GENETICS

CHROMOSOSMES:

Chromosome means Colored body. When coiled it stains darker and is clumped and is called ***Heterochromatin.*** When extended it is pale staining and is called ***Euchromatin .*** Chromosome is studied best in Metaphase. Its average size is 5μm.

Chromosomes are thick rod shaped structures with two sister chromatids connected at the Primary constriction called Centromere. It has 2 arms : p arm & q arm. There can be secondary constriction and Nucleolar organizer Region on certain chromosomes. Genes are located at specific positions on chromosomes called Gene locus. There are two types of chromosomes called Autosomes & Sex chromsomes . Normal number of chromosomes in an individual is 46. 44 autosomes and 2 sex chromosomes. The chromosomal constitution of an individual is called KARYOTYPE. A diagram representing the chromosomal arrangement is called IDEOGRAM. The process by which a KARYOTYPE is obtained is called KARYOTYPING. Chromosomes are obtained in METAPHASE. Normal karyotype of a female is 44 + XX OR 46, XX and male is 44+ XY OR 46, XY.

Classification of chromosomes is three types:

- Based on position of Centromere : Metacentric , Submetacentric , Acrocentric & Telocentric.
- Standard / Denver classification: Based on chromosomal features such as length of chromosome, position of centromere, relative length of arms chromosomes are divided into 7 groups: A → 1,2,3 .. B→ 4,5 .. C→ 6,7,8,9,10,11,12, X ..D→ 13,14,15 ..E→ 16,17,18 .. F→19,20 ..G→21,22, Y
- Paris Nomenclature: Both p arm and q arms are divided into of regions numbered 1,2 & 3. These regions are further subdivided into bands so as to give precise locations.

X chromosome: The X chromosome is one of the two sex-determining chromosomes, is found in both males and females. It is a part of the XY sex-determination system. The X chromosome is notably larger and has a more active euchromatin region. X chromosome is a submetacentric chromosome and it is placed in the group "C" in Denver classification.

Y chromosome : The Y chromosome is one of two sex chromosomes. Y is the sex-determining chromosome, since it is the presence or absence of Y that determines the male or female sex of offspring produced in sexual reproduction. The Y chromosome contains the gene SRY, which triggers testis development. The Y chromosome is passed only from father to son. It is an acrocentric chromosome placed in the "G" group in Denver classification.

KARYOTYPING : The process by which a KARYOTYPE is obtained is called KARYOTYPING. Chromosomes are obtained from somatic cells by culture. Rapidly dividing cells used like lymphocytes, fibroblasts, BM cells, chorionic villi & amniotic fluid cells. There are Short term & Long term cultures. Long term uses fibroblast & amniotic fluid. Usually lymphocytes from peripheral blood culture is used. Peripheral blood culture involves following steps:

1**. Collection of blood**: Blood is collected from peripheral vein under sterile conditions in a heparinised syringe.
2. **Planting:** Blood samples transferred to 2-3 culture vials per sample. Each vial contains: Culture medium – Commonly used media are HAM F10, TC199, RPMI, Fetal calf serum or human AB serum to nourish cultured cells, Phytohemagglutinin, a mitogenic agent which promote the rate of mitosis and Antibiotics , usually penicillin & streptomicin combination to prevents bacterial growth.
3**. Incubation**: Culture vials put into incubator at 37°C FOR 3 DAYS. During this period lymphocytes in the blood undergo mitosis.
4. **Harvesting**: 70 hrs after planting, COLCHICINE is added to culture. It arrests mitosis in metaphase by preventing formation of spindle tubules. Two hrs after addition of colchicines the contents are centrifuged for 5 minutes and the supernatant is discarded. Pellets containing cells are treated with hypotonic solution. Cells swell & chromatids separate.Cells are resuspended in fixative containing Glacial acetic acid & Methanol in 1: 3 proportion & changed successively 3 times in fixative. Cells suspended in half ml of fixative is dropped from height on chilled slides.
5. **Staining**: Staining is essential for Chromosome analysis. One of the following banding techniques are used:
a.G- banding: Slides are stained with Geimsa solution – routinely used.
b.Q- banding: Chromosomes stained with quinacrine mustard.
c.R- banding: Chromosome preprations are preheated in a buffer & stained with Geimsa. Gives a banding (R- banding) pattern reverse of Q or G – banding.
d.C- banding: This is selectively for staining centromeric regions or regions with secondary constrictions.
e.NOR staining: Ammoniacal silver is used as stain. Stains nucleolar organising regions.

FISH –New diagnostic procedure in which DNA probes are used.
APPLICATIONS OF KARYOTYPING:
1. Clinical diagnosis – Indicated in patients with congenital anomalies, mental retardation & ambiguous genitalia.
2. Gene mapping – Localization of human genes
3.Role in cancer – Detection of Pheladelphia chromosome in CML.
4.Repeated fetal loss – May reveal a chromosomal defect in any one partner.
5.Prenatal diagnosis – Analysis of cells in amniotic fluid or chorionic villous to reveal chromosomal abnormality in fetus.

SEX CHROMATIN

Barr & Bertram in 1949 found that some cells show a chromatin mass in their nuclei. It is observed in females interphase nuclei. This is called the **sex chromatin or barr body.** Cells containing this is called chromatin +ve & others, chromatin –ve. Barr body represents one of the two X chromosomes of female cell. This remains condensed & in inactive state throught interphase. Identification of sex chromatin can be done in any cell – skin, oral, vagina or urethral epithelium or blood cells. Buccal mucosa is commonly used. Scrapings from inner side of cheek is taken on a slide & smeared. It is fixed with alcohol & stained with Thionin. If cells are chromatin +ve sex is identified as female. Number of barr bodies in different karyotypes are: Normal male (XY) – No barr body, Normal female (XX) – One, Turner Syndrome(XO) – No barr body, Klinefelter's Synd. (XXY) – One, and Triple X Syndrome (XXX) – Two.

LYON'S HYPOTHESIS: Dr. Mary F Lyon stated her hypothesis about X inactivation in 1962. The processes of X inactivation is referred to as LYONIZATION.
LYON'S HYPOTHESIS states that: In female somatic cells only one X chromosome is active. Other inactive X appears as sex chromatin. Inactivation occurs in early embryonic life. Inactivation is random but fixed. Inactive X can be Maternal or Paternal – in different cells of same individual. Once a particular X chromosome becomes inactive then the daughter cells follow the decision. During mitosis the inactive X replicates late. Mechanism of inactivation is DNA methylation.

GENETIC DISORDERS

- Chromosomal disorders.
- Single gene disorders or Mendelian inheritance.
- Polygenic or Multifactorial inheritance.

CHROMOSOMAL DISORDERS

- Normal Chromosome number – 46XY / 46XX
- Chromosomal Aberration – Any deviation either in number or structure of chromosomes.
- Diploid – 2n (Normal)
- Haploid – n (Gamates)
- Polyploid – Multiple of n (Triploid-69, Tetraploid-92)
- Aneuploid – Any number which is not exactly a multiple of n (47 or 45)

- NUMERICAL ABBERATIONS: Can be Autosomal abnormalities or Sex chromosome abnormalities
- STRUCTURAL ABBERATIONS: Chromosomes have a missing portion or a portion which is represented twice. Another variety can be Chromosome breakage. Important structural abnormalities can be Deletion, Duplication, Inversion, Ring chromosome, Isochromosome or Translocation
- DIFFERENT CELL LINES (Mosaicism /chimaerism)

STRUCTURAL ABBERATIONS:

Deletion: The term "deletion" simply means that a part of a chromosome is missing or "deleted." A very small piece of a chromosome can contain many different genes. When genes are missing, there may be errors in the development of a baby, since some of the "instructions" are missing.
2 types: Microscopic deletion (Cri – Du – Chat) – Terminal deletion of short arm of chromosome 5, AND Submicroscopic deletion (Prader Willi) – Interstitial deletion or microdeletions.

Duplication: It means that a part of a chromosome is duplicated, or present in two copies. This results in having extra genetic material, even though the total number of chromosomes is usually normal. Since a very small piece of a chromosome can contain many different genes, the extra genes present in a duplication may cause those genes to not function properly.

Inversion: Single chromosome breaks at two points and rearranges itself by inverting its position.
2 types: Pericentric, in which there are two breakings, including centomere, at both long and short arms. After the breaking, the piece is merged again in the form of reverse. & Paracentric in which there is a break off, not including centomere, at either long or short arms. After the breaking, the piece is united again in the form of reverse.

Ring Chromososme: Terminal ends of both arms are lost and they subsequently fuse to form a closed circle. Ring chromosomes may form in cells following genetic damage by mutagens like radiation, but they may also arise spontaneously during development. Usually results in chromosomal mosaics.

Isochromosome: Incorrect splitting of centromere. Duplication of one entire chromosome arm & deletion of other chromosome arm.

Translocation: Exchange of genetic material between two chromosomes . It is of 2 types :
Reciprocal – Exchange of chromosome material distal to breaks. Amounts to a balanced translocation and no chromosome material is lost.
Robertsonian – It is observed between two homological or non-homological two acrocentric chromosomes is called centric translocation. On this rearrangement long and short arms of two different chromosomes merged with themselves. United short arms disappear and just two different long arms merged their centromeric areas are observed. These disappeared chromosome pieces of short arms do not make an effect because of having multiple copies of rRNA genes just like at acrocentric chromosomes. At this patients have 45 chromosomes. It is one of the genetic cause of Down's syndrome.

NUMERICAL ABBERATIONS:

It can be Polyploidy (having more than two complete sets of chromosomes.) or Aneuploidy (having a chromosome number that is not a multiple of the haploid number). It can be in autosomes or sex chromosomes.

Numerical anomalies of autosomes are trisomy.

DOWN'S SYNDROME: (MONGOLISM)

It is trisomy of autosome 21. Incidence is 1 in 700 live births. Males are more affected.

Clinical features:

- Mental retardation
- Poor growth, short stature, Hypotonia
- Small head circumference, Malformed low set ears, flat nose with low nasal bridge
- Eyes : Epicanthal folds, Mongoloid slant due to slopping palpebral fissure Protruding tongue with open mouth
- High arched palate, Delayed dentition
- Short broad hands, Simian crease
- Cardiovascular defects

Numerical anomalies of sex chromosomes can be Trisomy, Eg. XXY : Klinefelter syndrome and XXX : Super female OR Monosomy , Eg. XO : Turner's syndrome

TURNER'S SYNDROME:

It is Monosomy. Karyotype is 45,XO. Incidence is 1 in 5,000 – 10,000 live births.

Clinical features:

- Affected individual is a female
- Short stature
- Pterygium colli (Webbing of neck)
- Cubitus valgus
- Low posterior hair line
- Broad chest with widely spaced nipples Shield chest
- High arched palate
- Lymphedema over feet
- Cardiovascular anomalies: Coarctation of aorta
- Streak like gonads, ovaries contain only connective tissue with no follicles
- Primary amenorrhea
- Secondary sexual characters are absent
- Abnormalities of Urinary System

KLINEFELTER'S SYNDROME:

Trisomy. Karyotype is 47, XXY. Incidence 1 in 1000 New born males.

Clinical features

- Affected individual is a male
- Detected during adolescence or during treatment for infertility
- Thin , tall, long legs
- Poorly developed secondary sexual characters
- Small testis, gynecomastia.

SINGLE GENE DISORDERS OR MENDELIAN INHERITANCE.

Single gene disorders are due to a single mutant gene. Also called Mendelian disorders. The traits of inheritance are determined by a single gene. It follows Mendel's laws of inheritance. It is classified into : Autosomal inheritance and Sex-linked inheritance. Sex linked disorders are further classified into X-linked and Y-linked. Each of these are further divided into dominant and recessive.

AUTOSOMAL DOMINANT INHERITANCE:

Disorder manifests even in single dose. Heterozygotes also manifests. Some disorders d/t AD inheritance:

- Huntington's disease
- Myotonic dystrophy
- Hypercholesterolemia
- ABO & Rh blood groups
- Common baldness
- Polyctstic kidney
- Achondroplasia
- Congenital cataract

Characteristics of Autosomal dominant inheritance are: Males & females equally affected. Disorder is seen in every generation. An affected person will always have an affected parent. Affected person will have normal and abnormal offsprings in equal proportion. Normal offsprings do not transmit the disorder.

AUTOSOMAL RECESSIVE INHERITANCE:

Expression of the disorder occurs only in homozygous state. Common disorders inherited by this mode are:

- Sickle cell anemia
- Schizophrenia
- Cystic fibrosis
- Albinism
- Alkaptonuria & Phenylketonuria
- Spinal muscular dystrophy
- Blond hair

Characteristics of Autosomal recessive inheritance are: Expressed only in homozygous state. Heterozygous person does not express. They are perfectly healthy and are called as CARRIER. Males & females equally affected. Risk is about 25% (1 in 4). Consanguinity increases manifestation of recessive disorders.

Y – LINKED INHERITANCE:

Affected male transmits to all his sons. Daughters are unaffected. Only males affected. Females never transmit the trait. Eg. Hairy pinna – Y-linked trait.

X – LINKED INHERITANCE: It can be dominant or recessive.

X – LINKED RECESSIVE DISORDERS:

- Diabetes mellitus
- Haemophilia
- Colour blindness
- Duchenne Muscular Dystrophy
- G6PD deficiency

X – LINKED RECESSIVE INHERITANCE:

Predominantly males are affected. Females affected only if she is homozygous. Disorder transmitted through carrier females to their sons. Affected males do not transmit to their sons. They transmit through all their daughters to half of their sons. Affected males usually have normal parents.

X – LINKED DOMINANT DISORDERS:

- Vitamin D – resistant rickets
- Hypophosphatemia

X – LINKED DOMINANT INHERITANCE:

Resembles autosomal dominant inheritance.Expressed in heterozygous females as well as males.

MULTIFACTORIAL INHERITANCE

A multifactorial trait is defined as one that results from a combination of genetic as well as environmental factors. Each gene contributes a small additive effect to the final phenotype expressed. The overall variance of the expression of phenotype is the sum of genetic and environmental factors. Heritability provides information of the importance of genetic factors in the causation of disease. It is more common than Mendelian inheritance. It shows familial clustering without any definite pattern as in Mendelian inheritance. Incidence in close relatives of affected individuals is low (2 to 4%) compared to Mendelian inheritance. Examples: Medical diseases- Cardiac disease, diabetes mellitus, asthma; Congenital diseases- Neural Tube Defects, cleft lip and palate; Neuropsychiatric diseases- Schizophrenia

MUTATION AND POLYMORPHISM

Any change in sequence of genomic DNA is called mutation. In human genetics, the term mutation is reserved for DNA sequence changes that cause genetic diseases and are consequently rare with a population frequency less than 1%. DNA sequence variants that are more common in populations (frequency more than 1%) are conventionally described as polymorphism.

Single base substitution or point mutation- It is a single base change in DNA sequence. This alters the triplet code. Since the code is degenerate, all substitutions may not alter the amino acid, such mutations are called silent substitutions. If there is a change in the amino acid and final product, it is called missense mutation. A change in the triplet code to a stop codon, nonsense mutation, results in premature cessation of transcription. Deletion or insertion of one or more base pairs- it leads to an alteration in the reading frame of DNA strand and thus the amino acid sequence. This is called frame shift. If the mutation occurs at promoter site or splice site, it can alter regulation of transcription or translation.

GENETIC COUNSELLING

Definition: A process in which patients or their relatives at the risk of a genetic disorder are made aware of the consequences of the disorder, its transmission and the ways by which this can be prevented . Steps in genetic counseling are:

1.Accurate diagnosis:

- History: It should include present & relevant past history, Family history, Obstetric history and any history of Consanguinity.
- Pedigree analysis: Constructing a pedigree with proper interrogation is an indispensable step towards counselling
- Estimation of risk: Forms one of the most important aspect of genetic counseling. The following points to be taken into account: Mode of inheritance, Analysis of pedigree and results of various studies. After that probability should be worked out.

2. Advising the patient and family members: In this various factors are involved like, psychology of the patient, emotional stress, attitude of family members towards patient, educational, social & financial background of family members, ethical, moral & legal implications. Gaining confidence and good communication skill is also essential.

3. Management of the disorder – It aims naturally for prevention rather than cure. Treatment is directed towards minimizing the damage by early detection and preventing further irreversible damage.