest number of individuals needing medical attention because of genetic diseases.**
- **Up to 10% of newborn children will express a multifactorial disease at some time in their life.**
- **Examples: Atopic reactions, diabetes, cancer, spina bifida/anencephaly, pyloric stenosis, cleft lip, cleft palate, congenital hip dysplasia, club foot etc.**
- **Some occur more frequently in one sex .**
- **Environmental factors as well as genetic factors are involved.**
- POLYGENIC vs MULTIFACTORIAL TRAITS
- **Polygenic traits are regulated by more than one gene**
- **Traits can also be multifactorial, meaning they have an environmental component**
- **Traits like height, skin color, disease and behavior are all multifactorial traits**
- POLYGENIC TRAITS
- **In a polygenic trait the combined action of many genes produces a continuously varying trait**
- **Multiple genes that regulate height and skin color result in continuously varying traits that exhibit a range of possible phenotypes**
- POLYGENIC INHERITANCE: ***contribution of genes involved***
- The distribution of height in a population if it were determined by one locus with three alleles
- The distribution of height in a population if were determined by two loci, each with three alleles
- MULTIFACTORIAL INHERITANCE
- **Multifactorial inheritance underlies some of the more clinically important human traits including**

- **Heart disease**
    - **Stroke**
    - **Diabetes**
    - **Schiozphrenia**
- **MULTIFACTORIAL INHERITANCE**
  FEATURES
- **Most affected children have normal parents. This is true of diseases and quantitative traits.**
- **Recurrence risk increases with the number of affected children in a family.**
- **Recurrence risk increases with severity of the defect. A more severely affected parent is more likely to produce an affected child.**
- **Consanguinity slightly increases the risk for an affected child.**
- MULTIFACTORIAL INHERITANCE
  FEATURES
- **Risk of affected relatives falls off very quickly with the degree of relationship.**
- **If the two sexes have a different probability of being affected, the least likely sex, *if affected*, is the most likely sex to produce an affected offspring.**
- REGRESSION TO THE MEAN
- **Multifactorial inheritance was first studied by Galton**
- **Galton noticed that extremely tall fathers tended to have sons shorter than themselves, and extremely short fathers tended to have sons taller than themselves.**
- **"Tallness" or "shortness" didn't breed true like they did in Mendel's pea experiments. The offspring seemed to regress to the median.**
- **REGRESSION TO THE MEAN**
- **A representation of Galton's studies on the inheritance of height.**
- **If the son's height were determined only by the father's height, the correlation should be that of the solid line. The dashed line is what is observed. Galton called this "regression to mediocrity."**
- **THRESHOLD MODEL OF DISEASE**

- If multifactorial traits are quantitative traits with continuous distribution, how can they control diseases, such as cleft lip or spina bifida? One either has the disease or doesn't. There is no intermediate.
- As the number of multifactorial genes for the trait increases, the liability for the disease increases. When it reaches a threshold, the liability is so great that abnormality, what we call disease, results.
- **THRESHOLD MODEL OF DISEASE**
- The threshold model for multifactorial traits. Below the threshold the trait is not expressed. Individuals above the threshold have the disease.

• Repetitive DNA

- Repetitive DNA makes up between 20% to 50% of a Human Genome
- Despite its majority, scientists still do not know much about these "junk" DNA
- they are necessary and important to DNA synthesis.
- These repetitive DNA can exists anywhere along a DNA strand
- Interspersed repeats
- Interspersed repeats are repeated DNA sequences located at dispersed regions in a genome.
- They are also known as mobile elements or transposable elements.
- In mammals, the most common mobile elements are LINEs and SINEs.
- Repetitive DNA in humans, two main groups

  1) LINE (Long Interspersed Nuclear Element)
- **2) SINE (Short Interspersed Nuclear Element)**
- **LINE :**
- average length of 6500 base-pairs
- A human genome contains about 60,000 to 100,1000 L1 elements.
- SINE

- **SINE:**
- much shorter in length, 150 to 300 base-pairs in length.
- make up 5% of the Human DNA.
- In humans, the most abundant SINEs is the Alu family.
- A human genome contains about 700,000 to 1,000,000 Alu sites.
- Tandem Repeats
- Tandem repeats occur in DNA when **a pattern of two or more nucleotide bases is repeated** and the **repetitions are directly adjacent to each other.**
- An example would be:
  - **A-T-T-C-G-A-T-T-C-G-A-T-T-C-G**
  - in which the sequence A-T-T-C-G is repeated three times.
  - They include three subclasses: satellites, minisatellites and microsatellites.
  - Satellites
- The size of a satellite DNA ranges from 100 kb to over 1 Mb.
- Most satellites in humans or in other organisms are located at the centromere
- Minisatellites
- The size of a minisatellite ranges from 1 kb to 20 kb.
- One type of minisatellites is called **variable number of tandem repeats (VNTR)**.
- The number of repeats for a given minisatellite may differ between individuals. This feature is the basis of **DNA fingerprinting**.
- Another type of minisatellites is present in the telomere contains tandemly repeated sequence GGGTTA.
- Microsatellites
- Microsatellites are also known as **short tandem repeats** (STR), because a repeat unit consists of only 1 to 6 bp and the whole repetitive region spans less than 150 bp.
- Similar to minisatellites, the number of repeats for a given microsatellite may differ between individuals. Therefore, microsatellites can also be used for DNA fingerprinting.

• DNA POLYMORPHISM

- **Most of our DNA is identical to DNA of others.**
- **However, there are inherited regions of our DNA that can vary from person to person.**
- **Variations in DNA sequence between individuals are termed "polymorphisms".**
- **DNA polymorphism is very useful for DNA analysis.**
- DNA Polymorphisms
- What types of DNA polymorphism exist?
  - **RFLP: Restriction fragment-length polymorphism**
  - **VNTR: Variable number of tandem repeats**
    - **minisatellite**
  - **STR: Short tandem repeats**
    - **microsatellites**
  - **SNP: Single nucleotide polymorphism**
- Although there are many variations in methodology, the basic principal for detection of DNA variability is differences in the size of fragments
    - RFLP
      (Restriction Fragment Length Polymorphism)
- Restriction Enzymes (endonucleases)
- Cut DNA from any source at specific sequences in palindromes (DNA sequences that read the same (5' 3') on both strands).
- They cut within the molecule, they are often called restriction endonucleases.
- If RE cuts straight across the double helix it will produce "blunt" ends.
- If cuts in offset fashion with overhanging piece of single-stranded DNA called "sticky ends" because they are able to form base-pair with complementary sticky end.
- **Example: enzyme HaeIII cuts DNA wherever it encounters the sequence**
  - **5'GGCC3'**
    3'CCGG5'

- **The cut is made between the adjacent G and C.**
- Application of RFLP
- Screening human DNA for the presence of mutant genes
- DNA "fingerprinting"
- Screening for the sickle-cell gene
- Both genes in the patient encode **valine** in the 6th position of the beta chain (betaS) of the hemoglobin molecule.
- "Normal" beta chains (betaA) have **glutamic acid** at this position.
- The only difference between the two genes is the substitution of a T for an A in the middle position of codon 6.
- Screening for the sickle-cell gene
- Mutation abolishes a sequence (CTGAGG, which spans codons 5, 6, and 7) recognized and cut by ***mst*** enzyme
- When the **normal** gene (betaA) is digested with the enzyme and the fragments separated by electrophoresis, the probe binds to a **short** fragment (between the red arrows).
- However, the enzyme cannot cut the **sickle-cell gene** at this site, so the probe attaches to a much larger fragment (between the blue arrows).
- Screening for the sickle-cell gene
- Application of RFLP
- VNTR (Variable Number Tandem Repeat)
- **Hypervariable sections of DNA consist of a short sequence of bases known as a motif, repeated at a variable number of times**
- **example, the top chromosome includes 12 motifs and the lower chromosome 17 motifs. The number of repeats varies by individual from as few as 7 to more than 40.**
- VNTR
- Complex ("fingerprinting") VNTR
- STR (Short Tandem Repeats)
- STRs are repeated sequences of a few (usually four) nucleotides, e.g., TCATTCATTCATTCAT. They often occur in the untranslated parts of known genes

- The exact number of repeats (6, 7, 8, 9, etc.) varies in different people (and, often, in the gene on each chromosome; that is, people are often heterozygous for the marker).
- In the U.S., where 13 STRs — scattered over different chromosomes — are examined, the chance that two people picked at random have the same pattern is less than 1 in 1 **trillion**.
- DNA Polymorphisms
- Single nucleotide polymorphism or SNP
  - **Also a change in a single base pair, but does not necessarily cause a change in a restriction enzyme site**
  - **As compared to RFLP's, SNP's are known to be widely distributed across the genome, occurring approximately once in every 500-1000 bp**
  - **SNP's are identified by sequencing, allele-specific hybridization or allele-specific primer extension**
- DNA fingerprinting
- A method used to identify multilocus DNA banding patterns that are specific to an individual by exposing a sample of the person's DNA to molecular probes and various analytical techniques such as Southern blot analysis.
- DNA fingerprinting is often used to provide evidence in criminal law cases. Also called *genetic fingerprinting*

DNA LABORATORY TECHNIQUES

A brief review of technology

DNA isolation and fragmentation

DNA labeling and hybridization

DNA amplification:

Separation of DNA on basis of size (Electrophoresis/chromatography)

Identification of base sequence in DNA

DNA synthesis

Medical genetics handout: Prof. M. Kamal, BSMMU, May 2012

A brief review of technology

DNA isolation and fragmentation

Phenol-chloroform, salting-out, columns

Mechanical shearing, restriction enzymes

DNA labelling and hybridization

Radioisotopes, fluorescent dye

Denaturation to single-strand with heat

Reformation of complementary double-stranded DNA with slow cooling

A brief review of technology

DNA amplification:

Polymerase chain reaction

Recombinant technology

Electrophoresis

Separation of DNA on basis of size

Column chromatography increasingly replacing electrophoresis for high throughput applications

Identification of base sequence in DNA

Southern Blotting

Dot blot

DNA ISOLATION

DNA isolation is procedure to collect DNA for subsequent molecular or forensic analysis.

Basic steps in a DNA extraction:

Cell disruption or cell lysis,

Removing membrane lipids

Removing proteins

Precipitating the DNA

DNA pellet

DNA fragmentation

The DNA is broken into fragments of appropriate size either by

mechanical shearing (this generates blunt ended fragments),

sonication, or by

using a suitable **restriction endonuclease** for partial digestion of the DNA

DNA labelling and hybridization

Radioisotopes, fluorescent dye

Denaturation to single-strand with heat

Reformation of complementary double-stranded DNA with slow cooling

DNA-DNA hybridization

A technique in which single strand of DNA from one source is bound to a special filter to which is added a single strand of radioactively labelled DNA from a different source.

Complementary base pairing between homologous sections of the two DNAs results in double-stranded hybrid sections that remain bound to the filter, whereas single-strand sections are washed away.

DNA probe

A single-stranded DNA molecule used in laboratory experiments to detect the presence of a complementary sequence among a mixture of other singled-stranded DNA molecules.

DNA synthesis

Oligonucleotide synthesis

Gene synthesis

DNA amplification

Polymerase Chain Reaction

Recombinant DNA

Oligonucleotide synthesis

Oligonucleotide synthesis is the non-biological, chemical synthesis of defined short sequences of nucleic acids.

Synthesized oligonucleotides are single-stranded DNA molecules around 15-20 bases in length up to 160 to 200 bases.

They are most commonly used as primers for DNA sequencing and amplification, as probes for detecting complementary DNA or RNA

Gene synthesis

Gene synthesis is the process of synthesizing an artificially designed gene into a physical DNA sequence.

first demonstrated by Har Gobind Khorana in 1970 for a short artificial gene.

It has become an important tool in many fields of recombinant DNA technology including heterologous gene expression, vaccine development, gene therapy and molecular engineering.

DNA APLIFICATION

PCR (**Polymerase Chain Reaction)**

A *polymerase* is a naturally occurring enzyme that catalyzes the formation and repair of DNA (and RNA).

A heat-stable DNA polymerase enzyme extracted from the bacterium *Thermus aquaticus* is used

What is the *chain reaction*? molecular reproduction technology, the target DNA could be exponentially amplified.

Polymerase Chain Reaction

A technique by which many copies of a specific DNA sequence are produced starting from a few copies of a particular DNA sequence very rapidly

At least a portion of the sequence of the DNA molecule should be known.

Principle of the PCR

The cycling reactions

Denaturation at 94°C

Annealing at 54°C :

extension at 72°C

Reactants

a. DNA

b. primers: small pieces of DNA with base sequence homology to the ends of the DNA to be amplified

c. thermal stable DNA polymerase

d. deoxynucleotide triphosphates (dATP, dTTP, dGTP, dCTP)

Stages of the PCR cycle

Strand separation

The original DNA (the **target** DNA) is heated to 95^0C for 5 minutes and denatured.

It separates into two single strand lengths of DNA.

Stages of the PCR cycle

Primer binding

The solution is rapidly cooled to 54^0C to allow the primers to bind to the complimentary base sequences on each of the single strands of DNA.

This provides starting point for DNA replication

Stages of the PCR cycle

Strand synthesis

Solution is heated to 72^0C

The DNA polymerase catalyses the synthesis of complimentary strand for each of the single strands of DNA

Result is two identical double strands of DNA

Uses of PCR

Used whenever only very small samples of DNA are available.

DNA fingerprinting

Forensic science

Detect inherited disease

Monitor bone marrow transplant

Confirm animal pedigrees

PCR thermocycler

Three major steps in a PCR are repeated for 30 or 40 cycles.

Done on an automated cycler, which can heat and cool the tubes with the reaction mixture in a very short time.

After 30 cycles, single molecule of DNA has been amplified into more than a billion copies (2^{30} = 1.02 x 10^9).

DNA Sequencing

The most popular method for doing this is called the **dideoxy method**.

DNA is synthesized from four deoxynucleotide triphosphate. The top formula shows one of them: deoxythymidine triphosphate (dTTP). Each new nucleotide is added to the 3′ -OH group of the last nucleotide added.

the dideoxy method is also called the **chain termination method**.

Recombinant DNA

Recombinant DNA: New combinations/arrangements of DNA constructed in the laboratory

It has been created artificially from two or more sources incorporated into a single recombinant molecule.

Genetic Engineering: The design and construction of new combinations of genes (DNA)

Construction of recombinant DNA molecules

Gene of interest is isolated from appropriate organism

Gene is recombined with a vector (carrier) DNA molecule

Recombinant DNA is introduced into appropriate host cell

Recombinant DNA is expressed at high levels in host cell

Gene product may be purified for use in treatments (antibiotics, hormones, etc.)

Gene may confer new properties on host cell that carries recombinant DNA (herbicide-resistance, pest-resistance, ability to metabolize toxins, etc.)

Once a gene is cloned, its product may be produced in mass quantity

Some recombinant DNA products being used in human therapy

- Insulin for diabetics
- Factor VIII for males suffering from hemophilia A
- Factor IXor hemophilia B

- Human Growth factor**GH**)
- Erythropoietin (**EPO**) for treating anemia
- three types of interferons
- several interleukins
- (**GM-CSF**) for stimulating the bone marrow after a bone marrow transplant
- **HBsAg** to vaccinate against hepatitis B infection

SOUTHERN BLOTTING

This procedure allows detection of various DNA gene sequences, and is one of the most widely used procedures in molecular biology.

SOUTHERN BLOTTING

Restriction endonuclease cuts down target DNA into small pieces.

SOUTHERN BLOTTING

Agarose gel electrophoresis to separate DNA fragments

SOUTHERN BLOTTING

Transfer of DNA fragments from gel to nitrocellulose membrane

SOUTHERN BLOTTING

DNA fragments transferred to NCM

SOUTHERN BLOTTING

Radioactively labeled probe hybridizes to target DNA fragment

SOUTHERN BLOTTING

Exposed X-ray film shows position of the probe binding.

Dot blot

A Dot blot (or Slot blot) is a technique in molecular biology used to detect biomolecules.

It is a simplification of the northern blot, Southern blot, or western blot methods.

In a dot blot the biomolecules to be detected are **not first separated** by chromatography.

Instead, a mixture containing the molecule to be detected is applied directly on a membrane as a dot.

This is then followed by detection by either nucleotide probes

Dot blot in sickle cell

Dot blot results are shown for a normal control (AA),

a carrier for sickle (AS), and

sickle disease (SS).

The prenatal sample F has both an A and an S signal, indicating a heterozygote or carrier of the sickle gene.

Dot blot: advantages and disadvantages

The technique offers significant savings in time

However, it offers no information on the size of the target biomolecule.

Furthermore, if two molecules of different sizes are detected, they will still appear as a single dot.

Dot blots ***therefore can only confirm the presence or absence of a biomolecule or biomolecules*** which can be detected by the DNA probes or the antibody.

In situ hybridization

Chromosome in situ hybridisation

FISH fluorescence label direct or indirect

Tissue in situ hybridization

Chromosome in situ hybridization

Detection with fluorescence microscopy

Metaphase spreads à double hybridization spots (sister chromatids)

Resolution about 1 Megabase

Chromosome in situ hybridization

Tissue in-situ hybridization

In this procedure a labeled probe is hybridized agianst RNA/DNA in tissue sections

GENETIC TESTING

- **WHAT IS GENETIC TESTING?**
- Genetic testing is the examination of a person's
  - chromosomes,
  - DNA or
  - the biochemical product of a gene
- Results of these tests may
  - confirm or refute a suspected genetic condition
  - or possible predisposition to a condition.
- The DNA examination
- May involve the analysis of the gene itself (direct gene testing)
- Or of short segments of the DNA close to or within a gene (indirect gene tracking).
- **WHAT IS DIRECT GENE TESTING?**
- **When a gene has been located precisely on a chromosome**
- **Where the mutations are known**
- **The gene is examined directly for the presence or absence of mutation**
- **The test is very accurate.**
- **LIMITATIONS OF DIRECT GENETIC TESTING**
- Locus and mutation(s) may not be known.
- There may be many mutations over different length of the gene
- Other genes, environmental factors can affect the expression of the gene.
- **WHAT IS INDIRECT GENE DIAGNOSIS**
- Indirect gene diagnosis or gene tracking or linkage analysis is used when
  - mutation(s) in a gene have not yet been defined or

  - where the DNA region containing the gene is known but the gene itself has not been precisely located.

- Basis of indirect gene Dx.
- Polymorphic markers are special segments of DNA are located very close to the gene (genetic linkage) on the same chromosome.
- These segments nearly always travel with the gene when it is passed from parent to child
- These markers are different in different families.
- The marker may travel with either the correct copy or the mutated gene copy.
- The markers that are linked to the faulty or correct gene copies are
  - special to each family,
  - so this method of genetic testing can only be done within families.
- Indirect gene tracking is a “family test”.
- Principle of linkage analysis
- **The top diagram shows paternal (blue) and maternal (red) chromosomes aligned in a germ cell**
- **Three DNA sequences, labelled A, B and C. Capital letters-paternal alleles and lower case letters maternal alleles.**
- **The middle panel shows the physical process of recombination, which involves crossing over of DNA strands between the paired chromosomes.**
- **The bottom panel shows what happens when the crossover is resolved. The maternal and paternal alleles are mixed (recombined) and these mixed chromosomes are passed to the sperms or ovum.**
- **If A is the disease gene and B and C are genetic markers, recombination is likely to occur much more frequently between A and C than it is between A and B. This allows the disease gene to be mapped relative to the markers B and C.**
- **Gene tracking in a family with Duchenne muscular dystrophy where no mutation has been found in the affected proband III4. Analysis of markers A,B and C has enabled the construction of haplotypes: the affected haplotype is shown by an orange box.**
- **Prenatal diagnosis of genetic diseases**
- Purpose of prenatal diagnosis

- To detect abnormalities in fetal life and allow termination.
- Provide a range of informed choice to the couples at risk of having a child with abnormality
- Provide reassurance and reduce anxiety, especially among high-risk groups
- Purpose of prenatal diagnosis
- Allow couples at high risk to know that the presence or absence of the disorder could be confirmed by testing
- Allow the couples the option of appropriate management (psychological, pregnancy/delivery, postnatal)
- To enable prenatal treatment of the affected foetus
- Indications for prenatal diagnosis
- advanced maternal age
- previous child with a chromosome abnormality
- family history of a chromosome abnormality
- family history of single gene disorder
- family history of a neural tube defect or other congenital abnormalities
- abnormalities identified in pregnancy
- other risk factors (consanguinity, poor obst., history, maternal illnesses
- **Methods of prenatal diagnosis**
- ***Invasive:***
- Amniocentesis
- Chorionic villus sampling
- Cordocentesis
- Preimplatation genetic diagnosis
- Fetoscopy
- ***Non-invasive testing:***
- Maternal serum AFP

- Maternal serum screen
- Ultrasonography
- Isolation of fetal cells from maternal circulation
- Invasive methods of prenatal diagnosis
  Amniocentesis
- Aspiration of 10-20 ml of amniotic fluid through the abdominal wall under ultrasound guidance around the 16 weeks of gestation.
- In about 14 days there will be enough cells for chromosome analysis for biochemical or DNA studies some time a longer time is needed to grow more cells.
- Couples should be informed of the risk of abortions (0,5-1%) and the possibility of termination if wished.
- Amniocentesis procedure
- **Chorionic villus sampling**
- It enables diagnosis in first trimester (10-11 week of gest.) under ultrasound guidance by transcervical or transabdominal aspiration of chorionic villi
- These are fetal cells drived from the outer layer of trophoblast.
- Disadvantage:
  - higher risk of abortion (2-3%)
  - limb abnormalities if carried before the 9 weeks of gestation.
- Chorionic villus sampling procedure
- **Cordocentesis**
- Visualisation of the umbilical vessels by transabdominal ultrasound and enabling fetal blood sampling.
- It is usually used in the management of Rhesus isoimmunization and in some cases to solve the problem of mozaicism.
- Cordocentesis
- **Non-invasive methods of prenatal diagnosis**
  Maternal serum AFP
- Mostly done around the16 weeks of gestation.

- More specific for the diagnosis of NTD (95% of NTD can occur with out a history)
- Amniocentesis was used to confirm the diagnosis but with a good detailed ultrasound first and second degree can be diagnosed
- It has been found that by periconceptional supplementation with folic acid decrease the rate of occurrence of NTD and other abnormalities
- Non-invasive methods of prenatal diagnosis
  Maternal screening test
- It is now a standard practice to offer screening for NTD, Down's synd. and Edward synd.
- Using a blood sample obtained from the mother at the 16 (15-20) weeks of gestation
- It can diagnose up to 75% of NTD and 60-70% of Down's sy.
- Maternal screening test
- **Ultrasonography**
- It is used for obst. diagnosis as placental localisation and multiple preg. As well as for prenatal diagnosis of structural abnormalities which are not associated with known chromosome, biochemical, or molecular defects.
- It is a non invasive with no risk to the foetus or mother
- It is offered to those with a history of genetic disease
- **Ultrasonography**
- Detailed fetal anomaly scanning is offered also to all pregnant women around the 18 weeks of gest. as a screening procedure for structural anomalies (NTD and cardiac anomalies)
- It can identify features which suggest underlying chromosomal abnormality indicating amniocentesis.
- **Problems in prenatal diagnosis**
- Failure to obtain a sample or culture failure
- An ambiguous chromosome result
- An unexpected chromosome result
- The Human Genome Project aimed to sequence the human genome in order to track down the genes responsible for inherited disease in humans.
- There are six main objectives/areas of work of the Human Genome Project.

1. Human gene maps and mapping of human inherited diseases
2. Development of new DNA technologies
3. Sequencing of the human genome
4. Development of bioinformatics
5. Comparative genomics
6. Functional genomics

- Human Chromosome No.3
- A summary map
- Prenatal treatment
- In the most situations the diagnosis of prenatal abnormalities has a subsequent option of *termination of the pregnancy.*
- While this applies in most situations, there is cautious optimism that with the advent of gene therapy prenatal diagnosis will, in time, lead to effective *treatment in utero.*

1. **Treatment of genetic disease by conventional means** requires identification of the gene products and an understanding of the pathophysiology of the disease process.
2. **Gene therapy** can be defined as the replacement of a deficient gene product or correction of abnormal gene. Gene therapy can be carried out either ex vivo by treatment of cells or tissue from an affected individual in culture, with reintroduction into affected individual or in vivo **.**

- Treatment of genetic diseases
- Treatment of the autosomal recessive disorder - congenital adrenal hyperplasia (CAH).
- Affected female are borne with virilisation of the external genitalia.
- There is an evidence that this can be prevented by powerful steroid therapy at early gestational age.
- process of gene therapy
- Examples of gene therapy

Combined immunodeficiency

deficiency of the adenosine deaminase

bone marrow retrovirus

Cystic fibrosis

deficiency of the transmembrane reg. gene

liposomes fusing with epithelial cells

Haemophilia A

gene for factor VIII

liver tissue application into portal vein

Lung carcinoma

K - ras (onkogene) at 30-40% adenocarcinomas

instillation of the mirror gene coding transfer of RNA

block of the decoding p53 tum. suppressor gene at

50-70% of all carcinomas instillation of good work. gene's copy retrovirus - into tumour deposit

- ***Genetic Counselling***

- What is Genetic Counselling?
- Genetic counseling is the process by which patients or relatives, at **risk of an inherited disorder**, are advised of

  -the consequences/nature of the disorder

  -the probability of developing or transmitting the disorder

  -the options open in management and family planning in order to prevent, avoid or accommodate it.

- Genetic counseling involves
- evaluating family history and medical records
- genetic tests
- evaluating the results of this investigation
- helping parents understand and reach decisions about what to do next
- Role of the counsellor
- Assess the risk of a genetic disorder by researching a family's history and evaluating medical records.
- Weigh the medical, social and ethical decisions surrounding genetic testing.

- Provide support and information to help a person make a decision about testing.
- Role of the counsellor
- Interpret the results of genetic tests and medical data.
- Provide counseling or refer individuals and families to support services.
- Explain possible treatments or preventive measures.
- Discuss reproductive options.

THE END